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"From the century of the genome to the century of the organism: New theoretical approaches"

Guest editors: A.M. Soto & G. Longo

1. Preface

2. Longo G, Soto AM. ***Why do we need theories?*** Prog Biophys Mol Biol. 2016 Jul 4. pii: S0079-6107(16)30063-3. doi: 10.1016/j.pbiomolbio.2016.06.005. [Epub ahead of print] Review. PubMed PMID: 27390105.

3. Perret N, Longo G. ***Reductionist perspectives and the notion of information.*** Prog Biophys Mol Biol. 2016 Jul 22. pii: S0079-6107(16)30082-7. doi: 10.1016/j.pbiomolbio.2016.07.003. [Epub ahead of print] Review. PubMed PMID: 27458079.

5. Soto AM, Longo G, Montévil M, Sonnenschein C. ***The biological default state of cell proliferation with variation and motility, a fundamental principle for a theory of organisms.*** Prog Biophys Mol Biol. 2016 Jul 2. pii: S0079-6107(16)30078-5. doi: 10.1016/j.pbiomolbio.2016.06.006. Review. PubMed PMID: 27381480.

6. Mossio M, Montévil M, Longo G. ***Theoretical principles for biology: Organization***, Progress in Biophysics and Molecular Biology, Available online 10 August 2016, ISSN 0079-6107, http://dx.doi.org/10.1016/j.pbiomolbio.2016.07.005. (http://www.sciencedirect.com/science/article/pii/S007961071630089X).

7 Montévil M, Mossio M, Pocheville A, Longo G. ***Theoretical principles for biology: Variation***, Progress in Biophysics and Molecular Biology, Available online 13 August 2016, ISSN 0079-6107, http://dx.doi.org/10.1016/j.pbiomolbio.2016.08.005.

10. Ana M. Soto, Giuseppe Longo, Paul-Antoine Miquel, Maël Montevil, Matteo Mossio, Nicole Perret, Arnaud Pocheville, Carlos Sonnenschein, ***Toward a theory of organisms: Three founding principles in search of a useful integration***, Progress in Biophysics and Molecular Biology, Available online 4 August 2016, ISSN 0079-6107,

From the century of the genome to the century of the organism: New theoretical approaches

Special issue of Progress in Biophysics and Molecular Biology

Guest editors: A.M. Soto & G. Longo

Contents

Preface to

FROM THE CENTURY OF THE GENOME TO THE CENTURY OF THE ORGANISM: NEW THEORETICAL APPROACHES

This focused issue of *Progress in Biophysics and Molecular Biology* is entitled "From the century of the genome to the century of the organism: New theoretical approaches." It was developed during Ana M. Soto's tenure as Blaise Pascal Chair of Biology 2013-15 at the Ecole Normale Supérieure (ENS, Paris, France). Giuseppe Longo was the Pascal Chair host at the ENS. This ongoing theoretical work was also used as the content of a 10 session course attended by graduate students and post-graduates, which took place at the National Museum of Natural History and at the ENS. The attendants of the course encouraged the guest editors to make this material easily available, hence the origin of this PBMB issue.

The reason for such an issue of the journal is that biology in the 21st century will need such approaches as it tackles more complex interactions in organisms. Unravelling and understanding complexity is a very different kind of investigation from identifying the components of an organism, their structures and their chemical interactions, which formed the basis of successful biological research in the 20th century. Beyond the interactions of a few components, the behavior of complex networks becomes very difficult to predict from the behavior of individual components in isolation, and the behavior of the ensemble is often counter-intuitive. This fact has been understood since the work of great theoretical biologists since at least the 1950s (Bertalanffy, 1969; Weiss, 1970; Weiss, 1977). Rigorous development of theoretical approaches is therefore necessary.

Yet, during most of the twentieth century experimental and theoretical biologists lived separate lives. Very few experimental biologists read and studied the work of theoretical biologists. So, the two did not interact in the way they naturally do in other sciences.

For example, it is inconceivable that experiments in physics could be done without extensive mathematical theory being used to give quantitative and conceptual expression to the ideas that motivate the questions that experimentalists try to answer. It would be impossible for the physicists at the large hadron collider, for example, to search for what we call the Higgs boson without the theoretical background that can make sense of what the Higgs boson could be. The gigantic masses of data that come out of such experimentation would be an un-interpretable mass without the theory.

So, how did experimental biology apparently manage for so many years without such theoretical structures?

Actually, it didn't. The divorce was, in a sense, only apparent.

First, there was a general broad theory provided by the theory of evolution, which deals with phylogeny, a large time-scale phenomenon. But it lacks a theory of organisms, which will encompass one life cycle, from conception to death. The main long-term objective of the Pascal Chair research on theoretical biology has been and still is to elaborate a theory of organisms. The immediate objective was to identify principles that could be used to frame such a theory. To accomplish the latter, we formed a research group that we called the ORGANISM group. The members of the group had already been collaborating on theoretical issues with Soto, Longo, or both. For example, Sonnenschein and Soto (Sonnenschein & Soto, 1999) on the default state of proliferation and motility, Longo and Montévil (2014) on the principle of variation, and Mossio, Montévil and Longo on the principle of organization. In addition to this theoretical work, we had also collaborated on related issues such as the inadequacy of concepts derived from mathematical theories, like information, program and signalling (Longo, Miquel, Sonnenschein, Soto, 2012), and philosophical issues, such as downward causation and physicalism (Soto, Sonnenschein & Miquel, 2008). Central to the theoretical work of the ORGANISM group is the realization that there are differences between the inert and the living that

require theoretical development. Conservation laws and a priori phase-space are central to theoretical development in physics and to the mathematical elaboration of such theories. In biology, instead, ontogenesis and evolution are about relentless changes of symmetries, and the phase-space is being created along, rather than set a priori. The ORGANISM group expertise, in addition to theoretical biology, ranged from ecology and experimental biology (Pocheville, Sonnenschein, Soto), mathematics (Longo and Montévil), physics (Montévil), and philosophy (Miquel, Mossio and Perret).

The problem with the standard theory of evolution is that the formulation of the Neo-Darwinist Modern Synthesis ignored much of what the developing theories of complexity showed through using a strongly gene-centric approach. The gene-centric approach is important as a reductive procedure, but it is only one of the ways of studying and interpreting the functioning of organisms. Viewing organisms from the viewpoint of their functional phenotypes is equally important.

Second, there *was* theory in biology. In fact, there were many theories, and in many different forms. Moreover, these theories were used by experimental biologists. They were the ideas in the minds of experimental biologists. No science can be done without theoretical constructs. The so-called Central Dogma of Molecular Biology, for example, was an expression of the background of ideas that were circulating during the early heydays of molecular biology: that causation was one way (genes to phenotypes), and that inheritance was entirely attributable to DNA, by which an organism could be completely defined. This was a theory, except that it was not usually formulated as such. It was presented as fact, a *fait accompli*. Meanwhile the pages of journals of theoretical and mathematical biology continued to be filled with fascinating and difficult papers to which experimentalists, by and large, paid little or no attention.

We can call the theories that experimentalists had in mind implicit theories. Often they were not even recognised as theory. But that means that they were not properly developed as rigorous theories in the way that is common in physics. The consequence is that, just as physicists would not know what to do with the gigantic data pouring out of their colliders and telescopes without a structure of interpretative theory, biology has now hit up against exactly the same problem. There is therefore an essential incompleteness in biological theory that calls out to be filled.

The reason that there is no fully-developed current theory of biological organization lies in the multi-level nature of biological interactions, with lower level molecular processes just as dependent on higher-level organisation and processes, as they in their turn are dependent on the molecular processes (Soto et al 2008). The error of twentieth century biology was to assume far too readily that causation is one-way.

In an important book, *Perspectives on Organisms: Biological time, Symmetries and Singularities* (Longo & Montevil, 2014), the authors write, "the molecular level does not accommodate phenomena that occur typically at other *levels of organisation*." Denis Noble encountered this insight in 1960 when he was interpreting experimental data on cardiac potassium channels using mathematical modelling to reconstruct heart rhythm. The rhythm simply does not exist at the molecular level. The process occurs only when the molecules are constrained by the whole cardiac cell to be controlled by causation running in the opposite direction: from the cell to the molecular components. This insight is general. Of course, cells form an extremely important level of organisation, without which organisms with tissues, organs and whole-body systems would be impossible. But the other levels are also important in their own ways. Ultimately, even the environment can influence gene expression levels. Between the genes and the environment there is a whole organism whereby these levels of organisation are entangled. Organogenesis, for example, requires the reciprocal interaction between different tissues, a fact that inspired Soto and Sonnenschein to postulate the tissue organization field theory of carcinogenesis, whereby cancer is understood as a relational problem akin to organogenesis and tissue remodeling (Sonnenschein, & Soto, 1999). There is no *a priori* reason to privilege any one level in causation. This is the principle of biological relativity (Noble 2012).

The avoidance of engagement with theoretical work in biology was based largely on the assumption that analysis at the molecular level could be, and was in principle, complete. The articles in this issue of the journal seek to engage at one and the same time with experimentalists and with other theoreticians. They engage with experimentalists by suggesting possible experiments, and with theo-

reticians by exploring the boundaries of theoretical work, i.e. the metaphysics without which theory is impossible.

We now turn to the articles gathered together in this issue.

Longo & Soto: Why do we need theories? The authors present an overview of the role of theories in physics, as well as of the principles of construction and proof is used as a point of departure to identify differences between the observables in physics and those in biology. In contrast to physical objects, organisms are not generic but specific. They undergo incessant changes which represent the breaking of symmetries, and thus the opposite of conservation principles, a central component of physical theories. Additionally, while in physical theories the phase-space is set *a priori*, in biology it is not pre-determined, but generated along the way. These distinctions are fundamental for the construction of a theory of organisms.

Perret & Longo: Reductionist perspectives and the notion of information. This essay focuses on a critique of two stances that have dominated the practice of biological research in the second half of the 20^{th} century: physicalist reductionism, and the misuse of the notions of information, program and signal, which were transplanted from mathematical theories of information.

THREE PRINCIPLES FOR A THEORY OF ORGANISMS

I. Soto, Longo, Montévil & Sonnenschein: The biological default state of cell proliferation with variation and motility, a fundamental principle for a theory of organisms. Unlike physical objects living ones such as cells are characterized by agency (the capacity to initiate action), normativity (the capacity of generating their own rules) and individuation (the ability to change one's own organization). Agency is at the core of the default state. In analogy to Galileo's inertia, we propose a foundational principle, the biological default state. The biological default state is implied in Darwin's "descent with modification". Like the principle of inertia, the biological default state does not require an explanation; what require an explanation are departures from it (quiescence, lack of variation and lack of movement).

II. Mossio, Montévil & Longo: Theoretical principles for biology: organization. A succinct historical survey of the understanding of organization in the organicist tradition provides the bases for a specific characterization of organization in terms of the closure of constraints. Organization provides a framework for a systemic understanding of the notion of function. In the authors' framework, organization as a principle also provides a basis for biological stability.

III. Montévil, Mossio, Pocheville & Longo: Theoretical principles for biology: variation. The principle of variation extends Darwin's notion of random variation**.** In physics, objects are generic and evolve in well-defined phase spaces, whereas in biology, objects are specific and the phase space is not set *a priori*. Biological objects show randomness, historicity and contextuality. The principle of variation is expressed in terms of symmetry changes, where symmetries underlie the theoretical determination of the object.

Miquel and Hwang: Physical and biological individuation. Based on Simondon's work the authors start from the assumption that an individual is the result of individuation, and not with the classical philosophical claim according to which, individuation is a property of an individual. Individuation occurs in complex physical systems by the coupling between the system and its outside conditions. The system is not entirely defined by its structure at a given time because this structure will change and global emergent properties will appear. Thus physical individuation is defined both by the coupling of a physical system with its environment and by the diachronic dynamics taking place. Biological individuation is interpreted as a recursive procedure through which physical individuation is also acting in "its own theatre".

Montévil, Speroni, Sonnenschein & Soto: Modeling mammary organogenesis from biological first principles: cells and their physical constraints. The typical approach for mathematical modeling in biology is to apply mathematical tools and concepts which originated from theoretical principles in physics and computer sciences. Instead, the authors propose to construct a

mathematical model based on proper biological principles. Specifically, they use principles identified as fundamental for the elaboration of a theory of organisms, namely i) the default state of cells and ii) the principle of organization. Cells display agency and move and proliferate unless constrained; they exert mechanical forces that i) act on collagen fibers and ii) on other cells. As fibers organize, they constrain the cells on their ability to move and to proliferate. The model exhibits a circularity that can be interpreted in terms of a closure of constraints. Implementing the mathematical model shows that constraints to the default state are sufficient to explain ductal and acinar formation, and points to a target of future research.

Sonnenschein & Soto: Carcinogenesis explained within the context of a theory of organisms. The tissue organization field theory (TOFT) posits that cancer is a tissue-based disease whereby carcinogens (directly) and mutations in the germ-line (indirectly) alter the normal interactions between the diverse components of an organ, such as the stroma and its adjacent epithelium. The TOFT explicitly acknowledges that the default state of all cells is proliferation with variation and motility. When taking into consideration the principle of organization, the authors posit that carcinogenesis can be explained as a relational problem whereby the release of constraints created by cell interactions and the physical forces generated by cellular agency lead cells within a tissue to regain their default state of proliferation with variation and motility.

Soto, Longo, Miquel, Montévil, Mossio, Perret, Pocheville & Sonnenschein: Toward a theory of organisms: Three founding principles in search of a useful integration. Organisms are agents capable of making their own norms thus creating novelty and stability. The three principles for a theory of organisms (the default state of proliferation with variation and motility, the principle of variation and the principle of organization) provide understanding of the organism's ability to create novelty and stability and to coordinate these apparent counterparts. These principles profoundly change both biological observables and their determination with respect to the theoretical framework of physical theories. This radical change opens up the possibility of anchoring mathematical modeling in biologically proper principles.

We believe that these articles present the current state of play in developing a theory of organisms.

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Ana Soto (Editorial Board),
Giuseppe Longo (Guest Editor)
Denis Noble (Co-Editor in Chief)

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Why do we need theories?

Giuseppe Longo[a,b,*], Ana M. Soto[a,b]

[a] *Centre Cavaillès, République des Savoirs, CNRS USR3608, Collège de France et École Normale Supérieure, Paris, France*
[b] *Department of Integrative Physiology and Pathobiology, Tufts University School of Medicine, Boston, MA USA*

Abstract

Theories organize knowledge and construct objectivity by framing observations and experiments. The elaboration of theoretical principles is examined in the light of the rich interactions between physics and mathematics. These two disciplines share common principles of construction of concepts and of the proper objects of inquiry. Theory construction in physics relies on mathematical symmetries that preserve the key invariants observed and proposed by such theory; these invariants buttress the idea that the objects of physics are generic and thus interchangeable and they move along specific trajectories which are uniquely determined, in classical and relativistic physics.

In contrast to physics, biology is a historical science that centers on the changes that organisms experience while undergoing ontogenesis and phylogenesis. Biological objects, namely organisms, are not generic but specific; they are individuals. The incessant changes they undergo represent the breaking of symmetries, and thus the opposite of symmetry conservation, a central component of physical theories. This instability corresponds to the changes of the environment and the phenotypes.

Inspired by Galileo's principle of inertia, the "default state" of inert matter, we propose a "default state" for biological dynamics following Darwin's first principle, "descent with modification" that we transform into "proliferation with variation and motility" as a property that spans life, including cells in an organism. These dissimilarities between theories of the inert and of biology also apply to causality: biological causality is to be understood in relation to the distinctive role that constraints assume in this discipline. Consequently, the notion of cause will be reframed in a context where constraints to activity are seen as the core component of biological analyses. Finally, we assert that the radical materiality of life rules out distinctions such as "software vs. hardware."

Keywords: default state, mathematical symmetries, phase space, biological organization

> Nothing is more practical than a good theory
>
> Attributed to Ludwig Boltzmann.

1. Introduction

Broadly speaking, the aim of science is to improve our understanding of nature. Scientists seek this knowledge for its own sake and also for guiding us to act responsibly when using this knowledge. Given that the scientist does not have direct access to the world outside her and because the consequences of action are far from obvious, these are not easy tasks. Centuries ago the founders of mechanics were strongly committed to Christian faith, and thus circumvented this problem by believing and asserting that the infinite goodness and perfection of God justified the agreement between their theoretical reasoning, and the phenomena observed by them (Cottingham 2013). In other words, since God does not intend to deceive us, we, as Her creatures, can trust our own senses and rationality. Moreover, God could be viewed as a legislator both of nature and of human activities; thus, the notion of "law" could be extended from divine will and human societies, to the dynamics of nature. In the last 150 years scientists stopped relying on religion as a means to determine objectivity. Darwin's book "The origin of species" was a main contributor to this profound change in philosophical stance in science. From our perspective, this modern viewpoint implies that scientific objectivity should be conceived of as constructed by a human activity.

In spite of Descartes' Meditations, both physicists of yore and todays' practitioners put forward ideas and methods that are counterintuitive and usually contrary to common sense (Bachelard 2002; Wolpert 1994). The frame of reference we use as scientists is thus different than the one we all use in everyday situations, for example when we talk about "sunrise" and "sunset". Remarkably, com-

*Corresponding author
Email addresses: longo@ens.fr (Giuseppe Longo), ana.soto@tufts.edu (Ana M. Soto)
URL: http://www.di.ens.fr/users/longo/ (Giuseppe Longo)
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mon sense notions are useful in our everyday lives; this is probably why we still talk about the sunrise today, half a millennium after Copernicus proposed the notion of a heliocentric planetary system, a notion we are exposed to from childhood. This example also illustrates why the naïve perception that facts exist independent of any reference frame is incorrect. There is no observation devoid of theoretical content; sunrise and sunset refer to the sun rotating around the earth as in Ptolemy's theory. As put by the philosopher DC Dennett: "There is no such thing as philosophy-free science; there is only science whose philosophical baggage is taken on board without examination" (Dennett 1995).

Scientists purposely suspend the common sense world view used by all in our everyday life when constructing theories and contrasting them with experiments. Scientific theories provide organizing principles and construct objectivity by framing observations and experiments. Even research performed within the frame of one "wrong" theory sooner or later will result in the demise of such a theory, thus advancing our knowledge. This goes with one caveat, that the theory in question has to have clear enunciates that allow their demise by both theoretical and experimental considerations.

Physics provides the best example of why theory is central to the success of a scientific discipline. It also provides examples of how "wrong" theories such as the "luminiferous ether theory" which was conceived to explain the propagation of light, was useful in framing observations. A comment by H. Poincaré, published before the dismissal of the ether theory illustrates the role of theories: "Whether the ether exists or not matters little - let us leave that to the metaphysicians; what is essential for us is, that everything happens as if it existed, and that this hypothesis is found to be suitable for the explanation of phenomena. After all, have we any other reason for believing in the existence of material objects? That, too, is only a convenient hypothesis; only, it will never cease to be so, while some day, no doubt, the ether will be thrown aside as useless,"(Poincaré 1905). Indeed, the "luminiferous ether theory" ceased to be useful at the beginning of the 20^{th} century. Light was found to have both wave and particle properties; particles do not need a medium to travel. Moreover, the speed of light was supposed to be set with respect to the ether, but instead it was shown to be always the same in the 'vacuum', whatever the viewpoint of the observer is. This finding paved the way to special relativity.

2. Principles of conceptual construction and principles of proof in Mathematics, Physics and Biology

A brief excursion into Mathematics may help to clarify some general ideas about the foundation of natural sciences. Euclid's work is a permanent blend between constructions and proofs: Euclid traces lines, constructs plane figures and, by means of rotations and translations, gives proofs. Logic is also crucial to proof, as exemplified by proofs "per absurdum". Euclid proposes mathematical structures, of which the main one is the line with no thickness. Then, he builds on these structures by tracing, intersecting, rotating and translating. By means of these transformations, composite mathematical structures are obtained.

For more than two millennia from Euclid to Grothendieck, the proposal of new concepts and structures as well as the singling out of *"principles"* for these constructions, was at the core of mathematical activity. The construction of concepts and structures is followed by the development of suitable *principles of proofs* by means of logic. The job of these principles is to preserve the "meaning" of structures along proofs. For example, deriving by "modus ponens" (if A, and "A implies B", then B) preserves the "sense" (or truth) of the assumptions being examined. In a sense, principles of proof are formal transformations that preserve the mathematical meaning as an invariant of the proof[1].

The transfer of mathematical tools to another discipline should always take into consideration the origin and the constitutive dynamics of these tools. Specifically, these mathematical tools are far from neutral because they carry with them a specific organization of phenomena and a specific way of reasoning that cannot be separated (dissociated?) from them. Similarly, experimental tools such as sequencing techniques tend to force the search for answers to all kinds of biological questions in terms of sequences. Furthermore, animal models are far from neutral; S Gilbert discussed how the adoption of animal models that reproduce all year long in carefully controlled laboratory conditions obliterated the effects of the environment on the construction of the phenotype (Gilbert 2005). This omission resulted on the adoption of the idea of a developmental "program" totally contained in the genome. "Modern" biologists became oblivious to the previously entrenched notion that the environment plays a major role on the determination of phenotypes. In fact, polyphenism (one genome, multiple phenotypes) was discovered well before genetics entered the biological scene (Weismann 1875).

2.1. Principles of construction and proof in Mathematics and Physics

The deep link between Mathematics and Physics is due to their shared principles of construction. The concepts of Mathematics are used to single out physical concepts and objects. In Physics, the notions of speed and acceleration became scientific when forced into a mathematical construction by applying differential calculus and limit concepts to them (derivation and integration). It is the mathematical writing of equations that produces the stability of the physical concepts of energy or momentum. These

[1] The differences between principles of construction and of proof as well as those between generic and specific objects are discussed in detail elsewhere (Bailly and Longo 2011; Longo and Montévil 2014).

concepts may be characterized as invariants in the equations of movement under time or space transformations, respectively [Noether's Theorems, 1918, see (Bailly and Longo 2011; Longo and Montévil 2014)]. In other words, the concepts of Physics acquire stability when they are treated by the same methods and the same principles of construction used for mathematical concepts. Moreover, the objects of Mathematics, namely, the defined concepts, are "generic", exactly like the objects of Physics. That is, they are invariant of experiences and theory under suitable transformations: a line or a Hilbert space in geometry, a stone or an electron in Galileo's or Bohr's experiences are all invariant or symmetric with respect to replacement by another mathematical or physical object of the same type.

Some objects of Physics are "first" mathematical ones: an electron is a solution of Dirac's equation. Anti-matter is the negative solution, which originally had no physical meaning. Sacharov and Feynman dared to interpret this purely mathematical solution by some peculiar empirical evidence: the disappearing of a particle and the production of twice its energy as gamma-ray and called the "invisible" interacting particle a positron, and this gave the concept of anti-matter. This is a paradigmatic case of the intertwining of Mathematics and Physics. However, the transfer of such an extraordinary methodology into another discipline, like Biology, may either not make sense at all, or result in a surprising meaning that should be examined closely. In summary, exact mathematical invariances and the transformations that are mostly defined by means of equations play an identical constructive role in Mathematics and Physics: they propose or single out objects and show the sense in which they are "generic". This is all grounded on the fact that these two disciplines share similar "principles of conceptual construction".

While Mathematics and Physics share principles of construction they differ regarding the "principles of proof". On the one hand, in Mathematics, these "principles of proof" are of logical-formal nature and they make it possible to preserve meaning (or truth) in deductions. In Physics, as a natural science, on the other, proofs are grounded on experiences, in the broad sense of observations and experiments.

2.2. Principles of construction and proof in Biology

Physics and Biology share the principles of empirical proof, but they radically depart from the physico-mathematical practice regarding the principles of construction. Objects and concepts do not share the same "perfect" stability and interchangeability of those in Mathematics and Physics, a stability and a genericity which is defined by the mathematical invariance with respect to intended transformations. For instance, the *states* (speed, energy level) of an electron may change over time, but the invariances and the transformations that define its *properties* are stable (mass, for example). Physico-mathematical objects and concepts have no intrinsic or objective history[2].

The historical (phylogenetic, ontogenetic) formation of a biological object is instead crucial: each organism originates from a pre-existing one. The understanding of the evolutionary and ontogenetic path of a given organism is crucial to its scientific description as a biological object. Moreover, history produces the "specificity" of an organism and the organs within it. That is, each biological object is the result of an historical development which makes it specific and, in a sense, unique. This uniqueness poses problems for scientists, because all scientific analyses require some level of generality. The inherent specificity makes it necessary to determine the best level of general description of a biological object. In conclusion, while Mathematics and Physics share the same construction principles and deal with generic objects, with no history, Biology can neither rely on the same construction principles nor on the genericity of the objects; yet, like in Physics the proof principles are empirical.

2.3. The role of mathematical symmetries and invariants

When discussing construction principles we mentioned the stability of physical objects which depends on the preservation of invariants under transformations. For example, all circles are similar, and the ratio of the circumference to the diameter, π, is invariant.

In modern Physics, "symmetries" are transformations preserving the key invariants observed and proposed by the intended theory. In short, the conservation of these quantities is grounded on the idea that the "laws" of Physics are the same at different positions and times. The types of symmetries usually referred to in Biology are a subset of those in Mathematics; for example, symmetry with respect to an axis on a plane. Those symmetries represent specific cases of transformation such as a space rotation preserving the properties of the geometric structure under examination.

In Physics, changes of symmetry may force a change in theory; as an example, in classical mechanics time is reversible, while in thermodynamics time is oriented (Table 1). In other instances, a theory can accommodate a single change of symmetries, like the theory of critical phase transitions. This is exemplified by the passage from water vapor to snowflake, namely, the appearance of a new observable, snow. This phenomenon is called a phase transition and occurs at a point named the critical point. Since ontogenesis and phylogenesis are characterized by

[2]Cosmology is an exception to this, with the Big Bang as a limit case. Yet, this is exactly where the encounter of non-unified theories (quantum and relativistic fields) poses major problems to any attempt to consistently give historicity to physical objects: major physical constants are claimed to change in the first few nanoseconds after the Big Bang, but the physical constants remain stable afterwards. This is very far from biological histories, such as phylogenesis and ontogenesis: changing objects, functions, and observables is their permanent state of affairs.

	Physical world			Biological world
	Linear / equilibrium physics	*Classical thermodynamics*	*Far from-equilibrium / self-organization physics*	
Time	No arrow of time	Arrow of time	Arrow of time	Arrow of time (adds a biological level of irreversibility)
Conservation	Conservation principles (energy, momentum, etc)	Conservation and introduction of a non-conservation principle (production of entropy)	Conservation and a basic non-conservation principle associated to randomness in the self- organization process	Non-conservation principle, new possibilities
Description space	Stable	Microscopic: stable Macroscopic: shrinks over time	Microscopic: stable. Macroscopic: increases over time (emergence, yet causally reducible)	Not stable over time (emergence) *
Mathematical symmetries	Stable symmetries	The system gets more symmetric over time (measured by entropy increase)	Simple symmetry breaking (the system becomes less symmetric on the basis of former symmetries)	Ubiquitous symmetry changes.
Framing principle	Conservation of energy	Increase of entropy	Identical iterations (at the statistical level)	Non identical iteration.
Historicity	No (past and future are equivalent)	No (peculiar features from the past are destroyed by the dynamics)	No (a few features are akin to historicity but the framework is ahistorical and the objects are spontaneous)	Fully historical systems (objects are historical and not spontaneous)
Default state	Uniform rectilinear movement	Stationary state with maximal entropy (equilibrium)	Stationary state under constant flows (non-equilibrium)	Proliferation with variation and motility

Table 1: *From physics to biology: A comparison of fundamental principles for theory construction* [adapted from (Longo et al. 2015)].

the formation of new objects and symmetries, the theory of critical phase transitions is relevant to theory building in Biology. However, unlike in Physics, where the new object appears at and beyond the critical point, in Biology changes occur relentlessly.

2.4. Phase Spaces

The invention of phase spaces in Physics, that is, of the spaces of pertinent observables and parameters, has a rich history. There is neither space, nor mathematized plane in Greek geometry; this is a geometry of figures and of lines, manipulated by translations and rotations. Infinity is implicit, like in the notion of "line with no thickness" or it is *potential*, like in the "prime number theorem" in which an algorithm is given that, for any collection of prime numbers, constructs a larger one.

A different notion of infinity was generated in paintings in Italy at the end of the Middle Ages. It originated from a theological debate which specified the positive content of God's *actual* infinity instead of just potential infinity as the only conceptually possible one (Zellini 2005). This newly established concept of infinity moved into paintings under the form of *perspective*: in Annunciation paintings in the 14th century, the projective point is a symbolic form of the presence of the infinity of God (Figure 1), (Arasse 1999; Longo 2011; Longo 2010). In the 15th century, Piero della Francesca, Ghiberti, Alberti and others, invented a general technique from this pictorial construction, a "practical" version of projective geometry. In turn, in the 17^{th} century, Desargues turned it into the full glory of a mathematical theory.

To continue this short history of infinity, as Kant beautifully philosophized, the infinite spaces of Descartes and Desargues provided the very "conditions of possibility" for doing Physics. In other words, the *a priori* awareness (or the "positing") of space (and time) were the necessary preliminaries for framing Newton's equations. Yet, 19^{th} century Physics went further. The complete determination of a physical process may only be given by also specifying the pertinent observables. So, Hamilton, Poincaré, Gibbs and others explicitly referred to the choice of "what needs to be measured", possibly an invariant quantity of the intended process. In this way, two major invariants were added in the specification of space (or time): namely, momentum (in conjunction to space) and energy (as conjugated to time). Then, momentum *and* space or energy *and* time provided the fundamental phase spaces for physical analyses. This boosted the modern splendor of equational descriptions in Physics: once given the appropriate phase space, equations or functions describe the dynamics.

Figure 1: Lorenzetti, Ambrogio, *L'Annonciation,* 1344. Pinacoteca Nazionale, - Wikipedia.org, CC-PD-Art. A column, solid near the ground is attenuated towards the top where it overlaps and hides the vanishing axis of perspective at infinity, an explicit reference to God. In 1344, this was an extraordinary innovation: a rigorously drawn projective space. And then, by the effect of the geometry of this floor that goes from man to God, a new space is deployed: God is present in the story being told, albeit hidden, far away at infinity. The Madonna has a new human dimension: her solid, three-dimensional body accompanies the expression of a nascent humanism. Perspective introduces God as the actual limit,at infinity, therefore as the limit of a space which encompasses everything, including the human spaces which are renewed. All of the first paintings with "prospettiva" will be annunciations, this unique locus of the meeting between infinitude and finitude according to Catholic theology (From (Longo 2011)).

The phase space is the space of all possible states of a physical system. The procedure which requires that the phase space be a "condition of possibility" and thus a priori for constructing physical knowledge is still at the core of all forms of mathematization in Physics. That is, theoretical Physics is advanced by first positing the phase space of the possible dynamics, a task that may be rather abstract. We may compare this task with that of the painters mentioned above: before placing objects, the pictorial space was organized by means of perspective, the practical application of projective geometry. In classical mechanics, the phase space contains all possible positions of all the objects in the system and their momenta in order to determine the future behavior of that system.

Often, the hard part for the theoretical physicist is to invent the right phase space. In quantum Physics, for instance, the choice of Hilbert's spaces allowed Schrödinger to give an equational description of quantum dynamics, as the dynamics of an amplitude of probability. Another example is the choice of the frame of Connes' non-commutative geometry with the purpose of unification of quantum mechanics with relativistic theories. We stress again that the key point in this very powerful approach to physical dynamics is the pre-definition of the pertinent phase space.

The *a priori* choosing the phase space applies also in relativity theory. Energy and matter modify the metrics and thus the curvature of space, but neither the topology nor the dimension of the intended Riemannian spaces where Relativity Theory is analyzed. The resulting phase space, with the key observables, energy and momentum, does not change. This powerful procedure may be viewed as a strong form of separation of space and time from physical matter; admittedly, this represents a convenient dualism. Again, like in Italian renaissance paintings, the space is drawn before objects and humans are placed in it (de Risi 2012)[3].

The previous narrative generates a basic question in the quest to gain an understanding of biological phenomena. Namely, is there a way to construct *a priori* a phase space for organisms as is done in Physics? Here we arrive at one of the many challenges biological objects pose to scientists. During ontogenesis the appearance of an animal changes radically. Change is even greater through phylogenesis; this change encompasses the phenotypic diversity of the living world from unicellular organisms to butterflies, whales and humans. Is it wise to imagine a "phase space" that would contain all possible phylogenetic trajectories? This query brings to memory SJ Gould's idea of whether in replaying the "tape of life" we would end up with the same "tree-of-life" that we know and of which we are a part of. From the very contingency of life, his answer was a resounding "no" (Gould 1990).

We know from ecological developmental Biology that living beings are co-determined by their ancestry and their macro- and micro-environment. Reciprocally, organisms shape their environment. In short, evolution is about change along a hereditary history, and these changes represent a change of observables and changes of symmetries. All these factors make it apparent that there is no predetermined phase space (Longo et al. 2012). That is, the conditions of possibility for the emergence of new objects are generated along the way. Among the many examples of this type of event, the appearance of the ossicles of the inner ear in mammals which originated from jaw bones in reptilians is a rather dramatic one.

3. Causality: Theoretical versus differential causes

In classical mechanics, which deals with phenomena at an intermediate scale like objects of our everyday life (balls, bridges, trucks), it is relatively straight-forward to identify a theoretical cause. According to the principle of

[3]The separation of space from the objects inhabiting it is a sort of dualism that is also central to theories of information and computer sciences. Information or software is strictly separated from the hardware in all current theories of Information: in Biology, the use of information metaphors would make the material structure of organisms irrelevant to evolution (Gouyon et al. 2002).

inertia, if no force[4] modifies the state and properties of an object, the object conserves its state and properties. A ***theoretical cause*** would then be a force that modifies the state and properties of the object in question[5].

In contrast to the inert, biological entities are able to generate action (agentivity); they move and reproduce. This inherent ability of biological entities poses challenges to the classical notion of theoretical cause. In Chapter 5, we address this issue and propose the notion of a "default state" which represents the equivalent of inertia in mechanics. Put simply, the biological default state is what cells do when placed in an environment appropriate for maintaining flows of matter and energy. In these conditions, they move, proliferate and generate variation. Under these circumstances, we assert that the default state is a ***theoretical cause.*** Anything that affects the default state is a ***constraint***.

Constraint is a term that has been used in evolutionary Biology to indicate factors that limit the production of phenotypic variants. In our view of the organism, a constraint is a factor that will change the range of "possibles". A negative constraint will narrow down the range of possibles. For example, during rodent perinatal development, estrogens masculinize the hypothalamus, thus narrowing the repertoire of possibles to just the negative feedback, while in the absence of estrogens the hypothalamus expresses both positive and negative feedback. A constraint could also hinder one possible while enabling another. For example, the bottom of a tissue culture flask blocks the displacement of the cells below this surface but allows the cells to "crawl" along this surface.

When a perturbation is introduced into a biological system, for example, when one group of animals is treated with a hormone and another group of comparable animals with the vehicle alone, a difference in the behavior of the system is observed. We call this perturbation a ***differential cause.*** The difference in treatment provoked the modification in the system's behavior in a contextual manner, whereas a theoretical cause represents an invariant with respect to all pertinent contexts. In order to learn about the theoretical cause underlying the differential cause we need to find out how the latter affects the constraints on the system; for this to be achieved, we need to rely on a suitable theoretical frame.

4. Rooting biological knowledge in the specificity and materiality of life

Can the practice of postulating the phase space be transferred from Physics to Biology? It all depends on the preferred observables. If one considers phenotypes and organisms as pertinent objects of analysis, there is no way to consider them as time or space invariants. Indeed, in Biology we follow Darwin's approach, which is based on a non-conservation principle for phenotypes: that is, "descent with modification". Thus, in our view, there is no way to pre-define the phase space. Kant was right: there is no way to follow Newton's path to turn the analysis of organisms into a science. We need, instead, brand new principles and ideas. The strong form of dualism ingrained in Physics seems unsuitable for Biology and to the absolute materiality of life. Life is based on the *actual* materials living objects are made from, which includes a particular DNA, particular RNAs, proteins and membranes, just to mention some of the cell's components. There is no way to dissociate the actual materials from which living organisms are made from the functions these organisms fulfill[6]. When dealing with computers, however, the "software" is independent of the hardware. This radical difference between the inert and the living makes the transplantation from the mathematical and physical sciencesBiology unsuitable due to the fact that they do not contemplate the fundamental materiality of living entities.

Another important difference between Physics and Biology was alluded to above, namely, that in the latter the pertinent observables, phenotypes and organisms, are specific while in the former, objects are generic. Additionally, these biological observables continually change as a consequence of their material internal dynamics and of the interaction of organisms in contingent ecosystems. Yet, "organization" remains. Once we postulate organization as the invariant structure common to all organisms, an obvious question comes to mind: would it ever be possible to mathematically formalize this postulated "invariant"?

While searching for a way to deal with this postulate, we acknowledge that the best empirical solution to the challenge posed by the specificity of biological objects subject to continual changes is to adopt an extension of common practices in experimental Biology. These practices aim at decreasing variation as much as possible among the objects being studied to render them more "generic". For example, cloning of cells and developing animal strains by sister-brother pairing renders these biological objects more alike, comparable to monozygotic twins. However, the theoretical relevance of this common practice has not been made explicit by the practitioners. We propose the construction of a suitable experimental context where the best level of generality is obtained. In view of its resemblance to required transformations to preserve invariance when inventing a new concept or structure in Mathematics, Maël Montévil called these procedures "symmetrization." Empirical symmetrization in the context of proper

[4] In physics, a force is any interaction that, when unopposed, will change the motion of an object.

[5] However, in the small-scale world of quantum mechanics inert matter poses new challenges to causality, like quantum entanglement.

[6] In the last half of the 20th century we witnessed the replacement of certain *organ* functions by engineered devices that are useful in the short run, for example, dialysis machines and mechanical hearts. However, they do not substitute for the actual biological organ. In the long run, organ transplants are the best solution to overcome organ failure. Paradoxically, while organs can be replaced with mechanical devices, cells cannot.

theoretical principles may be an effective way to advance Biology while waiting for the development of appropriate mathematical tools to formalize the theoretical concepts we intend to develop.

5. From "Physics" to "nature" and toward an autonomous Biology

The Greek word from which the scientific discipline we call "Physics" originated from what today we call nature, including live objects such as plants and animals. In fact, Aristotle's Physics comprised both the inanimate and the living. "Nature", the latin word, originally meant "birth" as well as "beget", notions that evoke life. Although it was also synonymous with Physics, in ancient times the shift in meaning reveals a change of scope of the science. The mathematization of the world view and the origin of mechanics excluded out of the realm of "hard" science, for the most part the biological as well as the most distinct human characteristic, the mind.

Scientists interested in what we now call Biology (the term was introduced independently by Lamarck, Treviranus and others in the early 19$^{\text{th}}$ century) tended to polarize themselves into two main currents: vitalism and physicalism. The vitalists proclaimed the independence of Biology from Physics while the physicalists expected to reduce Biology to Physics.

We mentioned above that the dualism inherent in physical disciplines from Descartes to Information Theory is inimical to the constitutive "materialism" of the living, and we have succinctly explained why theories from one discipline cannot be automatically applied to another discipline. We also explored the main differences between Physics and Biology; this analysis was not meant to provoke a feeling of "Physics-envy", but to the contrary, it made us feel re-invigorated by the challenge posed by Biology. Philosophers, particularly those from the "Continental" tradition have long observed the differences between these two disciplines, and the radical difference between alive and inert (Kant 2000; Canguilhem 2008; Bergson 2007). That is, the agency and normativity of the living and the process of individuation (which will be addressed in this issue by Paul-Antoine Miquel and Su-Young Hwang). We biologists need to address the relentless change of the living objects and their individuality, their incessant change of symmetries, and their creativity. The Mathematics to formalize such an enterprise are yet to be developed. The challenge of tackling biological problems before such mathematization is truly invigorating, and history tells us that it has already begun. Biologists have already gone a long way guided by evolutionary theory, a theory of relentless change which is itself being reconstructed.[7]

[7] Darwin's theory of evolution underwent changes, a major one as the "modern synthesis" in the 20$^{\text{th}}$ century and it is now undergoing major critical reconstruction (Noble et al. 2014).

The task now before us is to build a theory of organisms comprising the entire life cycle. From our perspective, such theory-building task requires a multidisciplinary perspective, encompassing philosophy, Mathematics, Physics and Biology. This PBMB issue is a preliminary attempt through our own multidisciplinary effort towards a theory of organisms.

6. Conclusions

Altogether, we propose that the articulation between organisms and Mathematics is not equivalent to that of inert objects and Physics. This is mostly due to the historicity, variability and contextuality of organisms and cells. These are summarized by a very relevant conceptual duality: the genericity of physical objects and the specificity of their trajectories, in contrast to the specificity of biological objects and the genericity of their possible trajectories. The basic principles that we thus propose for Biology are different in nature but compatible with relevant physical principles. Mathematical models which are necessary to understand complex, non-linear interactions need to be grounded on robust biological principles. Finally, a theory of organisms eventually should be able to lead us towards this most human characteristic, the mind, which was excluded from the scientific realm at the dawn of the scientific revolution.

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Reductionist perspectives and the notion of information

Nicole Perret[a,*], Giuseppe Longo[a,b]

[a] *Centre Cavaillès, République des Savoirs, CNRS USR3608, Collège de France et École Normale Supérieure, Paris, France*
[b] *Department of Integrative Physiology and Pathobiology, Tufts University School of Medicine, Boston, MA USA*

Abstract

Reductionism is the dominant stance of biology. According to this perspective, biological phenomena have to fit with physical explanations. Some biologists thought that the introduction of the idea of program was a sound way to overcome both physicalism and reductionism. We argue instead that the introduction of information theory into biology did not liberate biology from reductionism. We argue that the adoption of information in biology is an erroneous transposition from a specific mathematical domain to one where it does not belong. Indeed, the mathematical framework of the information theory is too rigid and discrete to fit with biological phenomena. Therefore, information in biology represents an inappropriate metaphor. Then, we make explicit the use of metaphors and the choice of explanation mode. We argue that the choice of explanation is not neutral. Furthermore, the use of metaphors in science becomes dangerous when they take the place of theories and they lose their paradoxical content.

Keywords: information, metaphor, reduction, entropy, complexity, mathematical invariance

> To think that the genome completely determines the organism is almost as absurd as thinking that the pipes in a large cathedral organ determine what the organist plays.
>
> D. A. Noble "Theory of biological relativity", Interface Focus. 2012

1. Introduction

For at least two centuries two distinct and even antagonistic stances co-existed in biology. In current terminology, we refer to them as reductionism and organicism. The former is going to be addressed in this article while the latter is addressed in Mossio et al (this issue). The history of physics shows that a new phenomenon has always engendered new observables and principles. For instance, Galileo proposed momentum and its principle of conservation (inertia); thermodynamics studies trajectories in a relevant phase space: pressure, volume, temperature. A new observable, entropy, has greatly enriched physics by providing a principle that can be applied to any form of energy transformation: the second principle of thermodynamics[1]. Does biology operate similarly regarding the choice its observables and invariants? At the beginning of the 20th century, the central goal was to find observables and principles to understand the phenomenon of reproduction resulting in the hereditary transmission of phenotypes. In this context, the search for a proper observable specific to biology headed toward the notions of encoding and of program that are at the core of the theories of information.

In this paper, we argue that information is problematic for biology for at least three of the reasons that we will analyze here. First of all, we claim that the transposition of the mathematical theory of information into the biological field is scientifically erroneous. To this aim, in the first section, we analyze the incompatibility between the information sciences and the biological object.

The second reason is related to a general problem of reductionism. According to the distinguished biologist Ernst Mayr, the information field provides an anti-reductionist framework for biology. Despite this viewpoint, we argue that applying the theory of information to biology belongs to a reductionist attitude. In the second section, we analyze this reductionist approach, and we point out the relationship with determinism. We show that the reductionist stance hides the general idea according to which classical determinism is the regular form of scientific knowledge and that this is also true when information theory is applied to biology. For the reasons analyzed in the first section, it will be clear that the deterministic theoretical framework is inappropriate for biological theorizing.

Faced with this kind of criticism, oftentimes biologists defend the use of the idea of information, as well as the concepts of signal and program, as *just* useful metaphors

*Corresponding author
Email addresses: nicole.perret@ens.fr (Nicole Perret), longo@ens.fr (Giuseppe Longo)
URL: http://www.di.ens.fr/users/longo/ (Giuseppe Longo)

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[1] See Soto & Longo (this issue), see also Bailly and Longo, 2011; Chibbaro et al., 2015.

or fruitful ways with which to approach a phenomenon by using an image borrowed from common sense. That is why, in the last and conclusive section, we analyze the general use of metaphors and common sense in science, and we show their dangerous consequences. This is particularly the case of the idea of information and the genetic program in biology because, as we will explain, here we face a dead metaphor.

2. Information Sciences and Biology

The use of the concept of information in biology appeared in the middle of the 20$^{\text{th}}$ century, but it is related to what happened at the beginning of that century when the possibility of isolating chromosomes was coupled with the new concept of the mendelian gene as a functional unit of recombination. Mendel's writings did not directly imply this concept, which appeared after 1900 when Hugo de Vries, Erich von Taschermark, and Carl Correns "rediscovered" Mendel (see Pichot, 1999). Johannsen (1911), then, replaced the term *mendelian factor* with the term *gene* and suggested the consequent distinction between genotype and phenotype (see Moss, 2004). Therefore, the idea of associating a phenotype with a segment of these chromosomes appeared. Schrödinger (1944) then, realized that this association was not well founded in a law: "It seems neither adequate nor possible to dissect into discrete 'properties' the pattern of an organism which is essentially a unity, a 'whole'. Now, [...] a pair of ancestors are different in a certain well-defined respect [...] we locate in the chromosome the seat of this difference. Difference of properties, to my view, is really the fundamental concept rather than the property itself" (Schrödinger, 1944, p. 10). This great physicist understood that differential analysis does not allow for the deduction of a *law* in the physical sense. In order to obtain a law in the proper sense, it would be necessary to propose a direct causal link, between the *wild gene* and the normal phenotype. He introduced the notion of encoding, borrowed from the new sciences of coding, in order to provide a theoretical framework and establish this hypothetical correlation. In other words, the fact that a mutation modifies the structure of an enzyme does not allow for the deduction of a direct *one gene - one enzyme* correlation[2] (following Beadle & Tatum, 1941). The notion of information was introduced as a theoretical framework providing this direct causal link. However, it is scientifically inexcusable to adopt this notion in biology without clarifying its usage with respect to at least two of the fields which make rigorous use of it. These fields are information elaboration (Turing-Kolmogorov), (Turing, 1936) or algorithmic theory of information (see Calude, 1994; Davis, 1958), and information transmission (Shannon-Brillouin), (Brillouin, 1962, Shannon 1948).

The Central Dogma of molecular biology (Crick & Watson, 1953; Crick, 1970) suggested that the description of the chemical structure of the DNA molecule represents well the core of the informational/algorithmic view of biological phenomena. The idea here is that the expression from nucleic acid to protein is a unidirectional flow of information. Which information theory is involved here? Despite the different scientific implications of these theories, there is a significant confusion in biology which is rarely clarified. Maynard Smith (2000) explicitly refers to information elaboration (Turing-Kolmogorov) and to information transmission (Shannon-Brillouin) while emphasizing the relevance of the *latter* in biology. However, in the same text, he explains how molecular encoding can work as a short "recipe" (his wording) for generating complex, but organized (ordered) objects. The analogy is then the recipe for describing a circle by the three parameters which determine it. This recipe is less complex and contains less information than a point by point description of the circle. On the contrary, a totally disordered set can only be described point by point, as it does not obey as a rule. Now, this notion of a short (compressed) program for an organized object and of (maximal) informational complexity of disorder is that of Kolmogorov, and it is *covariant* ("it grows together") with entropy (total disorder has maximal entropy). Note instead that, according to Shannon and Brillouin, complexity, as covariant with the quantity of information, is *contra-variant* with entropy and is in fact negentropy (it has the opposite sign and, thus, information decreases when entropy increases). This is also how physicists describe it, for sound reasons internal to the theory of "transmission of information" which thus differs greatly from Kolmogorov's one, a theory of "elaboration of information" (Longo, Miquel, Sonnenschein, & Soto, 2012).

Another important difficulty lies in the fact that information in the two senses explored above deals with the realm of the discrete. Now, in this discrete framework, that is a precise concept in mathematics, only the dynamics of the discrete parts are relevant for the explanation of the entire system. In biology these discrete parts are molecules, so molecules alone forcibly and fully retain the researchers' attention. In this context it would be very hard to integrate, as a *positive* contribution to the expression of information, others events such as torsion, pressure (see for example (Lesne & Victor, 2006; Farge 2003), the dynamics of contact, geometries and relative distances, which all causally contribute to gene expression. A computer (Turing) or a cable (Shannon) does not receive a positive contribution from these observables which are better understood using continuous mathematics. In fact, in both the mathematicalabove theories of information, grounded on the treatment of discrete sequences of signs, such material dynamics can only cause an increase in noise[3]. On the

[2] Which we now know to be an erroneous bijective correspondence.

[3] It would be possible and interesting to provide a theory of bio-information by integrating the theory of continua. Control theory,

contrary, in biology we need a theory that includes such dynamics in a causal sense because they have a specific role in the gene expression and in the morphological constitution of the organism. In short, biological processes, which take place in an organism simultaneously, are coupled to continuous dynamics that unfold in physical space and time.

Furthermore, in both theories of information, the flow of information does not depend on the material that carries the information. Crick refers to the Morse code when addressing genetic information. Now, the nice idea behind this practice of Morse-type encoding is that it depends neither on its form nor on the material which conveys it. It is possible to transmit Morse code through smoke clouds, light flashes, electric impulses, and so on. It is then possible to encode the signals using different pitches, different materials, colors, etc. This independence of the encoding from its material embodiment is actually at the core of the two great theories of information of the 20th century that we mentioned. In other words, information is an invariant with respect to the transformations pertaining to the physical medium or to the form of encoding. In fact, these transformations of material, leaving the informational content invariant, can be performed on the most complex of our informational machines, the computer. Specifically, if your machine is dying, due to the age of the material, you can transfer, via cable or wifi, the operating system, the compilers, all programs, onto another machine[4]. This radical *software vs. hardware* dualism of the Turing Machine is at the core of all contemporary computer science. With their differences, as discussed above, these two theories share the same radical properties of well-theorized invariance, giving rise to central theorems for both theories.

What about biology? At which level would it be possible to find the fundamental invariant of information? Do we have another way to encode such "genetic information" than through the DNA and RNA? Are there other forms of transmission and elaboration of this "information", otherwise than the *specific* molecular cascades active within the cell? Is it possible to encode the informational "content" of these molecules, carried in DNA, by using different materials, such as wood, metal, or a different chemistry, or beeps, flashes, octets, pairs of colors? Would this transposition alone be able to generate living organisms? Evidently not: there only exists the physico-chemical materiality of DNA and RNA with their very specific roles in biological phenomena. Biological dynamics radically depend on their materiality, and this is far from the independence of matter proper to digital information theories. Moreover, DNA or RNA are not "rigid" and this is essential to biological processes: for instance, redundancy or, better yet, Edelman's degeneracy is omnipresent (Edelman, Gally, 2001). In short, it is not even possible here to speak of physical invariance/stability: the physical chemistries of DNA and RNA do not allow themselves to be set once and for ever. By definition they change while being conserved.

Rather, as proposed in Mossio et al. (this issue), the proper biological observable is "material organization". From the structure of DNA to neuronal dynamics, biological activities exist solely in their highly organized physical, chemical and biological materiality. Quite the opposite of information, the polyvalent use of a given material is a core property in biology. Would it be possible to relate information to 'processes' instead of 'materials' (molecules)? Would this save the notion of information for biology? We reject this option. Biological processes, as shown in chapter 4, are subordinated to their material realization and to the organization of the living. They can not be considered independent from them nor as "informational invariants", in the scientific sense of this term.

In conclusion, there is no reason for the physico-chemical trace of a history, such as DNA, to be considered as "information". A rock shaped by its history of rolling along the current of a stream does not transfer information by hitting, deforming, or breaking other rocks. Information is in the mind of the geologist who studies its origin, not in the rock itself nor in its dynamics, which, if we choose to use such a word, may transmit a form or chemical structures.

3. Reductionism, determinism and mechanistic explanations in biology

Ernst Mayr (1961) thought that applying to biology the notion of a genetic program, borrowed from information theories, was a good way to provide an autonomous description of biology with respect to physics. The genetic program was, in his mind, an anti-reductionist approach. Here we show that, on the contrary, applying information theory to biology belongs to a form of reductionism and the theoretical consequences enhance our position according to which information is not an appropriate notion for biology.

Reductionism is a philosophical attitude commonly distinguished into three main types: ontological, methodological and epistemic reductionism (See Brigandt & Love, 2014). Ontological reductionism, called physicalism, is a general statement according to which there are no such things *separated* from physical (chemical) things, so that nothing but molecules constitutes an organism. Ontological reduction does not necessarily mean that the explanation of the physical level provides a complete explanation of the entire organism. It is more a form of *antisupernaturalism* that refuses any supernatural causes in biology such as intelligent design or vital forces. Methodological reductionism claims that the best way to explain a complex sys-

for instance, deals with information by differential equations in a continuum, not to mention the new productive area of Information Geometry (Barbaresco, Djafari, 2015), entirely ignored in (molecular) biology.

[4] In Manchester, during the 100th anniversary of Turing's birth (2012), students built a Turing machine made of Lego blocks ... and it works, it is Turing complete (albeit somewhat slow).

tem, such as an organism, is to access its lowest possible level; that is the molecular and biochemical one. Finally, epistemic reductionism is the idea that the knowledge of one scientific domain can be reduced to what is supposed to be a more fundamental body of scientific knowledge. This attitude claims the possibility of *translating* a group of scientific theories into another that is intended to be the primary. These three philosophical postures share the idea that physics is the fundamental level of a scientific representation of nature. In all the three cases the reduction goes from biology to physics. In other words, physics *is* nature and biology has to be, to different extents, subordinated to physics.

Classical geneticists, as well as early molecular biologists, were strongly committed to the idea that the living can be entirely explained by its physical and chemical dynamics. Here the three philosophical attitudes of reductionism, ontological, methodological and epistemological, converge. In fact, the physical and chemical dynamics are supposed to be the ontological composition of the living as well as the proper epistemic level of description and also the only way to understand the complexity of the living. This can be partially understood through a historical event. During the 19th century, there was a debate between biologists of two different schools of thought: the vitalists and the mechanists. Mechanicism focused on the research of objectivity for biology in order to provide a scientific foundation for this discipline otherwise vulnerable to metaphysical implications[5]. This need pushed numerous biologists to look at physics as a proper scientific model of description and analysis. In this context, a very peculiar form of reductionism surfaced in biological research. This reductionism groups the idea that the elementary components can explain the complex with the idea that biology has to be reduced into the language and the laws of chemistry and physics (See Rosenberg, 1985). Francis Crick, for instance, thought that explaining the living by its elementary dynamics corresponds to the conviction that biology is subordinated to physics (See Feltz, 1995). In this context, the Central Dogma is not only reductionist but strongly deterministic. The unidirectional flow of information from genes to protein, coupled with the strong specificity of the "one gene one enzyme" statement (Beadle & Tatum, 1941), belong to a view of the organism as a highly predetermined and predictable system. Of course, from time to time, some "noise", as said explicitly in (Monod, 1970), may add some randomness to the determination of the dynamics. Indeed, the level of reduction is not physics in general, but classical mechanics, a deterministic field. The theoretical framework that served as a model to biologists at this time was the strongly deterministic one of classical mechanics (the cell is a "Cartesian mechanism" for (Monod, 1970)). Here reductionism and determinism overlap and converge in a strong commitment: nature intrinsically corresponds to a necessary order; this order can be expressed by mechanistic causes that represent the universal law of the phenomenon. Through these laws, phenomena must be predicted. The relationship between reductionism, mechanistic explanation and determinism in biology is the consequence of transferring the theoretical framework of classical mechanics to biology. These three aspects are strongly related and it is hard to distinguish one from the other in the context of classical molecular biology. Ernst Mayr's thought is emblematic of this conceptual relationship, enriched by his attempt to stress the singularity of biology. According to Mayr, as mentioned above, the notion of a genetic program is the best way to establish the epistemological autonomy of biology with respect to physics (Mayr, 1961). The genetic program is inspired by the theory of elaboration information, which is a mathematical, not a physical theory. From Mayr's perspective, this would represent a methodological anti-reductionism because the physical level would not be directly involved in the description of the living. This paradigm, though, uses the same deterministic structure as that of classical mechanics: when the "causal relations" are analyzed in informational cascades, they follow exactly the early deterministic paradigms, in both Turing's and Shannon's approaches. That is, the transfer of this paradigm preserves many of the reductionist and physicalist consequences related to this classical reference to physics, starting from the Laplacian characteristic of the system itself. That means that the organism is a deterministic system in which it is in principle possible to predict the future dynamics (it is "Laplacian"), both in ontogenesis and in phylogenesis, by knowing the present state of the "determining" elements of the system: DNA sequences and genes. The rest provides at most "conditions of possibilities" and noise, as rigourously spelled out in (Mondo, 1970). This is more generally true in the neo-darwinian approach, as strongly represented by Mayr. In fact, according to neo-darwinism, natural selection applies exclusively to the DNA level that has to preserve a deterministic structure. Even if natural selection can not be reduced to a Laplacian mechanistic process that includes all the ancestors of the organism and their environments, the level where natural selection applies is deterministic. Otherwise said, the fact that randomness is considered does not allow to depart from Laplacian determinism. After all, Laplace wass aware that randomness exist, that is why he studied probability. But in his theoretical framework, randomness appears as an external perturbation of deterministic dynamics (necessity) and it opposes to determination: it is "noise" (Monod, 1970). In the neo-darwinian frame, determinism is necessary for natural selection to apply. That is, Evolution preserves the deterministic molecular processes and is "the result of noise" plus selection (Monod, 1970).

The deterministic (Laplacian) nature of the coding and decoding processes was perfectly clear to Schroedinger, when he first proposed the notion of encoding for biology : "In calling the structure of the chromosome fibers a

[5] Vitalism was considered too close to finalism. See (Canguilhem 1968).

code-script we mean that the all-penetrating mind, once conceived by Laplace, to which every causal connection lay immediately open, could tell from their structure whether the egg would develop, under suitable conditions, into a black cock or into a speckled hen, into a fly or a maize plant, a rhododendron, a beetle, a mouse or a woman." (Schrödinger, 1944, 7)[6].

In conclusion, applying information theory to biology is not free from the attitude that tries to reduce complex biological systems to deterministic systems. On the contrary, it is grounded in this attitude and is responsible for imposing a too strong deterministic account for the living. As Turing observed in 1950, his Logical Computing Machine is "Laplacian ... as prediction is always possible". Similarly, in a Shannonian frame, the deterministic transmission of coded information must be predictable, modulo some noise. Thus, the informational reductionism brought us back not only to physics, but to a theory of determination that opposes determination to noise and that is largely superseded, even in classical physics, by the modern theory of dynamical systems, since Poincaré (1892). This theory integrates randomness as part of physical determination and understands it as unpredictability of (non-linear) deterministic dynamics, by a fine analysis of the interplay between measurement and non-linearity, see (Calude, Longo, 2015) for a synthetic frame for randomness in natural sciences.

4. Conclusion: Metaphors and common sense in science

Some metaphors are useful in science; they can guide and inspire scientific research in a deep way. In physics, for example, the Galilean metaphor of « the world as a book written in mathematical language by God » (See Galilei, 1957) is a very inspired metaphysical metaphor that guided the scientific revolution. It never became a way of explanation. That means that the Galilean scientific framework never appeals to entities like God or books in its formal conditions. It is *just a metaphor*, in the real sense of an abstract idea that inspires the gesture and the curiosity of the scientific attitude – even by a somewhat absurd or paradoxical reference (indeed, the universe does not have the structure of a book). However, metaphors become dangerous when they take the place of theories, and they lose their paradoxical content. Fresh metaphors in science are metaphors that have not been reduced to the common sense and reveal a new way of seeing. On the contrary, dead metaphors are metaphors that have lost their paradoxical references, and they literally take the place of the formal conditions of a theoretical framework. In this case, they become dangerous for the sciences because they crystallize a conservative thought into the common sense. This is the case for the metaphor of the genetic program related to information. This metaphor does not inspire research but replaces the theoretical framework in order to support the entire differential method and the reductionist attitude (see Davies, 2009; Longo & Tendero, 2007). In other words, the strong causal correspondence between genotype and phenotype is not proved by the differential method as we have shown above. On the contrary, by the reference to programmed informational dynamics, a Laplacian determination is supposed in order to maintain the general idea of this strong and unidirectional correlation, as in the common interpretation of Crick's Central Dogma. It is amazing to observe, as we did, that the founder of the "coding paradigm", Schrödinger, was perfectly aware of this, since 1944. Later, the metaphor in question became a theory because the theoretical framework appeals to entities related to this metaphor in order to justify and build itself: in the DNA there must be a true program, otherwise the strong correlation fails. This is also a problem related to the use of common sense in science. According to the French philosopher Gaston Bachelard, the uncritical acceptance of common sense is a serious issue for science. This is because, in general, common sense hides an entire *package* of metaphysical assumptions.

The general usage of determinism and predictability in biology is a clear example of this problem. Indeed, as we mentioned, even when methodological reductionism was questioned, as it was the case for Mayr, determinism remains the general model of scientific knowledge and inspires the metaphor of the genetic program. As a matter of fact, any programmable process is deterministic and predictable: it is Laplacian, as we know since Turing. Thus, the metaphysical *package* of classical determinism, namely the idea of a highly predetermined and predictable system built on the dynamics of the discrete and elementary parts, was transferred entirely to biological research. The scientific practice then tried to justify and confirm this general idea by data, starting by the search for steroespecific macromolecular interactions (they are required to transmit and elaborate information) and complete autoregulation of genes by genes. As Gaston Bachelard said very well: *There comes a time when the mind's preference is for what confirms its knowledge rather than what contradicts it, for answers rather than questions. The conservative instinct then dominates and intellectual growth stops.* (Bachelard, 2002, p. 25)

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[6] Turing (1950) also explicitly acknowledges that his Machine, the founding mathematical structure for programming, is "Laplacian".

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The biological default state of cell proliferation with variation and motility, a fundamental principle for a theory of organisms

Ana M. Soto[a,b,*], Giuseppe Longo[a,b], Maël Montévil[c,d], Carlos Sonnenschein[a,e,b]

[a] *Centre Cavaillès, République des Savoirs, CNRS USR3608, Collège de France et École Normale Supérieure, Paris, France*
[b] *Department of Integrative Physiology and Pathobiology, Tufts University School of Medicine, Boston, MA USA*
[c] *Laboratoire "Matière et Systèmes Complexes" (MSC), UMR 7057 CNRS, Université Paris 7 Diderot, 75205 Paris Cedex 13, France*
[d] *Institut d'Histoire et de Philosophie des Sciences et des Techniques (IHPST) - UMR 8590, 13, rue du Four, 75006 Paris, France*
[e] *Institut d'Études Avancées de Nantes, France.*

Abstract

The principle of inertia is central to the modern scientific revolution. By postulating this principle Galileo at once identified a pertinent physical observable (momentum) and a conservation law (momentum conservation). He then could scientifically analyze what modifies inertial movement: gravitation and friction. Inertia, the default state in mechanics, represented a major theoretical commitment: there is no need to explain uniform rectilinear motion, rather, there is a need to explain departures from it. By analogy, we propose a biological default state of proliferation with variation and motility. From this theoretical commitment, what requires explanation is proliferative quiescence, lack of variation, lack of movement. That proliferation is the default state is axiomatic for biologists studying unicellular organisms. Moreover, it is implied in Darwin's "descent with modification". Although a "default state" is a theoretical construct and a limit case that does not need to be instantiated, conditions that closely resemble unrestrained cell proliferation are readily obtained experimentally. We will illustrate theoretical and experimental consequences of applying and of ignoring this principle.

Keywords: default state, theory, organicism, emergence, mathematical symmetries, biological organization

> ...we should supplement Virchow's well-known tenet of the cell theory: "Omnis cellula e cellula," by its counterpart: "Omnis organisatio ex organisatione." If the former denies spontaneous generation of living matter, the latter denies spontaneous generation of organization. In admitting this, we merely paraphrase what Whitman has called the "continuity of organization." But within these specified limits the cell, even in development, is still, as Schwann has as said, an individual.
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> Weiss, P. (1940). *The problem of cell individuality in development.* The American Naturalist, 74:34-46

1. Introduction

Biologists and philosophers have long pondered the differences between inert matter and living entities. Rather than concentrating on this type of comparison, we will mention some compelling characteristics of the living that should be taken into consideration when addressing biological phenomena. They are: agency (the capacity to initiate action[1]), normativity (the capacity of generating their own rules), individuation (the ability to change one's own organization), the propensity to become sick, and the return to health. In this regard, Bichat referring to physical deformities stated: "Whereas monsters are still living beings, there is no distinction between normal and pathological in physics and mechanics[2]". The distinction between the normal and the pathological holds for living beings alone". Inspired by Canguilhem, we will add that the opposite of pathological is not "normal" but "healthy" (Canguilhem 1991). This is illustrated by the fact that individuals experiencing *situs inversus totalis* (heart in the right side, liver in the left side) may be perfectly healthy without being normal.

*Corresponding author
Email addresses: ana.soto@tufts.edu (Ana M. Soto), longo@ens.fr (Giuseppe Longo), mael.montevil@gmail.com (Maël Montévil), carlos.sonnenschein@tufts.edu (Carlos Sonnenschein)
URL: http://www.di.ens.fr/users/longo/ (Giuseppe Longo), http://montevil.theobio.org (Maël Montévil)

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[1] These definitions of agency, normativity and individuality are chosen because they are brief and broadly useful. They have been discussed more extensively (Burge 2009; Moreno and Mossio 2015) and PA Miquel this issue)

[2] Quoted by (Canguilhem 2008) page 90

There are differences between the inert and the alive, and thus between the sciences that study them (Longo and Soto, this issue). In this regard, it is pointless to try to fit biology into physics, as one would when thinking that because a prebiotic world preceded the advent of life, life would represent a particular case of the physical "world". In fact, scientists do not directly deal with the "real world" but with scientific disciplines constructed by the human mind to understand such a world. Hence, when we refer to the physical or biological, we are referring to the disciplines that address inert and living matter, respectively. Thus, we can only talk about the coherence between the two disciplines. That is, living matter "obeys" the laws of physics, but additional principles and observables may be necessary to understand organisms. When biology is interpreted as "extended physics" the inert state of matter can be considered as a special case or a singularity of the living state of matter. In this case, physics is biology when all organisms are ignored or dead. In science, similar conceptual transitions already exist: after Riemann, Euclidian Geometry instead of being considered the ultimate foundation of mathematics has been viewed as a special case, a singularity: Riemann's geometry on space of no curvature (that is, curvature 0).

Before the 20$^{\text{th}}$ century, biologists often explicitly stated the philosophical bases for their observations, experiments and theories. Two examples of this practice are Blumenbach's correspondence with Kant about a "formative force" (Lenoir 1982) and Darwin's explicit mention of being influenced by Whewell (Ruse 1975). In the preceding articles of this issue we have addressed the role of theory on the choice of the observables and the construction of objectivity, particularly the founding role of Galileo's inertia in classical mechanics. This principle represents a limit case: if no cause (a force) modifies the properties of an object, the object conserves its properties. In the rigorous mathematical sense, this is a limit or asymptotic case since there are always frictions and gravitational forces and no physical body can be exactly identified to a point-mass moving on a Euclidean straight line. For didactic purposes we use the term "default state" (borrowed from computer science) to denote a state that applies when "no action is taken". In short, the default state is what happens when nothing is done to the intended object or system in question. Galileo's choice of inertia as a fundamental theoretical postulate was counter-intuitive because objects present in our immediate surroundings are subject to forces that hinder the manifestation of such a state. The counter-intuitiveness of Galilean inertia is illustrated by the fact that Kepler and Leibnitz thought that the opposite was true, namely, that "The globe [meaning a planet] has a natural inertia or stillness, for which it remains at rest in every place, where it is posed alone [quoted in: (Bussotti 2015)].

The crucial point is that accepting inertia as a postulate implies that *we do not need to explain uniform rectilinear motion, rather, we need to explain departures from it.* The usefulness of this postulate remains uncontested in classical mechanics. In fact, 300 years after Galileo, this counter-intuitive postulate was buttressed by E Noether's theorems; they provided a deeper understanding of inertia by justifying conservation properties of energy and momentum on the basis of time and space symmetries, respectively (van Fraassen 1989). Ever since, symmetries (and their breaking) acquired an even more fundamental role in physics.

In short, the conservation of these symmetries is based on the idea that the 'laws' of physics are the same at different positions and times. In spite of the advance due to Noether's theorem, the notion of symmetries is already used in Archimedes' law of the lever: equal weights at equal distances are in equilibrium. This article proposes a biological default state which would play a comparable useful role in organismal biology.

2. Existing biological theories

Biology has one comprehensive theory, the theory of evolution which encompasses the time-scale of phylogenesis and is based on two principles, i) reproduction with modification, and ii) natural selection. In contrast, a theory of organisms encompassing the time-scale of a life cycle has yet to be formulated. The theoretical wealth of biology is manifested by the various theories that address important but more restricted areas of biology, such as the cell theory, the chromosome theory, the germ theory of disease, etc. Among those, the one relevant to this chapter is cell theory, which postulates that cells i) are the basic unit of life, ii) are made from pre-existing cells, and iii) that organisms are made up of one or more cells and extracellular matrices, which are made by cells.

The cell theory is central to both ontogenesis and phylogenesis. Regarding the former, multicellular organisms develop from a zygote, that is at the same time a cell and an organism (Soto et al. 2008). Regarding phylogenesis, all existing living organisms are believed to have a common unicellular ancestor. Using cell theory as a starting point we postulate a biological default state as a step towards building a theory of organisms and their ontogenesis.

3. The biological default state

Let's assume for the sake of argument that we could observe the moment that life emerged from the pre-biotic soup.... What would have been the properties of this first cell? Is it reasonable to infer that it would do pretty much the same as unicellular organisms do today? Indeed, microbiologists agree that unicellular organisms spontaneously proliferate as long as their milieu provides sufficient nutrients and appropriate ranges of pH, temperature and pressure. They would also agree that motility is commonplace in unicellular prokaryotes and eukaryotes; by motility we mean the ability to initiate movement. Motility is perhaps the most obvious instantiation of agency,

i.e., the characteristic that makes the intuitive distinction between alive and inert[3].

In biology, we propose a ***default state of proliferation with variation and motility***, which is common to all prokaryotic and eukaryotic cells, meaning all those that are unicellular organisms and those that form part of multicellular ones. *In other words, paralleling the concept of inertia in classical mechanics, proliferation, variation and motility, require no explanation in biology. On the contrary, hindrances to the expression of default state, namely, proliferative quiescence, lack of variation, and lack of movement require an explanation.* There is, however, a fundamental difference between the default state in mechanics and in biology. While the former is about invariance (of momentum in particular) and conservation of symmetries (of space-time), the latter is about symmetry changes.[4] These differences between theories of the inert and of the living are discussed in greater detail in Longo and Soto, this issue, and (Longo et al. 2015).

3.1. Proliferation

As mentioned above, a "default state" is a theoretical construct, a limit case, and thus does not require experimental confirmation. However, this fact does not mean that it lacks an experimental correlate. Galileo conceptualized the principle of inertia through experimentation using ramps. He gave sufficient evidence to justify the hypothesis that the Aristotelian ideas where every motion requires a moving force and where the tendency of objects is to remain at rest were wrong. Based on the experimental observations whereby Galileo was changing the influence of gravity and friction on the motion of an object, he dared to imagine a "limit" case where no forces were acting upon the object. Inertia is not a figment of the imagination; we can experience it when riding a vehicle that suddenly and forcefully stops. Similarly, in biology there are natural and experimental conditions that closely resemble ***unrestrained cell proliferation***; these are instantiated in prokaryotes and unicellular eukaryotes, like yeast, when growing in a nutrient-rich environment, and by cells from multicellular eukaryotes when placed in culture conditions in a nutrient-rich medium. We posit that from LUCA (the Last Universal Common Ancestor) on, proliferation has been retained as the default state with the advent of multicellular organisms (metaphyta and metazoa). This conclusion is supported by the conservation of cell cycle components throughout eukaryotes (Sonnenschein and Soto 1999) and by experimental evidence (Sonnenschein and Soto 1999; Soto and Sonnenschein 1985; Sonnenschein et al. 1996; Leitch et al. 2010; Ying et al. 2008).

The default state is exemplified by the behavior of estrogen-responsive cells like those in the mammary gland. When given to a sexually immature animal, estrogen will induce the growth of the ductal tree of the mammary gland. This effect was interpreted as evidence that estrogen induces the proliferation of the epithelial cells that form the ductal tree. However, when removed from the organism, these cells proliferate maximally in the absence of estrogen. Also, when estrogen-free blood serum is added to the culture medium, it induces a dose-dependent inhibition of cell proliferation, which is manifested as a cell cycle arrest in the Go-G1 phase of the cell cycle. Only after this inhibition takes place, is estrogen necessary to overcome such inhibition (Figure 3.1) (Sonnenschein et al. 1996); indeed, estrogen neutralizes the action of the serum-borne inhibitor. The default state of proliferation has been adopted advantageously as a fundamental principle in theories of carcinogenesis and of development (Sonnenschein and Soto 1999; Soto and Sonnenschein 2010; Minelli 2011).

3.2. Variation

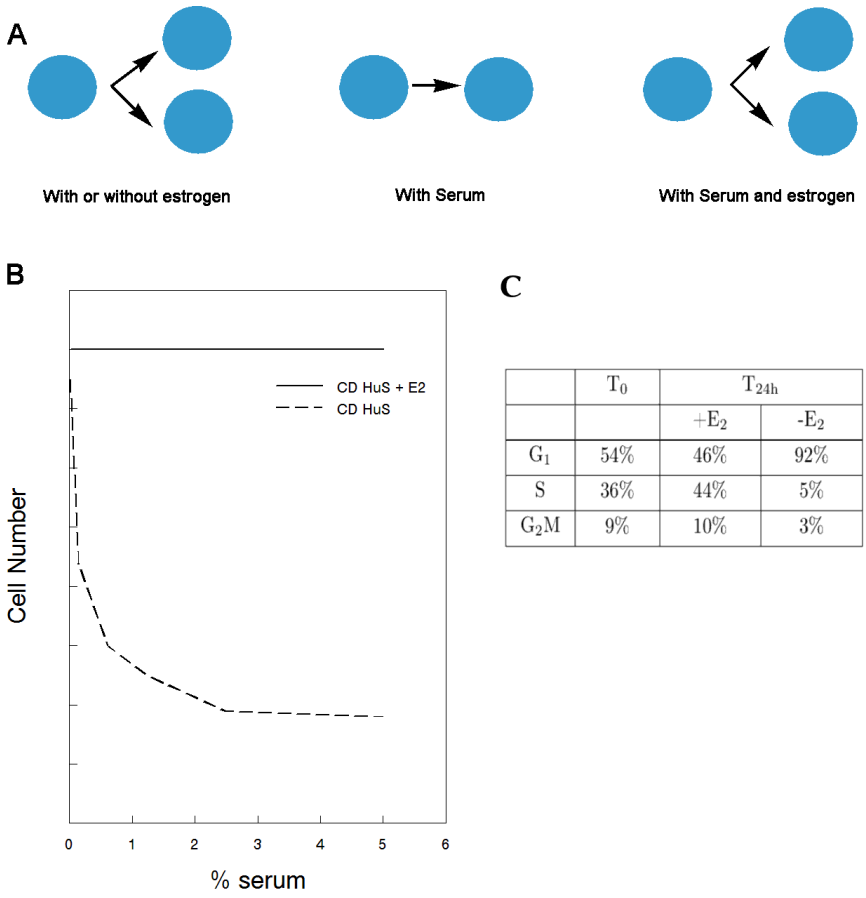
Variation, an integral part of the biological default state, is readily generated with each cell division. It manifests itself as the unequal distribution of macromolecules and organelles following cell division, and it is related to the low number of these intracellular components (Huh and Paulsson 2011). Additional variation is generated by the inherent stochasticity of gene expression which leads to intrinsic cell-to-cell variation of mRNA and protein levels (Kupiec 1983; Taniguchi et al. 2010; Tyagi 2010; Marinov et al. 2014; Raj and Oudenaarden 2008). Another source of variation is generated by somatic mutations and aneuploidy, that, contrary to conventional wisdom suggesting that these events only occur in cells in a neoplastic state, were described in cells of normal mammalian organs, like kidney, liver and brain (Martin et al. 1996; Rehen et al. 2001). In this new context, aneuploidy is seen as a common and advantageous outcome; near 50% of liver cells are aneuploid and probably because of it livers are better adapted to toxic injury (Duncan et al. 2012; Rehen et al. 2005). Variation is also generated at supracellular levels of organization (Montévil et al, this issue), like during branching morphogenesis. We have referred to this supracellular source of variation when positing the framing principle of non-identical iteration of morphogenetic processes (Longo et al. 2015); Montévil et al, this issue; Montévil, Speroni and Soto, this issue).

3.3. Motility

Motility, the third component of the biological default state, encompasses intracellular, cellular, tissue and organismic non-random movements (Stebbings 2001). From gliding to swarming or swimming, the motility of microorganisms immediately suggests the idea of agency, and in

[3] Inert definition: having no inherent power of action, motion, or resistance (http://dictionary.reference.com/browse/inert).

[4] Theoretical symmetries are transformations that do not change the intended aspect of an object (or mathematically of an equation). For example, the equation of classical gravitation does not depend on the time or location of the objects considered, only their mass and relative distance matter. Theoretical symmetries have a fundamental role in physics making possible its formalization by using mathematical tools and concepts (van Fraassen 1989; Bailly and Longo 2011; Montevil et al. this issue).



C

	T_0	T_{24h}	
		$+E_2$	$-E_2$
G_1	54%	46%	92%
S	36%	44%	5%
G_2M	9%	10%	3%

Figure 1: Experimental examples of the default state.
Panel A: Schematic view. Left, the blue estrogen-target cells proliferate in serumless medium regardless of the presence of estrogen. Middle, cells are constrained from proliferating by serum. Right, estrogen cancels the serum inhibition and cells proliferate.
Panel B: Schematic representation of serum inhibition. Cells proliferate maximally in the absence of serum supplement when similar numbers of estrogen-target cells are cultured in a defined medium containing nutrients. The addition of estrogen-free human serum resulted in a dose-dependent inhibition of cell proliferation. Addition of estrogen does increase cell numbers in serumless conditions; instead it neutralizes the inhibitory effect of serum (---- with estrogen, ———- without estrogen).
Panel C: Effect of serum inhibitor (recombinant serum albumin) on the cell cycle profile of estrogen target MCF7 cells at 24h. Cells in medium containing HAS are predominately arrested in G1. Almost half of the cells in media containing HSA and estrogen are undergoing DNA synthesis (S phase of the cycle).

fact, the organism uses these movements to migrate to more suitable environments (Jarrell and McBride 2008). To do so, they use sensors for attractants and repellents. Motility is not synonymous with locomotion. For example, plants that are attached to the ground by their roots cannot move from one location to another one, but they can make their parts move, as when growing towards a source of light. Flowers and leaves open and close in response to light (van Doorn and van Meeteren 2003), and like animal cells, can move organelles using actin and myosin (Ueda et al. 2010). In summary, like the mechanical default state, the biological one is a limit case which is theoretically derived from actual experimental observations.

4. 4 The usefulness of the concept of inertia and default state in biology

4.1. The Hardy-Weinberg equilibrium

The introduction of inertia by Galileo, a simple and universal principle which applies to both celestial bodies like planets and stars and to terrestrial ones, like apples and cannon balls, was reformulated by Newton as the first law of motion. In addition to the indisputable founding theoretical value of such a principle in its realm of classical mechanics, it inspired evolutionary biologists to develop their own founding principle. Indeed, early in the 20th century population geneticists formulated a principle that allowed them to study the effect of several "forces", namely mutation, selection, mate choice, on the allelic frequencies of target populations. This is the Hardy-Weinberg equilibrium principle which states "that allele and genotype frequencies in a population will remain constant from gen-

eration to generation in the absence of other evolutionary influences". In other words, Hardy–Weinberg equilibrium describes an ideal condition against which the effects of these forces can be analyzed (Edwards 1977).

Unlike Newton's law, the Hardy-Weinberg equilibrium does not constitute a founding principle of biology, but an epistemic tool to study the factors that will negate such equilibrium, like selection. Loosely related to this use, epidemiologists who as population geneticists deal with large populations and statistics, took from the latter the idea of a null hypothesis representing the possible outcome that chance is only responsible for the observed results. Again, the epistemic value of these tools is that it fixes a "no-change" hypothetical condition against which to study change.

4.2. The Zero Force Evolutionary Law

Quite recently, some evolutionary biologists criticized the Hardy-Weinberg equilibrium when taken as a founding principle. More precisely, Brandon and McShea stated that quite to the contrary of the stasis represented by Hardy-Weinberg, their view of "the zero-force evolutionary law" is the *constitutive* tendency for diversity and complexity to increase (Brandon and McShea 2012). In contraposition to the Hardy-Weinberg equilibrium, by adopting the zero-force evolutionary law what requires explanation is stasis. By claiming that the "...default condition of evolutionary systems is change, and change of a particular sort—increase of diversity and complexity", these authors elevate their "zero-force evolutionary law" to a natural condition, that is, a situation for which there is empirical evidence (Brandon and McShea 2012; Gouvêa 2015). We are now full circle back to the point where we described inertia as a limit case derived from empirical evidence. Additionally, this zero-force evolutionary law, like the biological default state, is about ***change.***

4.3. The Zero Force Evolutionary Law: a consequence of the default state

We consider that the "zero-force evolutionary law" is not the biological first law. Instead, the "zero-force evolutionary law" is the consequence of the biological default state, which is the generator of intrinsic variation[5]. The general tendency of biological evolution towards an increase of the average complexity is compatible with the fact that some species have lost appendages, structures or organs and become less complex under various complexity measurements. We consider this fact as a *consequence* of a more general phenomenon, which was proposed by S.-J. Gould (Gould 1996) and closely analyzed in (Bailly and Longo 2009) and (Longo and Montévil 2014). Gould proposed a "left wall" of minimal organismal complexity, such as that of bacteria, beyond which life is not possible (Figure 4.3).

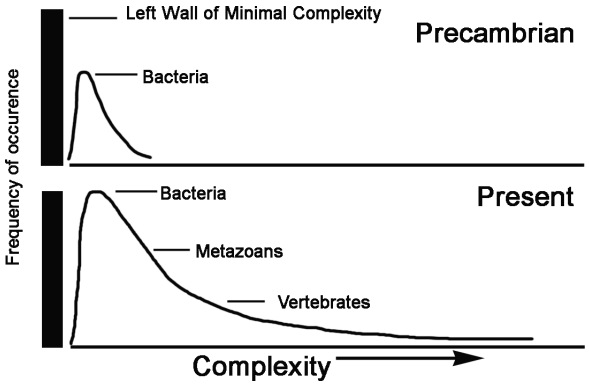


Figure 2: S.J. Gould proposed a wall of minimal complexity to the left of which life is not possible. Proliferation increases the biomass while creating diversity. The asymmetry resulting from the left wall results in increased average complexity.

From this initial stage (proliferation with variation), the expression of the default state results in the increase of the biomass while creating diversity. Like a gas exploding against a wall, the *diffusion* of the biomass generated by the default state is asymmetric, resulting in increased average complexity. This means that there is no need for any sort of evolutionary pressure towards higher complexity. Indeed, the curve proposed by Gould as a preliminary mathematical description of this spreading of life may be fully reconstructed with a diffusion equation that includes the dynamics of asymmetric boundary conditions (the left wall)(Bailly and Longo 2009; Longo and Montévil 2014). This is done by assuming that evolution follows a variability law, which is a consequence of the default state.. As a *consequence* of the original asymmetry and the default state, complexity can only increase on average, with no need to assume this increase as a principle. In this way, two very simple assumptions produce a strong consequence. [6]. Indeed, this structure of reasoning also applies to the evolution of other organismal quantities, such as body mass, as long as the ancestor organism has a low value for this quantity when compared to their descendants. Thus, the "diffusion" following from the instantiation of the default state will result in an average increase of the considered quantity over evolution. In particular, the default state justifies the diffusion equation used to model

[5] It is worth noting that the authors of this Chapter independently arrived at the conclusion that the "zero force evolutionary law" is not a principle. While Soto and Sonnenschein proposed that the generation of variation by the default state is the condition of possibility for the zero-force evolutionary law, Longo and Montévil derived the increasing diversity and "complexity" in evolution from the asymmetric random diffusion principle they postulated (Longo and Montévil 2014), p229)."

[6] In (Bailly and Longo 2009) a detailed, yet preliminary, measure of organismal complexity is formalized, which refines Gould's informal scheme, and set the basis for the proposal of a "hallmark" of cancer in (Longo et al. 2015). As a matter of fact, cancer seems to be the only pathology where decreasing functionality (of organs) is correlated to increasing complexity (of tissues: folding, fractal structures, increasing number of lumena).

the evolution (and overall increase) of the mass of mammals in combination with a selective pressure against this increase (Clauset and Redner 2009). It follows that the same reasoning would apply *mutatis mutandis* to different measures of complexity, provided they follow the above assumptions. Again, the default state principle has more generality than the "zero-force evolutionary law"; meaning that the latter may be understood as a consequence of the former.

5. From inertia to operational definitions

Given that evolutionary biologists used the principle of inertia in the first decade of the 20th century, why is it that organismal biologists have yet to develop comparable theoretical constructs? We attribute this lack of theoretical thinking in organismal biology to the belief expressed by many biologists in the first half of the 20th century that facts "speak for themselves" (see Perret et al in this issue), and later, to the adoption of the mathematical theory of information without critical examination. This brought the metaphorical use of the concepts of *information*, *program* and *signal* to biology hindering its progress (Longo et al. 2012). Regarding the former, organismal biologists tend to believe that they observe the "real world" and thus that data are objective. Contrary to this belief, data are theory laden, and thus one should examine the hidden philosophical content of "data". Another important factor in this discussion is that, lacking global theories, operational thinking plus dubious common-sense beliefs become the substitute for theories. In operationalism, scientific terms are defined by the experimental operations which determine their applicability (Hull 1968).

5.1. The operational origins of hormones and growth factors

Surgical removal of the gonads results in atrophy of the accessory sex organs (uterus, prostate). This non-controversial fact prompted the search for "factors" secreted by gonads that made the accessory organs grow in size, due to an increase of their cell number and in the deposition of extracellular matrix. Administration of gonadal extracts resulted in the reversal of this atrophy and in due turn, the substances that produced these trophic effects were identified. They were named "hormones", and were defined operationally as the substances that, in their bioassays, induced the growth of the target organs. The operational nature of this definition was soon forgotten and it became a "fact" that hormones directly stimulate proliferation., Despite evidence to the contrary, this notion remains engrained among specialists (Sonnenschein and Soto 1999).

The concept of "growth factor" appeared in the early 20th century when biologists, having succeeded in propagating bacteria in medical laboratories using meat broth and other complex extracts and body fluids, turned to the study of bacterial nutrition. Any substance that improved bacterial propagation was called a "growth factor". In modern microbiology textbooks, growth factors are defined as substances required in small amounts by unicellular organisms because they fulfill specific roles in the biosynthesis of the organism's own components. A growth factor is necessary when a metabolic pathway is missing or is blocked. In this context growth factors are purines and pyrimidines, amino acids and vitamins.

At the time when several groups attempted to develop methods to culture cells isolated from metazoan organisms, research on bacterial metabolism and nutrition was flourishing. Among those groups, Margaret and Warren H. Lewis at Johns Hopkins University empirically created artificial conditions of life while wishing to have control over these cells. For the Lewises, cells were not agents. Instead, they thought that in order to grow the cells needed to be "stimulated" to proliferate as if they were as passive as inert objects. In hindsight, we now know that when freshly isolated cells fail to thrive it is not due to them being quiescent but because they die. Slowly but inexorably, the operational concept of "growth factor" became established within the field of tissue culture as a specific "signal" to induce a passive cell to proliferate (Sonnenschein et al. 2013).

The idea of a "program" in biology reinforced the view that cells need to receive "information" or "signals" in order to proliferate and to move. When applying this thinking to the initial cell at the beginning of life what or who would be the purveyor of such stimuli? From our perspective, cell culture represents a state of de-emergence, whereby the cells that form part of an organism are "liberated" from the constraints imposed by that organism. Under extra-organismic (in culture) conditions, these cells regain properties that mimic those of the unicellular organisms from which the multi-celled organism eventually evolved. This brings up the relevance of placing cell and tissue culture under an evolutionary perspective. The pioneers of tissue culture failed to apply evolutionary theory when venturing into quasi-artificial life (Maienschein 1983). In hindsight, this was a squandered opportunity to recognize that in the quasi-artificial life of the culture flask, metazoan cells behave as unicellular organisms, and thus exert their constitutive ability to proliferate and move, properties that enabled the LUCA to generate all the diversity of life on earth that we recognize today.

5.2. From operational definitions to "the law of the land"

As mentioned above, microbiologists accept as fact that unicellular organisms constitutively proliferate in the presence of nutrients (proliferation is their default state). Obviously, cells in multicellular organisms do not exhibit unconstrained cell proliferation. Below we transcribe the standard explanation for this difference from a widely used textbook. "Unicellular organisms tend to grow and divide as fast as they can, and their rate of proliferation

depends largely on the availability of nutrients in the environment. The cells of a multicellular organism, however, divide only when the organism needs more cells. Thus, for an animal cell to proliferate, it must receive stimulatory extracellular signals, in the form of mitogens, from other cells, usually its neighbors. Mitogens overcome intracellular braking mechanisms that block progress through the cell cycle."(Alberts et al. 2014)

From the above analysis about inertia and a biological default state, what exactly is objectionable in the just quoted textbook account of this difference? The quotation acknowledges that unicellular organisms have proliferation as their default state. Next, it moves to multicellular organisms and, it states the obvious: that cells in multicellular organisms do not proliferate despite plenty of nutrients being available. From there, while using common sense, the sense that Galileo systematically disregarded, the quotation claims as a fact that animal cells are quiescent and need stimuli, i.e. signals to proliferate. This option implies a reversal of the default state taking place with the advent of multicellularity. However, no explanation is given about the acknowledged fact that metaphyta conserved proliferation as the default state, or that the cell cycle components are conserved through evolution; altogether, these pieces of evidence strongly suggest that there was no change of default state in the cells of multicellular organisms. The concept that the default state could be constrained in animals, namely, that an additional layer of regulation emerged during the advent of multicellularity, was not contemplated by the authors of the textbook referred to above.

Since the introduction of the concept of a biological default state operating in all cells (Soto and Sonnenschein 1991), researchers dealing with the phenomenon of lymphocyte quiescence found that quiescence is an induced state, namely that proliferation is actively constrained. Separately, other researchers concluded that embryonic stem cells proliferate constitutively, a phenomenon they called "ground state" (Ying et al. 2008; Leitch et al. 2010). In both cases, proliferation as a default state was interpreted as a peculiarity of the particular experimental model being investigated. The absence of a bold attempt to generalize these findings to all cells is probably due to a dominant perception among biologists that there are neither laws nor rules in biology. Finally, and most fundamentally, in the absence of a global theoretical framework that constructs objectivity and determines the proper observables, organismal biology appears as less intelligible given that new results create more contradictions that happily coexist and are never discarded.

6. The biological default state links ontogenesis to phylogenesis

The biological default state is a founding principle upon which a theory of organisms and of their ontogenesis can be constructed. It takes into consideration the agency of organisms manifested as the constitutive ability to reproduce and generate movement. Equally important, the biological default state ties the source of variation together with its transmission at each proliferative event. Each cell division thus represents a symmetry change that generates two non-identical daughter cells.

A founding principle for a theory of organisms that addresses ontogenesis needs to be compatible with the theory of evolution, which addresses phylogenesis. Below we address three points in common between these theories, namely, constitutive reproduction/ proliferation, variation and historicity.

6.1. Darwin's limit case and the default state

In the Origin of Species, Darwin stated: "...There is no exception to the rule that every organic being naturally increases at so high a rate, that, if not destroyed, the earth would soon be covered by the progeny of a single pair" (Darwin 1859). According to Darwin's theory, reproduction is linked to modification: in his own words, "descent with modification". Reproduction with variation is intrinsic to organisms regardless of whether they are unicellular or multicellular (Sonnenschein and Soto 1999; Soto and Sonnenschein 2011). Darwin's narrative implies that reproduction with variation is a *default state* and he describes it as a limit case. In fact, because reproduction and proliferation are the same event in asexual reproduction of unicellular organisms, this default state represents a common postulate for the theories of evolution and organisms.

6.2. Change, symmetry breaks, and historicity

The theory of evolution addresses the generation of incessant change (variation in our words, modification in Darwin's) upon which natural selection operates; the result is phenotypic diversity. The incessant changes of life processes may be analyzed as *extended critical transitions (Bailly and Longo 2011; Longo and Montévil 2014)*. Under our theoretical approach, throughout its ontogeny, an organism may be understood as being in a permanent transition with all the main signatures of criticality, such as changes of symmetries and the formation of a new global structure (Longo et al. 2015). In an organism, each cell division changes local symmetries because each of those divisions forces new local and global correlations. These changes yield variability and adaptability to organisms. In the context of evolution, the advent of new functions and organs are additional examples of symmetry changes.

Far-from-equilibrium, self-organizing physical systems have been used as a starting point to understand complex biological organization. These physical systems are understood by the analysis of their instantaneous flows. Indeed, the shape of a flame can be calculated from the flows of matter that go through it, whereas the shape of an organism cannot. Far from equilibrium systems appear spontaneously and can be analyzed independently. In contrast, organisms are not spontaneous but historical; that

is, they are a consequence of the reproductive activity of a pre-existing organism. Organization cannot be deduced from flows operating within and upon organisms; instead, understanding biological organization requires a historical analysis, and this applies to the time-scale of ontogenesis as well as the one of phylogenesis (Longo et al. 2015).

Finally, the recently proposed "zero-force evolutionary law" (Brandon and McShea 2012; Gouvêa 2015), namely the *constitutive* tendency for diversity and complexity to increase throughout evolution is not a default state or principle, but a derived property of the biological default state. The zero-force evolutionary law stresses increasing complexity and diversity. As we mentioned above in reference to Gould's work, this tendency may be seen as a consequence of i) the agency of living matter instantiated by the biological default state of proliferation with variation and motility, and of ii) natural selection, once this increase of diversity and complexity is analyzed in the global terms of an asymmetric diffusion from the least (bacterial) complexity.

7. Conclusions

The view proposed herein is anchored in the radical materiality of the living, whereby it is impossible to dissociate the actual materials from which living organisms are made of from the functions these organisms fulfill. This view is inimical to the strong dualism implied by the notion of program and information which manifests itself in the independence of the software from its material substrate, the hardware. On the contrary, cells can only be obtained by the proliferation of pre-existing cells which are made up of chemicals of a precise composition. Paraphrasing the epigraph by Paul Weiss, a theory of organisms should be based on the notions that all cells come from pre-existing cells, and that every biological organization comes from preexisting organization. These tenets rule out both the spontaneous generation of living matter and of biological organization. Instead, the cell is an agent and an individual endowed with normative capacity, even when residing in a multicellular organism. Indeed, every organism was once a cell, and in multicellular organisms undergoing embryonic development, the zygote resulting from the union of a female and male gamete is both a cell and an organism. Thus, organisms are the consequence of the inherent variability generated by proliferation, motility and self-organization. Their morphogenesis would then be the result of the default state plus physical constraints, like gravity, and those generated and imposed by the organism itself, such as physical ones like muscular tension, tissue rigidity and compliance, and chemical ones such as the molecular particularities of amino-acids, proteins and nucleic acids.

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Theoretical principles for biology: organization

Matteo Mossio[a,*], Maël Montévil[b,a], Giuseppe Longo[c,d]

[a] *Institut d'Histoire et de Philosophie des Sciences et des Techniques (IHPST) - UMR 8590, 13, rue du Four, 75006 Paris, France*
[b] *Laboratoire "Matière et Systèmes Complexes" (MSC), UMR 7057 CNRS, Université Paris 7 Diderot, 75205 Paris Cedex 13, France*
[c] *Centre Cavaillès, République des Savoirs, CNRS USR3608, Collège de France et École Normale Supérieure, Paris, France*
[d] *Department of Integrative Physiology and Pathobiology, Tufts University School of Medicine, Boston, MA USA*

Abstract

In the search of a theory of biological organisms, we propose to adopt organization as a theoretical principle. Organization constitutes an overarching hypothesis that frames the intelligibility of biological objects, by characterizing their relevant aspects. After a succinct historical survey on the understanding of organization in the organicist tradition, we offer a specific characterization in terms of closure of constraints. We then discuss some implications of the adoption of organization as a principle and, in particular, we focus on how it fosters an original approach to biological stability, as well as and its interplay with variation.

Keywords: Theoretical principle, organization, constraints, closure, stability, organicism

> The physiologist and the physician must never forget that the living being comprises an organism and an individuality. [...] Indeed, when we wish to ascribe to a physiological quality its value ad true significance, we must always refer to this whole and draw our final conclusions only in relation to its effects in the whole.
>
> Bernard, 1865/1984, quoted and translated by Wolfe, 2010.

1. Introduction

For the past five decades, most of biological research has been framed on the hypothesis that biological organisms are essentially determined by genetic information[1], and the molecular mechanisms through which such information is expressed. This hypothesis – which we refer to here as genocentrism – acknowledges of course that a variety of causal factors (e.g. physical, environmental...) concur in enabling the development and functioning of biological organisms. Yet, among these factors, genetic ones would have a special status, insofar as they determine the *distinctive* features of biological phenomena. In particular, protein synthesis, (and thereby biological functions) results from the expression of genetic information. According to a genocentric perspective, therefore, what makes biological systems specific with respect to other natural systems is ultimately the fact that they would be the result of the expression of genetic information.

Understood in this way, genocentrism carries on a form of explanatory reductionism insofar as biological phenomena are assumed to be adequately explained[2] by appealing to genetic information. In particular, the concept of organism loses centrality in biological sciences (Laubichler, 2000) because of its supposed derivability from genes: organisms would be, under adequate conditions, the *result* of the expression of genetic information through development.

The research program framed on genocentrism has undergone a spectacular development, remarkably represented by the Human Genome Project, which was declared complete in 2003. Recently, however, experimental evidence is increasingly challenging the idea that genetic information determines biological functions: in particular, gene expression is subject to massive variability, which suggests

*Corresponding author
Email addresses: matteo.mossio@univ-paris1.fr (Matteo Mossio), mael.montevil@gmail.com (Maël Montévil), longo@ens.fr (Giuseppe Longo)
URL: http://montevil.theobio.org (Maël Montévil), http://www.di.ens.fr/users/longo/ (Giuseppe Longo)

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[1] Note that we do not aim to discuss the notion of genetic information here; see Perret & Longo (2016) and Longo et al. (2012a) for a critical analysis.

[2] The notion of "theoretical determination" should not be confused with "determinism". Determinism corresponds to the assumption that the perfect knowledge of a given situation at a given time entails its future descriptions. Theoretical determination is the framework for understanding the changes of the intended object, and this framework can be not deterministic, as is the case in quantum mechanics, for example. Genocentrism rather corresponds to an assumption of "completeness" of the DNA as a code for development.

that DNA *under*determines functional proteins and, in the end, the very organization of the organism. Far from being mere "noise", variation is increasingly conceived as an inherent dimension of gene expression (Lestas et al., 2010; Dueck et al., 2016). Moreover, experimental biology shows not only that gene expression is variable, but even inherently stochastic (Ray & van Oudenaarden, 2008; Kupiec & Sonigo, 2000)[3].

As a matter of fact, the accumulation of experimental evidence at odds with genocentrism has induced a progressive renewal of interest in more integrative accounts, which aim at complementing genes with other determinants of biological phenomena. A main example of this trend is Systems Biology (Kitano, 2002) that elaborates mathematical and computational models on large, multi-scale molecular networks, whose dynamics cannot be determined by genetic information and which, in turn, control the activity of genetic templates.

In the search for integrative accounts, a specific theoretical option consists in claiming that the relevant level of description at which Biology should be framed is that of the organism: the alternative to genocentrism would therefore be *organicism* (Gilbert & Sarkar, 2000; Ruiz-Mirazo et al, 2000; Soto & Sonnenschein, 2005). From an organicist perspective, organisms are the main object of biological science because they are the systems that underlie biological phenomena and – crucially – they cannot be reduced to more fundamental biological entities (such as the genes or other inert components of the organism).

The elaboration of a theory of biological organisms requires dealing with their distinctive complexity, which in turn requires taking into account a number of dimensions, including individuation (see Clarke, 2011; Miquel, 2016), agency (Barandiaran et al., 2009; Arnellos & Moreno, 2015; Soto et al, 2016), regulation (Bich et al. 2015), adaptivity (Di Paolo, 2005), historicity (Ruiz-Mirazo & Moreno, 2012; Longo & Montévil, 2011; 2014), ... and cognition (Thompson, 2007). In this paper and in Montévil et al. (2016), we take a theoretical step toward a Biology of Organisms by arguing that organisms are governed by two theoretical principles: *organization* and *variation*. All biological organisms, in all their diversity and richness of forms and kinds, meet two general principles without exceptions: they are organized, and their organization undergoes variation.

As theoretical principles, organization and variation constitute overarching hypotheses that frame the intelligibility of the objects within the biological domain. Taken together, they characterize the relevant aspects of biological objects, that are measurable observables, relations and changes. To better grasp their nature, a relevant comparison can be made with the role of space and time in Physics, ever since Newton and Kant. One may consider space and time as "conditions of possibility" for constructing physical knowledge; in more modern terms, positing *a priori* the phase space (i.e. the list of pertinent observables and parameters) allows us to spell out a complete determination of the intended processes in physical theories, by equations or evolution functions. Analogously, the ambitious aim of this work is to single-out the principles to be posited as *a priori* conceptual tools for the intelligibility of ontogenesis.

In the general discussion of Montévil et al. (2016), we further elaborate on the status of organization and variation as theoretical principles. One important implication of this strategy is that, although the two principles are supposed to lay the foundations of a biology of organisms, their domain of application is not necessarily restricted to the latter. Indeed, the set of systems that comply with the two principles – and can therefore be taken, by definition, as *biological* systems – is presumably larger than that of organisms. For instance, it has been recently argued (Nunes-Neto et al., 2014) that ecosystems might be described as organized systems by appealing to the same organization principle we are presenting herein. Accordingly, if they were shown to comply with both the organization and variation principles, ecosystems might be conceived of as biological systems, although not necessarily as organisms (Moreno & Mossio, 2015). In other words, we submit that biology is the science of systems meeting the principles of organization and variation, organisms being a specific, particularly relevant, class of biological systems. In the general discussion of Montévil et al. (2016), we further elaborate on the status of organization and variation as theoretical principles.

To characterize each principle, as well as their mutual relations, we elaborated in two distinct papers: the present one deals with organization, while Montévil et al. (2016) explores variation. Within our framework, the two principles are closely related, and each one is involved in the biological realization of the other. On the one hand, organization is a condition for variation, in the sense that the variation we focus on is that *of* the organization: relevant biological variation is that affecting organized systems and their parts. In addition, organization favors the propagation of variation because the mutual dependence between the parts enables the maintenance of changes. On the other hand, variation is a condition for the maintenance and adaptation of organisms over time, as well as the appearance of functional innovations. Biological organization would neither display its current complexity, nor would it last, unless it varied, both during phylogenesis and ontogenesis.

[3] This perspective broadened theoretical determination proper to genocentrism, although it mostly continued to attribute a central role to genes in ontogenesis. In short, "stochastic gene expression" is an increasingly relevant perspective, which modifies the role of randomness in molecular biology, as this moves from "noise" to a form of "functional randomness", while preserving the genocentric perspective. In Montévil et al. (this issue), we further discuss this issue and show how our analysis of organismal constraints may also propose a tentative understanding of the role of genome and the way its stochastic expression is canalized within and by the organism.

2. A primer on the relationship between organization and variation

The principle of organization focuses on the specific complexity of biological systems. Organization refers to the differentiation of functional roles (i.e. division of labor) among the parts of a system and, at the same time, to their integration and coordination as a whole. Furthermore, organization involves a generative dimension in the form of a mutual dependence, such that the very activity *and* existence of each organized part depends on its mutual relationship with the others. As we recall in section 3, these ideas have a long history in both biology and philosophy of biology. In section 4, we provide a characterization of the central features of the very notion of biological organization.

One central implication of proposing a theoretical characterization of biological organization is that this modifies the role of genes as determinants of biological systems. Genes are no longer supposed to be the fundamental causes of biological functions and complexity. Genes (or, more appropriately in our framework, DNA) are certainly *constituents* of biological organization, and they undoubtedly play an indispensable role in the development and functioning of biological systems, as templates for the synthesis of functional macromolecules. Moreover, DNA may be considered as a trace of history: without DNA, biological complexity would not exist. Yet, the expression of genes does not determine the organization, and organization can by no means be understood as its result. Rather, just as for any functional part, the *expression of genes presupposes that the system is organized*[4] (see also Moreno & Mossio, 2015, chapter 6).

As discussed in section 4, the realization of organization involves the conservation of relevant biological aspects, which in turn are associated with the maintenance or reestablishment of local and global theoretical symmetries. In this respect, as we argue in section 5.1, organization constitutes the fundamental ground of biological stability, both at the ontogenetic and phylogenetic scale. Biological organization tends to maintain itself and, thereby, to counter and remove potentially deleterious variations, while preserving useful variations.

At the same time, biological organization undergoes variation, our second theoretical principle. Based on continual symmetry changes (breaking and reconstruction), variation refers to various kinds of changes. Over time, the conserved aspects of organisms *do* vary, and these variations can be *within* the organization (some flows may change quantitatively) or *of* the organization itself (functions can change qualitatively). In Montévil et al. (2016), we discuss how the principle of variation enables us to frame biological objects as *specific* objects, endowed with historicity, contextuality and variability (see also Longo et al., 2015). In section 5.2, we discuss another aspect of the relationship between organization and variation, i.e. the fact that the former can, in the appropriate circumstances, favor and enhance the propagation of local variation to the whole system. Accordingly, section 5 as a whole emphasizes the twofold role of the principle of organization in enabling both stability (section 5.1) and variation (section 5.2): a conceptual tension and complementarity with which a theory of biological organisms should deal in depth in the future. In the conclusion, we sum up the main ideas of the paper, and open some future research directions as, in particular, the conceptual connection between the principle of organization and the principle of the biological default state proposed by Soto et al. (2016).

We next focus on the organization principle, by briefly addressing its history as it pertains to the biological domain.

3. Biological organization: a historical perspective

In the history of biology and philosophy of biology, the organicist tradition has advocated an understanding of biological systems as organized systems (Wolfe, 2010). As Gilbert and Sarkar (2000) explain, organicism constitutes a middle ground between reductionist perspectives and non-naturalist ones. The former assume that the whole can be reduced to its parts (for instance the genes), while the latter appeal to non-natural entities[5]. Others conceive organicism as a tradition that relies on both bottom-up and top-down structures of determination (Soto and Sonnenschein, 2005; Noble, 2006; Soto et al., 2008).

Two preliminary remarks are relevant here. First, until quite recently, organicism had not yet elaborated a coherent and integrated theoretical framework; rather, it has had a multifarious perspective, in which the very notion of organization has not been spelled out in precise theoretical terms. More recently, the situation has improved, and organization is being conceived in more specific terms. Our own proposal in this paper is directly reminiscent of, and grounded in, some of these recent developments.

Second, the organicist[6] tradition has been traced back to Aristotle's conception of the teleological dependence of

[4] Our understanding of the concept of gene converges with what Lenny Moss (2003) labels "Gene-D", the developmental resource that templates amino acid sequences for proteins. "Gene-D" is in contrast to the "Gene-P", the preformationist gene concept, which serves as an instrumental predictor of phenotypic outcomes.

[5] Gilbert and Sarkar refer to *vitalism* as the typical example of a non-naturalist perspective. In fact, although this understanding of vitalism has become quite typical in the 20th century (see also section 2.1 below, about Bertalanffy's vision), it does not apply to all vitalist accounts. As a matter of fact, some of the most important vitalist schools, such as that of Montpellier in the 18th century (Wolfe & Terada, 2008), explicitly reject and criticize the appeal to non-natural entities to explain living phenomena (see Cimino & Duchesneau, 1997).

[6] The term 'organicism' has begun to be used in its contemporary meaning around the end of 19$^{\text{th}}$ century (Peterson, 2010). Its use in relation to previous authors is therefore somewhat questionable.

the parts to the whole organism (Aristotle, 2002) and, in more recent times, to Leibniz's notion of 'organic machines of nature' (Fichant, 2003) that he opposed to Stahl's animistic perspective in their famous controversy (Duchesneau, 1995). However, we will restrict the present overview to the last two centuries, in line with the view that the scientific study of biological organization was significantly oriented by Kant's contribution (Lenoir, 1982).

3.1. From Kant to Weiss

In his *Critique of Judgment* (1790/1987), Kant explicitly describes biological systems as systems characterized by the specific way in which their parts are organized, by the distinctive relationship between the parts and the whole. Kant claims that, unlike any other kind of system, the parts of biological systems do not and cannot exist by themselves, but only insofar as they constitute an organized whole which, in turn, is itself a condition for their own existence and functioning[7]. Accordingly, biological systems are able to organize themselves – to *self-organize* – and can thereby be characterized as "natural purposes", that is, as entities whose constituents are inherently subordinated to the whole organization.

The Kantian focus on biological organization had continuity in the (mostly Continental) Biology of the 19$^{\text{th}}$ century, notably in the work of Goethe (1995) and Cuvier (1817). Cuvier's principle of the "condition of existence", for instance, claims that "the different parts of each being must be coordinated in such a way as to render possible the existence of the being as a whole" (1817 i., 6, quoted and translated by Reiss, 2005). By implying that the different parts are linked and coordinated, Cuvier's principle grounds and guides his empirical investigations in comparative anatomy and paleontology (Cuvier, 1805; see also Huneman, 2006, for an analysis).

Kant's and Cuvier's perspectives further influenced the so-called German "teleomechanists" (Lenoir, 1982) and in particular – to mention two of their prominent figures – Müller's physiology (1837/1840) and von Baer's embryology (1828). They both consider that, as Huneman writes "the proper object of life sciences should be a set of parts organizing itself as a whole, the development and the functioning of this specific kind of entity being the proper field of, respectively, embryology and physiology" (Huneman, 2010: 342).

Claude Bernard explicitly invokes Cuvier's holistic view, and claims that biological systems are to be conceived as organized entities, whose parts are interdependent and mutually generative. In his words, "The physiologist and the physician must never forget that the living being comprises an organism and an individuality . . . If we decompose the living organism into its various parts, it is only for the sake of experimental analysis, not for them to be understood separately. Indeed, when we wish to ascribe to a physiological quality its value ad true significance, we must always refer to this whole and draw our final conclusions only in relation to its effects in the whole" (Bernard 1865/1984, II, ii, § 1, 137, quoted and translated by Wolfe, 2010). Bernard's main focus is on the contribution of the organized parts - that must be investigated through the experimental method – to the conservation of the *internal milieu,* in spite of the continuous variations taking place in the *external milieu* [8].

An important moment in the history of the scientific treatment of biological organization is represented by the "Theoretical Biology Club", founded in Cambridge by a group of researchers that included Woodger, Needham, Waddington and von Bertalanffy (Etxeberria & Umerez, 2006; Peterson, 2010). The Theoretical Biology Club promotes a scientific organicist perspective for biology, and undergoes a rigorous conceptual and theoretical treatment of various dimensions of the very idea of organization, including the analysis of internal relations (Woodger, 1929) and hierarchies (Needham, 1937). A particularly relevant contribution is due to von Bertalanffy (1952), who conceived biological systems as thermodynamically open systems. Biological systems are organized, and their organization goes along with thermodynamic *openness*, i.e. the fact that they continuously exchange energy and matter with the surroundings. Initially used by Bertalanffy as an argument against both vitalism (but see footnote 5) and mechanism, the thermodynamic openness of biological systems – as we will discuss –plays a crucial role in the subsequent elaborations of the notion of biological organization.

To complete this quick overview of pioneering approaches, it is worth recalling that the notion of organization has played a central role in the organicist perspective that permeated embryology in the first half of the 20$^{\text{th}}$ century. In particular, Paul Weiss refers to organization as the "coordinating principle" (Weiss, 1963: 190) that characterizes biological systems beyond local components and processes, and that grounds their stability in the face of internal or external perturbations (see Bich & Arnellos, 2013, for a discussion of Weiss' ideas in relation to the organicist tradition). One of the central goals of this paper is precisely to focus on organization as a principle of stability, as fore-

[7] The generative nature of closure seems to adequately encompass one of the main differences between biological systems on the one hand, and artifacts and other categories of natural systems on the other. Intuitively, it seems correct that those situations in which the existence of the parts depends on that of the whole system are indeed characteristic of biological organisms. The parts of a rock do not dissolve if the whole is broken into pieces, just as the components of a computer do not disintegrate if the whole machine is disassembled.

[8] Indeed, Bernard is rather mentioned for his emphasis on the constancy of the internal milieu than for the vindication of the organizational nature of biological systems. Actually, as is often recalled, his work paved the way for the development of the idea of homeostasis by Cannon (1929) and, later on, by First-Order Cybernetics (Wiener, 1948; Ashby, 1956). Homeostasis, however, designates a general systemic capacity that does not specifically apply to biological systems. In this paper, hence, we do not discuss it. See Mossio & Bich (2014) for additional remarks on this point.

seen by Weiss (see section 5.1 below).

3.2. From Piaget to Kauffman

In the second half of the 20th century, the conceptualization and scientific treatment of biological organization entered into a new phase, characterized by an increasing coherence and theoretical refinement. A milestone in this tradition is the account put forward by Jean Piaget (Piaget, 1967), whose core idea is to integrate into a single coherent picture two inherent dimensions of biological systems: thermodynamic *openness* and organizational *closure*. On the one hand, as emphasized by Bertalanffy, organisms are thermodynamically open (dissipative) systems, traversed by a continuous flow of matter and energy. On the other hand, they realize closure, i.e. a mutual dependence between a set of constituents which maintain each other through their interactions and which could not exist in isolation.

In Piaget's view, closure captures a fundamental aspect of the very idea of "organization", through the association between division of labor and mutual dependence that it implies. In other words, biological organisms are organized precisely because they realize closure. The centrality of closure and its connection to organization, as well as its distinction from (and, yet, complementarity to) thermodynamic openness, have become givens in most subsequent accounts of biological organization (Letelier et al., 2011).

One of the best known accounts of biological organization is the one centered on the concept of *autopoiesis* (Varela et al., 1974; Varela, 1979) which, among other aspects, places heavy emphasis on the generative dimension of closure: biological systems determine themselves in the sense that they "make themselves" (auto-poiein). Precisely because of their dissipative nature, the components of biological organisms undergo degradation over time; the whole system preserves its coherence and identity only insofar as it maintains and stabilizes not just some internal states or processes, but the autopoietic system itself as an organized unity. In spite of its qualities, however, we have argued elsewhere (Montévil & Mossio, 2015) that a central weakness of the concept of autopoiesis is that it does not provide a sufficiently explicit characterization of closure. Biological systems are at the same time thermodynamically open and organizationally closed, but no details are given regarding how the two dimensions are interrelated, what constituents are involved in closure, and at what level of description. In the absence of such specifications, it remains unclear in what precise sense closure would constitute a causal regime that distinctively characterizes biological organization[9].

A concerted attempt to answer this question has been made by Robert Rosen. In *Life Itself* (Rosen, 1991), Rosen reinterprets the Aristotelian categories of causality, and claims that the distinction between closure and openness should be grounded on a distinction between efficient cause and material cause[10]. By relying on this distinction, Rosen's central thesis is that: "a material system is an organism [a living system] if, and only if, it is closed to efficient causation" (Rosen, 1991, p. 244). In turn, a natural system is closed to efficient causation if, and only if, all components having the status of efficient causes within the system are materially produced by the system itself. What matters here is that closure is located at the level of efficient causes: what constitutes the organization is the set of efficient causes subject to closure, and its maintenance (and stability) is the maintenance of the closed network of efficient causes.

Although Rosen's account represents a clear step forward in the theoretical understanding of organization, we think that it still remains too abstract, and therefore hardly applicable as a guiding principle for biological theorizing, modeling and experimentation. Rosen defines closure as involving efficient causes but, without additional specifications, it might be difficult to identify efficient causes in the system: what entities actually play the role of efficient causes in a biological system? To deal with this issue, decisive insights have emerged from more recent literature that elaborates on the thermodynamic grounding of biological systems (Bickhard, 2000; Christensen and Hooker, 2000; Moreno & Ruiz-Mirazo, 1999) and the relations between closure and openness. In particular, Stuart Kauffman (Kauffman, 2000) argues[11] that biological organization implies a circular relationship between work and constraints, in the form of what he labels a "work-constraint (W-C) cycle". When a (W-C) cycle is realized, constraints that apply to the system are produced and maintained by the system itself. Hence, the system needs to use the work generated by the constraints in order to generate those very constraints, by establishing a mutual relationship – a cycle – between constraints and work.

The understanding of the principle of organization that we put forward in this paper lies at the intersection between Rosen's and Kauffman's proposals, and elaborates

[9] Without a precise characterization, the idea of a thermodynamically open system in which the parts depend on each other for their own maintenance does not seem to apply distinctively to the biological domain. Let us mention an example that is frequently referred to in this kind of debate, namely, the hydrologic cycle. In this case, a set of water structures (e.g. clouds, rain, springs, rivers, seas, etc.) generate a cycle of causal relations in which each contributes to the maintenance of the others, and is in turn maintained by the others. Clouds generate rain, which (contributes to) generates a spring, which gives rise to a river, which (contributes to) generates a lake, which regenerates clouds, and so on.

[10] Let us consider an abstract mapping f between the sets A and B, so that f: A $=\gg$ B. If we interpret the mapping in causal terms, and look for the causes of B, Rosen claims (and develops a detailed conceptual and formal justification that we will not repeat here) that A is the material cause of B, while f is the efficient cause.

[11] Kauffman has proposed retrieving the classic idea of "work cycle" (in the sense of the Carnot machine), and to apply it in the context of self-maintaining biochemical reactions. Kauffman's approach is based on Atkins' ideas about work, conceived as a "constrained release of energy" (Atkins, 1984).

on the idea of « self-construction » put forward by Kepa Ruiz-Mirazo and Alvaro Moreno in their analysis of basic autonomy (Ruiz-Mirazo and Moreno, 2004). In the following section, our central thesis is that closure should be specifically understood as *closure of constraints*, a regime of causation which is, at the same time, distinct from and inherently related to the underlying causal regime of thermodynamic openness.

4. Biological organization as closure of constraints

By relying and elaborating on the biological and philosophical tradition outlined in the previous section, we submit that biological organization is to be understood as a *closure* of *constraints.* In other words, claiming that biological systems are "organized" means, in a theoretical precise sense, that some of its constituents acting as constraints realize a regime of mutual dependence between them, which we label 'closure'.

As mentioned above, the concept of closure relates to that of openness. Only thermodynamically open systems can possibly comply with the organization principle, although not *any* thermodynamically open system does. As all open systems, indeed, be they physical or chemical, biological systems are traversed by a flow of energy and matter, which takes the form of processes and reactions occurring in open thermodynamic conditions. In this respect, organisms do not qualitatively differ from other natural open systems. In turn, the characteristic feature of biological systems is that the thermodynamic flow is constrained and canalized by their constitutive constraints, which realize a specific form of mutual dependence – closure – between them.

The understanding of biological organization in terms of closure relies therefore on a distinction between processes (and reactions) and constraints exerted on the former. Let us then turn to this distinction.

4.1. Processes and constraints

In the complex dynamics taking place in biological organisms, different *parts* can be observed and distinguished. Parts are specific structures that play a role in controlling the dynamics, while remaining essentially unaltered by them. In the case of a mammal, an intuitive example is the vascular system, which while transporting blood is not altered by the blood flow. At a much lower level of description, another example is provided by enzymes that change the kinetics of a chemical reaction without being consumed. We propose to characterize the general notion of a biological part in terms of the more precise one of 'constraint' and, thereby, to ground the distinction between thermodynamic openness and organizational closure on a distinction between processes and constraints.

Broadly speaking, processes refer to all those transformations (typically physical processes, chemical reactions, etc.) that occur in biological systems and involve the alteration, consumption, production and/or constitution of entities. Constraints, in turn, refer to entities that, while acting upon these processes, can be said (in some appropriate sense) to remain unaffected by them. A variety of entities can play the role of constraints in an organism, be it in the form of boundary conditions, parameters, restrictions on the configuration space, etc.... In some cases, constraints are exerted by external physical forces and fields, which are essential for life as we know it: for instance, gravitation canalizes development (Bizzarri et al., 2015). In other cases (which, as mentioned, are of paramount importance in the biological domain), constraints are exerted by specific material structures within the organism.

In all situations, constraints contribute to determining the behavior of the system (be it physical, chemical or biological), by reducing the degrees of freedom of the processes and dynamics on which they act.

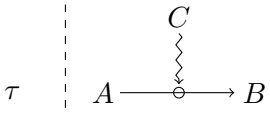
We suggest defining constraints as entities that exhibit a *symmetry* with respect to a process (or a set of processes) that they help stabilize. In general terms, a symmetry is a transformation that does not change the relevant aspects of an object: symmetries and conservation (of energy, momentum, electrical charges, etc.) are therefore complementary concepts (van Fraassen, 1989). Applied to the notion of constraint, this means defining constraints as entities that can exert a causal influence *because of* some symmetrical (conserved) aspect with regards to the target process.

More precisely, given a process A=≫B (A becomes B), C is a constraint on A=≫B, at a specific time scale τ, if and only if two conditions are fulfilled:

I/ C exerts a causal role on the target process. In formal terms, we express this by stating that the processes A=≫B and A_C=≫B_C (i.e. A=≫B under the influence of C) are asymmetric (different) at a time scale τ[12].

II/ C is not altered by (i.e. is conserved through) the target process, at the scale at which the latter takes place. More formally: a temporal symmetry (a conservation property) is associated with all aspects of $C_{A=\gg B}$ with respect to the process A_C=≫B_C, at time scale τ.

The situation which fulfills conditions I-II will be expressed as $C(A=\gg B)\tau$ or, in a graphical form, as:



Let us go back the two biological examples of constraints mentioned above, the vascular system and an enzyme in a mammal, and show that they meet both conditions.

Consider first the vascular system. On the one hand, there is a difference between the flow of oxygen under the

[12]We note $C_{A=\gg B}$ those aspects of C which play a role in the above asymmetry between A=≫B and A_C=≫B_C at time scale τ. In what follows, we generically use the notation C instead of $C_{A=\gg B}$ whenever this does not give rise to confusion.

influence of the vascular system ($A_C{=}{\gg}B_C$) or in its absence ($A{=}{\gg}B$) since, for instance, $A_C{=}{\gg}B_C$ occurs as a transport of oxygen canalized to the neighborhood of every cell where diffusion will occur, whereas $A{=}{\gg}B$ would only have a diffusive form. Consequently, the situation $A_C{=}{\gg}B_C$ fulfills condition I, with the vascular system playing a causal role in the flow of oxygen. On the other hand, a temporal symmetry is associated with the vascular system C with respect to the transformation $A_C{=}{\gg}B_C$ since, among other things, the spatial structure of the vascular system is conserved at the time scale required to accomplish the transport of oxygen molecules from the lungs to the cells. Hence, the situation fulfills conditions II, which means that the relevant aspects $C_{A=\gg B}$ (here, the spatial structure) are conserved during the process of oxygen transport.

Consider now an enzyme. There is an asymmetry between a chemical reaction when considered under the influence of an enzyme ($A_C{=}{\gg}B_C$) and when not ($A{=}{\gg}B$) since, typically, $A_C{=}{\gg}B_C$ occurs faster than $A{=}{\gg}B$. Similarly, a temporal symmetry is associated with the configuration of an enzyme, which is conserved during the reaction while reactants do not. Note that at time scales shorter than τ, an enzyme does undergo alterations insofar as it binds to the substrate. The symmetry is respected only by considering the whole process at τ, when the enzyme unbinds and returns to its initial configuration.

Since they meet the two conditions, both the vascular system (with respect to oxygen transport) and enzymes (with respect to chemical reactions) act as constraints within the organism.

A crucial remark is that each condition is met only at the relevant time scale and, in particular, that the time scale τ at which conditions I and II must be fulfilled is the same. A constraint, to be such, must conserve its relevant aspects at the same time scale at which its causal action is exerted, even though changes and alterations may occur at shorter and/or longer time scales. Indeed, it is precisely because of their conservation that constraints are able to exert their causal power. Consider again our two examples. The structure of the organism's vasculature does not change at those time scales at which it channels the flow of oxygen; yet, the structure of the vasculature does change at longer time scales due to the effects, for example, of neovascularization. The same holds true for enzymes, which are conserved at the time scale of catalysis, while decaying and randomly disintegrating at longer scales. Moreover, as mentioned above, enzymes also undergo alterations at shorter time scales (since they bind with the substrate and loose or gain electrons, protons, etc.) and are then restored when catalysis is achieved.

More generally, a given entity cannot be qualified as a constraint *in abstracto*, insofar as its conserved aspects (and their causal powers) can only be assessed in relation to a specific process *and* the relevant time scale at which it occurs. This context- and scale-dependence are, in our view, general features of constraints. For example, a protein may be used differently in different biological contexts: crystallins, the structural proteins that confer transparency to the vertebrate lens, also act as enzymes when expressed in other organs (Rao et al., 1992).

The central outcome of the theoretical distinction between constraints and processes is a distinction between – to use a philosophical jargon – two regimes of causation. For a given effect B of a process or reaction, one can theoretically distinguish, at the relevant time scale, between two causes (or, as Rosen put it, two answers to the question "why B?"): the inputs or reactants A that are altered and consumed through the process, and the constraints C, which are conserved through that very process. Constraints constitute a distinct kind of causes insofar as they are not reduced to the thermodynamic flow, and to the material inputs or reactants.

4.2. Dependence and closure

In Physics and Chemistry, constraints are usually introduced as *independent* determinations whose existence and maintenance does not depend on the dynamics on which they act. The classical example of the inclined plane illustrates this situation well: the inclined plane acts as a constraint on the process (the sliding or rolling of an object on it), whereas the constrained process does not exert a causal role in generating and maintaining the plane itself.

In a fundamental sense, we submit that biology as a science is about those circumstances in which the constrained process *does* play a role in determining the conditions of existence of (some of) the constraints exerted on them. More specifically, there are situations in which the existence of a set of constraints collectively depends on the actions that they exert on the processes and dynamics. When this occurs, the set of mutually dependent constraints can be said to realize closure and therefore to be organized.

We can now characterize the principle of organization more precisely:

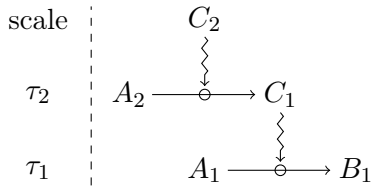
The principle of organization states that biological systems realize a closure of constraints.

The organization of constraints realizing closure achieves a form of "self-determination", in the precise sense that the conditions of existence of the constraints subject to closure (that we label "constitutive", see footnote 21 below) are determined within the organization itself. Before discussing closure as such, let us first have a look to the idea of the "dependence" between constraints.

As discussed above, constraints are defined as entities that, at the relevant time scale, exhibit conservation (a symmetry) with respect to the process on which they act. Yet, constraints are also subject to degradation at longer time scales, and must be replaced, repaired or maintained. For instance, the cells that constitute the vascular system must be nourished, and enzymes undergo degradation over time, and must be replaced. When the maintenance of

a constitutive constraint depends (also) on the action of another constraint, a relationship of dependence is established between the two.

A bit more formally, let us consider a constrained process $C_1(A_1 =\gg B_1)\tau_1$. Because of condition II, there is a time symmetry at scale τ_1 associated with C_1, which concerns those aspects that are relevant for the constrained process. At the same time C_1 is, by hypothesis, the product of another constrained process $C_2(A_2 =\gg C_1)\tau_2$, at a different time scale. At scale τ_2, C_2 plays the role of constraint, whereas C_1 does not, since it is the product of the process $C_2(A_2 =\gg C_1)$. Schematically:



This situation establishes a dependence relationship between constraints in which constraint C_1 depends on constraint C_2. In this situation, we say that C_1 is *dependent* on C_2, and that C_2 is *generative* for C_1.

In organisms, the dependence between constraints is ubiquitous. As an example, let us consider the production of an enzyme. As discussed above, an enzyme acts as a constraint on the reaction it catalyzes. In turn, enzymes are themselves produced by and within the cell, through the transcription and translation processes: messenger RNA is synthesized, ribosomes build the primary sequence of the future protein on the basis of the messenger RNA sequence, without consuming it. Since the ribosomes and the mRNA play a causal role while being conserved during these processes, they both act as constraints (at specific time scales) on the production of the enzyme. Consequently, the relationship between the enzyme, the ribosomes and the mRNA can be pertinently described as dependence between constraints (in which the enzyme depends on both ribosomes and mRNA). Of course, other constraints are involved in the process of producing a functional protein, for example, alternative splicing of RNA, post-translational modifications and folding

At a different level of description, another example of dependence is that between the vascular system and other systems or organs in the body, for instance the gut. The vascular system constrains the transport of nutrients (for instance amino-acids and oxygen) to the cells, while being conserved at the relevant time scales. In turn, the epithelial cells lining the mucosa of the small intestine constrain the transport of nutrients into the blood, while being conserved at specific time scales (the life time of these cells spans a few days). The relationship between the vascular system and the gut is dependence between constraints[13].

[13] As we will discuss below, the relationship exists also the other way around, the vascular system being dependent on the gut. That is precisely what the principle of organization is about.

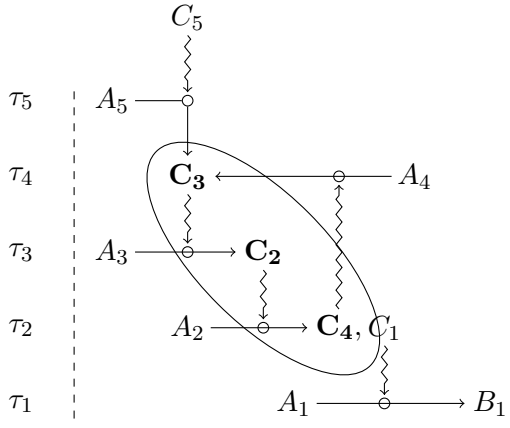
In a general sense, dependence between constraints underlies any "repair mechanisms" at work in the organism: in addition to the wide-ranging literature on DNA repair (Friedberg et al., 1995), this also includes the repair or, better, reconstruction[14] of all kinds of parts of an organism (Wang et al., 2009; Bergamini, 2006). Reconstruction requires the existence of a part (C_1) that is conserved while the main process occurs (i.e. its alteration is negligible at the relevant scale, τ_1), even though it may be altered in the long run (τ_2). The maintenance of the system's organization, on the other hand, requires, at time scale τ_2, the existence of a second subsystem (C_2) in charge of maintaining C_1 through the adequate canalization of a process (or a set of processes) $A_2 =\gg C_1$.

Let us now turn to closure, which we interpret as a specific case of mutual dependence between constraints. In the natural world, constraints may (and usually do) depend on other constraints, so that "chains" of dependences can be described. The specificity of biological systems consists of the fact that such chains of dependences realize complex networks of *mutual* dependences, usually at various levels of description. To express the idea more formally, we argue that a set of constraints C realizes closure if, for each constraint C_i belonging to C:

1. C_i depends directly[15] on at least one other constraint belonging to C (C_i is dependent);

2. There is at least one other constraint C_j belonging to C which depends on C_i (C_i is generative)[16].

As an abstract illustration of closure, consider the following network of dependent constraints:



[14] In organs, repair means replacement of cells by *new* cells. Accordingly, we consider that "reconstruction" (with variation) is more appropriate than "repair" into a previous state.

[15] The relationship of dependence that is relevant for biological closure is a *direct* one. i.e. a situation in which, considering the different processes that occur at τ_2 and contribute to maintaining a relevant aspect of C_1 that depends on C_2, none of them follows the one constrained by C_2, in physical time. This specification is necessary because the definition given above would otherwise apply to a wide range of relationships between constraints, including those in which the enabling and dependent constraints are linked through very long chain of processes. In this case, the concept of dependence would include many biologically irrelevant situations.

[16] See Montévil & Mossio (2015) for additional specifications of this definition (which are not required for the purposes of this paper).

In this diagram, C_1, C_2, C_3, C_4 and C_5 satisfy, *ex hypothesi*, the definition of constraint at τ_1, τ_2, τ_3, τ_4 and τ_5 respectively. Furthermore, C_1, C_2, C_3 and C_4 play the role of dependent constraints, while C_2, C_3, C_4 and C_5 are generative constraints. The subset of constraints which are both generating and dependent is then (C_2, C_3, C_4): this subset realizes closure, and is therefore organized.

The following illustration provides a more biologically oriented example. It represents in a highly simplified way the mutual dependence between the vascular system and the small intestine of a mammal, included in the overall closure of the organism. Both the vascular system and the small intestine act as constraints on the flow of nutrients. They realize closure by jointly contributing to maintaining their own cells. In particular, while the small intestine constrains the breaking down of food and the absorption of nutrients, the vascular system constrains their transport to all cells, including their own. Each individual cell of the small intestine, in addition to secreting enzymes that contribute to breaking down the food, realizes organizational closure itself, as any cell in the organism.

It is important to underline that this schematic illustration is by no means supposed to provide a *model* of closure, which would adequately capture the complexity of real biological systems. In particular, it represents the relations between two structures while most of other organs are not included. Rather, its aim is to express in a clear form some structural features of the principle of organization; the principle, in turn, should guide the further development of models of biological organization. Yet, some important implications can already be derived from this preliminary characterization.

First, we claim that constraints subject to closure define biological functions (Mossio et al., 2009; Saborido et al., 2011). Within this framework, performing a function means exerting a constraint on a target process or reaction. All kinds of biological structures and traits to which functions are usually ascribed satisfy the definition of constraint given above, albeit at various different temporal and spatial scales. In addition to the vascular system and enzymes, some intuitive examples include membrane pumps and channels (which constrain both the inward and outward flow of materials through the membrane) as well as organs (such as the heart which constrains the transformation of chemical energy into blood movement). The principle of organization grounds functionality within biological systems: constraints do not exert functions when taken in isolation, but only insofar as they are subject to closure.

Second, closure should be clearly distinguished from independence, insofar as a system that realizes closure is a physically open system, inherently coupled to the environment with which it exchanges energy and matter (Nicolis and Prigogine, 1977). This implies that closure is a context-dependent determination, to the extent that it is always realized with respect to a set of specific boundary conditions, which includes several external (and independent) constraints acting on the system (such as, for instance, constraint C5 in the abstract diagram above). Consequently, closure does not and should not include all the constraints with which the system may have a causal interaction, but rather only the subset of those that fulfill the requirements stated above[17].

Third, the principle of organization makes closure a general aspect of biological organisms that is constantly conserved during their lifespan. As we discuss and develop in Montévil et al. (2016), biological systems continuously undergo changes that may also result in the acquisition or loss of constitutive constraints and related functions; yet, whatever change must generate a network of dependencies that preserves closure. Different biological organisms realize different forms of closure, and even the same organism continuously modifies its own organization through time; but in all situations the very fact of realizing closure is conserved. We refer to this situation as the *non-identical iteration of morphogenetic processes* (Montévil et al., 2016; Longo et al., 2015), which refers to the dynamics of organs and, when these dynamics result in a functional change, to the overall organization.

5. Bringing variation into the picture

One central implication of adopting organization as a theoretical principle for biology concerns the understanding of the interplay between the *stability* and *variation* of biological phenomena.

On the one hand, we submit that the closure of constraints underlies the stability of biological systems (both at the individual and evolutionary scale), and determines the maintenance of their constitutive dynamics over time. On the other hand, organizational closure undergoes variation, as we argue at length in Montévil et al. (2016). One aspect on which we focus here is that closure, in the relevant situations, favors and enhances functional variation, i.e. variation of the organization itself. Next, we discuss both aspects in some details.

5.1. Organization grounds stability

One of the most astonishing features of biological organisms is the stability (i.e. their maintenance through

[17]This distinction between "constitutive" and "non-constitutive" constraints relies mainly on the definition of dependence established in the previous section. In fact, most external constraints do have causal interactions with the organism and, consequently, either affect it or are affected by it. Yet, even when it can be shown that a non-constitutive constraint interacts with the organism (in which case one may wonder whether or not it is subject to its closure), it should also be shown that, in accordance with the definition, the relationship of dependence is generative (and not only modulatory) direct and, moreover, concerns the relevant aspects as a result of which the entity satisfies the definition of constraint, at the relevant scale.

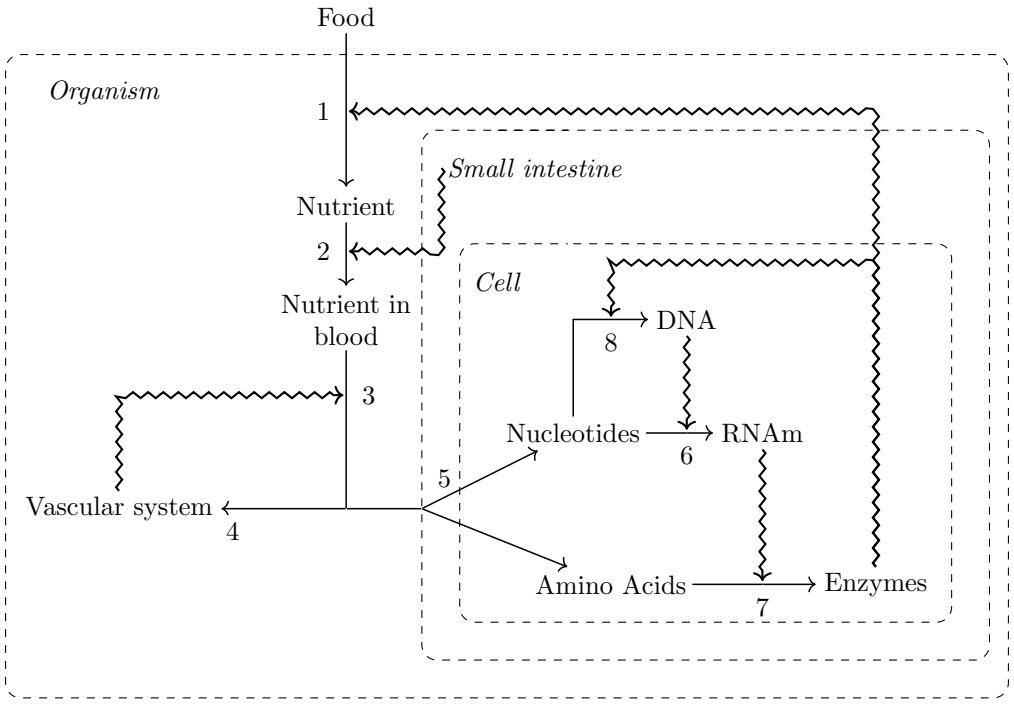


Figure 1: *Schematic illustration of the organizational closures between the vascular system and the small intestine in a mammal, and within a single cell.* After ingestion, food is broken down in the gut (1) and transformed into nutrients, which are absorbed into blood (2) through the mucosa of the small intestine. Nutrients are transported to all the cells of the organism through the vascular system (3). In particular, the absorbed nutrients feed the cells of the whole organism, including those of the vascular system (4), and those of the small intestine (5). Within each cell, the nutrients include nucleotides that are assembled into mRNA in accordance with DNA sequences (6). In turn, mRNA mediates the synthesis of enzymes from amino acids (which are also nutrients) (7). There are enzymes specifically involved in DNA repair mechanisms (8); as a result, DNA, RNA and enzymes realize organizational closure in the cell. A subset of the small intestine's cells secretes enzymes that contribute to the breaking down of food mentioned above (1). As a result, the vascular system and the small intestine realize closure by jointly contributing (through the transport and breaking down of nutrients, respectively) to maintaining their own cells. As discussed in the preceding sections, all entities from which a zigzag arrow originates are constraints, by hypothesis.

time[18]) that they exhibit, both at the individual and cross-generational scale, in spite of the huge complexity and delicate equilibrium of their constitutive dynamics.

What does explains biological stability? As in any other natural system, biological systems are subject to physicochemical factors, whose (stable) influence can contribute to explain the (stable) occurrence of some biological patterns. The focus on physical determinants is quite old in biology, D'Arcy Thompson's *On Growth and Form* (1917) being one of the most famous illustrations. In recent years, more precise work has been done in this field, for instance about the role of gravity on cell and tissue organization (Bizzarri et al., 2015). Another example is the analysis of the morphogenesis of the gut as a buckling process (Shyer et al., 2013).

Yet, physical factors apply to any kind of system, and are not usually considered sufficient to account for the stability of biological systems. It seems necessary therefore to appeal to some specifically *biological* sources of stability, one of them of course being DNA. An advocate of what we dubbed "genocentrism" in the introduction could claim that organisms are stable because, in addition to generic physical determinants, their behavior would be specifically controlled by the expression of the information contained in DNA, which is a highly stable molecule. In short, the stability of the DNA molecule would account for the stability of biological organisms.

The adoption of organization as a biological principle implies a significant departure from this view. Biological stability is due to the mutual interactions among constraints subject to closure: biological dynamics are stable because the organization of constraints is stable both at the individual and inter-generational scales. It is worth emphasizing that from the organizational perspective, DNA (interpreted as a constraint) of course plays a crucial role in determining stability. DNA is a fundamental physicochemical trace of a history, continually used by organisms as a template for the production of proteins. Yet, this role cannot be dissociated from the mutual dependences

[18] A logical distinction could be made between stability, understood as "maintenance through time", and regularity, which would rather refer to the "synchronic similarity", for instance, between individuals of the same species. We will not elaborate here on these distinctions and will just focus on stability. It is worth mentioning that both notions can be understood in the light to the idea of *genericity* of the object, as opposed to its specificity. See Montévil et al. (2016) for details.

between DNA and the whole set of constraints subject to organizational closure. Taken in isolation, indeed, DNA is totally inactive and undergoes degradation in a relatively short characteristic time (half-life estimated to 521 years, see Morten et al., 2012); moreover, its alterations, such as mutations, may be induced by ordinary metabolic activities and are present at each cellular replication. As an organized constraint, in turn, its structure is reconstructed over very long time scales as a component of organisms. The relative stability of DNA structure both during ontogenesis and phylogenesis in the long run is therefore a consequence of the biological activity of the whole organization[19].

The understanding of organization as the fundamental source of biological stability should distinguish between two dimensions, a local and a global one. In both cases, organization grounds stability through the relevant symmetries that it displays (of constraints and closure respectively), and the related conserved aspects.

On the one hand, each functional constraint subject to closure exerts a control over target processes and reactions. For instance, consider the sodium-potassium pumps in the cell membrane. The "pump" is a Na+/K+ -ATPase enzyme, which enables the active transport of sodium ions to the outside and of potassium ions to the cytoplasm. Both Na+ outward flow and K+ inward flow occur against the gradient, which means that they would not occur without the constraint exerted by the pump. As a result, the pump contributes to maintain the adequate concentrations of ions within the cell and, among other things, avoid water flows in by osmosis, which could possibly lead to cytolysis ("osmotic burst"). In this case, the stability of these specific inward and outward flows, which in turn results in the overall stability of ions concentrations, is due to the action of "local" constraints, which operate at specific temporal and spatial scales. Local constraints determine the behavior of processes and reactions, and avoid undergoing deleterious variation, which would undermine the overall functioning of the organism. In the case of the sodium-potassium pumps, for instance, the constraints (the pumps) avoid deleterious (i.e. too large) fluctuations of the internal concentration of sodium.

On the other hand, the conservation of each constraint holds, as mentioned, only at a given time scale τ, which means that, at longer time scales, they must be regenerated or repaired. If it were not the case, their role in stabilizing processes and reactions would be altered, and would eventually cease. Now, because of closure, the maintenance of each constraint is (among other things) dependent on the activity of other organized constraints. The sodium-potassium pumps, as well as the whole membrane, are maintained by a number of functions exerted in the cell cytoplasm. In particular, the pumps are produced and replaced in the same manner than other proteins, thanks to the constraints exerted by DNA and mRNA mentioned above (see also figure 1). Accordingly, the maintenance of each constitutive constraint beyond their characteristic time scale τ depends on the stability of the organizational closure.

As a fundamental biological property, organizational closure grounds the stability of functional constraints, by exerting a control over the variations that they undergo. Organization enables the maintenance of constitutive constraints, beyond their characteristic time scales, through the continuous re-establishment of their mutual dependences. In this respect, one might describe the overall stability of closure as the result of a kind of "organizational inertia". Because of the network of mutual dependencies, biological organization tends to remove variations affecting local constraints and to regenerate them in a fundamentally unaltered form. Such a tendency towards what we might call conservative *stabilization* would occur in those cases in which local variation does not affect the constraints in charge of generating the one (or set) being affected. Indeed, because of closure, generative constraints are themselves – directly or indirectly – dependent on the constraint undergoing variation. Conservative stability supposes therefore that those constraints in charge of re-establishing the dependent constraint are not themselves altered by the variation that they might remove. For example, variations may occur during the synthesis of enzymes, typically as "errors" in transcription or translation. Yet, if these variations do not alter the final outcome (the function), they are leveled by the organization and not sustained over time. In the following subsection, we will examine the role of closure for enhancing variation when this condition is not met.

The pivotal role of organization in maintaining biological stability should not be restricted to the functioning of an adult organism, or to a single individual. Indeed, it can be argued that organization grounds equally cross-generational stability, by playing for instance a crucial role in inheritance and developmental processes, understood as the set of "processes that explain this reliable reoccurrence of features within lineages" (Mameli, 2005). In recent years, in particular, an increasing quantity of experimental data has put strong emphasis on non-genetic inheritance, which includes epigenetic, ecological, behavioral and cultural processes (Bonduriansky, 2012). Non-genetic inheritance suggests that the stability of biological patterns through generations cannot be adequately explained by appealing uniquely to genetic factors. Rather, biological inheritance could be the result of the interplay between

[19] More generally, the principle of organization induces a departure from what could be dubbed a "biophysical posture" in biology, i.e. the idea that biological systems could be understood through the elaboration and composition of local models of functions and dynamics. Rashevsky (1954) has put strong emphasis on the importance of such a departure when he advocates the establishment of what he labels *relational* biology: "*We must look for a principle which connects the different physical phenomena involved and express the biological unity of the organism and of the organic world as a whole*". In accordance with an organicist perspective, the principle of organization does put the emphasis on the (mutual) relations between the parts, and not on their features considered in isolation.

a set of mutually dependent factors of different kinds, understood as constraints realizing an "extended" organizational closure (Pontarotti, 2015).

A detailed account of how the principle of organization underlies biological stability would go far beyond the scope and limits of this paper. What matters most for our present purposes is to put an emphasis on the general hypothesis that organization grounds stability of biological phenomena[20], which constitutes a substantial shift in focus from other approaches centered on genetic determinants or physical factors (or both of them). Organization controls the dynamics of the organism, and prevents deleterious variations that would threaten its very existence. Accordingly, there is an important sense in which organization, by grounding stability, *counters*, *canalizes and uses* variation.

Yet, the control exerted by the organization on variation is only one aspect of the intricate relations between the two theoretical principles. Next, we will consider how organization may *enhance* variation.

5.2. Organization propagates variation

The principle of variation that we propose for Biology– alongside organization – has fundamental implications. By complying with it, organisms undergo cascades of changes, which contribute to their specificity and, ultimately, to their historicity and contextuality (see also Longo & Montévil, 2014, and Longo et al., 2015). This leads to a central distinction between biological and physical objects the latter being, just as mathematical objects, generic and understandable in an ahistorical manner (see also Longo & Soto, 2016).

Biological systems undergo variation that can alter one or more constraints, or even their whole organization. As a result, the stability achieved by an organism is not just conservative but also, over individual and evolutionary time, *cumulative*, insofar as it keeps the track of functional innovations, and enables their preservation through time (see Montévil et al., 2016). Yet, the principle of organization does not merely ground stability, be it conservative or cumulative. Indeed, organization also (and somehow paradoxically) favors the propagation of functional innovation, and hence the increase of biological complexity.

What is the idea behind these claims? Broadly speaking, any system (be it a rock, a flame or a table) can undergo variation of a subset of its constitutive elements. A local variation can have different consequences on the global structure of the system, depending on the nature of the latter. In the case of a rock, a local fissure can result in the loss of a fragment, and in the modification of the global shape. In turn, a variation of some components of the flame (for example a small perturbation of its shape, or of the supply in combustibles), provided that it is compatible with the thermodynamic open state, does not affect at all the global behavior: the flame will keep behaving in the same way in spite of various possible modifications of its components. Lastly, a breakdown of one of the legs of the table might result in an alteration, or even extinction, of the global function of the table. A local variation can therefore induce various kinds of consequences in the global system's configuration or functioning. Yet, in all these cases a local variation cannot induce other local variations, which would result in a global reconfiguration of the system. Either the variation remains local, or it might result in the breakdown/disintegration of the entire system; but no global variation results from local variation.

In turn, one of the specific features of systems meeting the organization principle is the fact that local (functional) variation might induce global stable reorganizations. In the previous section, we mentioned that organizational closure may tend to remove a variation occurring to a local constraint in those cases in which such variation does not feed back into the generative constraints. But, of course, there is another possibility, in which the feedback does occur. In such a case, a local variation can possibly affect other constraints, their properties, and their activity, which in turn could affect other constraints, and so on. When a local variation affects an organized system, the variation can *propagate* through the various functional constraints and two outcomes are possible. Either the resulting system cannot realize closure anymore, in which case it is not viable and disintegrates. Or, it does realize a new closure through cumulative stability, in which case the functional innovations are integrated into the organization, and preserved. The propagation of variation through closure is our way of understanding Darwin's principle of "correlated variations"[21]. In this way, the organized system can explore what Kauffman (2000) calls "the adjacent possible" in the wide space of functional constraints. More precisely, a given organization does not entail such a change but it enables it, see (Longo et al., 2012b). In this respect, the generation of structural and organizational innovations constitutes a specifically biological form of randomness, leading to unpredictable organizational changes (Montévil et al., 2016).

The exploration of functional innovations and organizational variants, favored and enhanced by closure, may lead, in some circumstances, to the generation of increasingly complex structures, which could act as new constraints, generating more sophisticated and accurate functions. In brief, this process may lead to the increase of biological

[20] It is important to distinguish between the *stability* of biological phenomena and their *generation*. In this paper, we deal with the role of organization in understanding how biological phenomena are maintained through time, and *not* how they originated. The two issues are of course related and, as a matter of fact, there is a rich literature advocating an organization-centered view on the origins of life. Yet, we hold that the two issues can be treated separately.

[21] "I mean by this expression that the whole organization is so tied together during its growth and development, that when slight variations in any one part occur, and are accumulated through natural selection, other parts become modified" (Darwin, 1909-1914).

complexity, roughly conceived here as the degree of functional variety of an organized system.

6. Conclusions

We have claimed that the elaboration of a sound theory of biological organisms should adopt organization as a theoretical principle.

By elaborating on the long organicist tradition, we have put forward a specific understanding of the notion of organization, expressed in terms of closure of constraints. By relying on Montévil & Mossio (2015), we have proposed a diagrammatic description of closure, which provides a structured understanding of the principle. In this framework, biological organization refers to the mutual dependence (closure) between constraints, exerted on processes occurring in open thermodynamic conditions. Constraints are described as local symmetries, aspects that are conserved at the relevant time scale. Closure, in turn, is a global biological property, an overall determination that is conserved through ontogenetic and phylogenetic times.

We have mentioned some relevant implications deriving from the application of the principle of organization. In particular, we have discussed in some detail how it grounds biological stability and its interplay with variation, in an original (although complementary) way with respect to theoretical frameworks more centered on genes and/or physical factors.

Organization plays a twofold role with regard to stability and variation. In some conditions, closure can remove, integrate or average out variations and tends to conserve the ongoing network of mutual dependencies between functional constraints. In other conditions, the very same closure may promote the propagation of local variations to the whole organism: when this occurs, the resulting regime must realize a new stable organization, while integrating and preserving the functional innovations. Both situations are continually encountered in an organism, be it a unicellular or a multicellular one, since each metabolic cycle and each cell reproduction, in a metazoan, is a locus of possible change.

In this respect, a fundamental connection seems to exist between the principle of organization and the idea of the biological "default state" proposed by Soto et al. (2016). According to this idea, cells – both those that live autonomously as unicellular organisms and those that form part of multicellular ones – are by default in a state of proliferation with variation and motility. As a consequence, as they put it, "proliferation, variation and motility, require no explanation in biology. On the contrary, hindrances to the expression of default state, namely, proliferative quiescence, lack of variation, and lack of movement require an explanation" (Longo et al., 2015).

There are two important ways in which the principle of organization and the idea of the default state can be theoretically connected. On the one hand, it could be argued that proliferation with variation and motility are the default state *of* organized systems. Accordingly, the default state enriches organization by making explicit some of its most biologically relevant features and, reciprocally, organization grounds the default state by specifying the relevant class of natural systems to which it applies. On the other hand, the constraints constituting the organization certainly play a central role in explaining the observed departures from the default state. Under the effect of organized constraints, exerted both within their individual organization and by the multicellular organization of which they can be a part, cells can exhibit different degrees in their capacity to proliferate with variation and move, up to the extreme cases of proliferative quiescence, lack of variation or lack of movement. In a word, organized constraints control the default state, and the default state helps in the understanding of the nature of biological functions[22]. These theoretical considerations were used to model organogenesis on the bases of the default state and organizational closure (see Montevil et al, 2016).

The complex relations between stability and variation from an organizational perspective, outlined in this paper, should be investigated both theoretically and experimentally by a developing theory of organisms. Among other issues, a central one is certainly that regarding the description of different levels of organization, as well as their reciprocal relationship. Indeed, organized systems are not only typically constituted by a hierarchy of levels but, in addition, entities located at different levels interact with each other. A sound account of biological stability and variation should integrate these interactions in the picture.

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[22] These preliminary considerations would require a much more full-fledged analysis, which we leave for a future work. In Montévil et al. (2016), we mention the connection between the default state and the other theoretical principle that we propose for biology, i.e. variation.

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Theoretical principles for biology: Variation

Maël Montévil[a,b,*], Matteo Mossio[b], Arnaud Pocheville[c], Giuseppe Longo[d,e]

[a] *Laboratoire "Matière et Systèmes Complexes" (MSC), UMR 7057 CNRS, Université Paris 7 Diderot, Paris, France*
[b] *Institut d'Histoire et de Philosophie des Sciences et des Techniques (IHPST) - UMR 8590, 13, rue du Four, 75006 Paris, France*
[c] *Department of Philosophy and Charles Perkins Centre, The University of Sydney, Sydney, Australia*
[d] *Centre Cavaillès, République des Savoirs, CNRS USR3608, Collège de France et École Normale Supérieure, Paris, France*
[e] *Department of Integrative Physiology and Pathobiology, Tufts University School of Medicine, Boston, MA USA*

Abstract

Darwin introduced the concept that random variation generates new living forms. In this paper, we elaborate on Darwin's notion of random variation to propose that biological variation should be given the status of a fundamental theoretical principle in biology. We state that biological objects such as organisms are specific objects. Specific objects are special in that they are qualitatively different from each other. They can undergo unpredictable qualitative changes, some of which are not defined before they happen. We express the principle of variation in terms of symmetry changes, where symmetries underlie the theoretical determination of the object. We contrast the biological situation with the physical situation, where objects are generic (that is, different objects can be assumed to be identical) and evolve in well-defined state spaces. We derive several implications of the principle of variation, in particular, biological objects show randomness, historicity and contextuality. We elaborate on the articulation between this principle and the two other principles proposed in this special issue: the principle of default state and the principle of organization.

Keywords:
Variability, Historicity, Genericity, Biological randomness, Organization, Theory of organisms

> Since the beginning of physics, symmetry considerations have provided us with an extremely powerful and useful tool in our effort to understand nature. Gradually they have become the backbone of our theoretical formulation of physical laws.
>
> Tsung-Dao Lee

> The artificial products do not have any molecular dissymmetry; and I could not indicate the existence of a more profound separation between the products born under the influence of life and all the others.
>
> L. Pasteur

Contents

*Corresponding author
Email addresses: mael.montevil@gmail.com (Maël Montévil), matteo.mossio@univ-paris1.fr (Matteo Mossio), arnaud.pocheville@sydney.edu.au (Arnaud Pocheville), longo@ens.fr (Giuseppe Longo)
URL: http://montevil.theobio.org (Maël Montévil), http://www.di.ens.fr/users/longo/ (Giuseppe Longo)

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1. Introduction

A striking feature of living beings is their ability to change. All naturalists know that two individuals of the same species usually display important qualitative differences. All experimentalists know that two replicate experiments can give quite unexpectedly different results – even in the absence of any abnormality in the experimental setup.

Variation took a central role in biological reasoning in Darwin's book *The Origin of the Species* (1859) in which it served as a means to explain the current diversity of life, by virtue of the concept of "descent with modification" (Darwin, 1859, pp.119-124): organisms might show some differences from their parents, these differences might be heritable and, under some proper conditions, accumulate to form new lineages. Importantly, to Darwin, some of these variations would be "chance" variations, that is, changes that would be unrelated to the conditions of existence of the organisms, and even unpredictable (Darwin, 1859, p.p 131, 314)[1]. In so doing, Darwin introduced contingency and historicity into biological thinking: accidents would happen along life's trajectory, which would at the same time be unpredictable, unrepeatable, and have long lasting effects (Gould, 2002, p. 1334).

In this paper, we elaborate on the Darwinian idea of "chance" variation. We argue that variation should be given the status of a principle in biology, and in particular organismal biology. Informally, the principle of variation states that biological objects (such as organisms) continually undergo modifications. Some of these variations have functional repercussions, which we discuss with precise concepts in section 3). Moreover, whatever the mathematical frame used to describe an object, unpredictable variations are nevertheless possible: the principle of variation thus implies that the existence of exceptions is the rule in biology. However, a proper biological theory cannot be a mere catalog of exceptions. Accommodating the changes biological organisms undergo during their lives (ontogenesis), as well as during evolution (phylogenesis), in a general theory is a specific challenge raised by biological systems, in particular in contrast to physical theorizing.

In physics, theoretical definitions enable us to discuss abstractly and adequately the behavior of objects (such as the trajectory in space of a punctual object of mass m in classical mechanics, or the behavior of quantum objects as a vector in a Hilbert space in Quantum Mechanics). Such a theoretical framework does not (yet) exist for the biology of organisms and our proposal aims at contributing to the elaboration of the "biological counterpart" of the theoretical frameworks and abstract objects at work in physics.

It is worth emphasizing that, although we will elaborate on the concept of variation by analogy with and in contrast to the physico-mathematical perspective, we by no means advocate a physico-mathematical treatment of biological phenomena. Rather, we think that biology in general, and the biology of organisms in particular, requires a significant change of perspective with respect to the physical viewpoints and methodologies. Typically, physics provide an ahistorical understanding of the phenomena studied[2]. In contrast to physics and in line with the theory of evolution, we argue that historicity is an essential feature of biological phenomena and that biological historicity stem from the principle of variation.

The principle of variation is related to the other principles put forward in this special issue: the biological default state (proliferation with variation and motility), and the principle of organization. The default state is described as a primary generator of variation; when a cell divides, it generates two non-identical cells (Soto et al., 2016). The principle of variation specifies the nature of the difference between these cells. The principle of organization is a way to interpret biological functions as a property stemming from the role that parts play in the maintaining of a system (Mossio et al., 2016, 2009; Montévil & Mossio, 2015). According to this principle, a biologically relevant part (constraint) both depends on and maintains other parts of the organism, thus forming a mutual dependence (labeled "closure" for historical reasons). In Mossio et al. (2016), variation and organization are discussed as two intertwined principles: organization is a condition for variation and favors its propagation, whereas variation is a condition for the maintenance and adaptation of biological organization and for the generation of functional innovations. In section 4 of this text, we argue that any relevant variation is a variation of an organization.

Biological variation occurs at all levels of organization, from the molecular level to large scale structures and functions (West-Eberhard, 2003; Dueck et al., 2016). Single cell observations on one side and high throughput technologies on the other enable biologists to observe both inter-cellular and inter-individual variations, which have received an increasing amount of attention (Elowitz et al., 2002; Collective, 2005; Rivenbark et al., 2013). There

[1] This concept of chance variation contrasts sharply with, for instance, the concept of variation of Lamarck (1809) another father of theoretical biology. To Lamarck, variations would be directed by the conditions of existence. This directedness entails that if the conditions of existence re-occur in time, evolution is repeatable and thus, ahistorical (Gould, 2002, p. 191). Other 19$^{\text{th}}$ century writers would advocate that variation would be so canalized (by the properties of the organisms) as to direct evolution (when evolution was acknowledged). See e.g. Bowler (2005; Pocheville & Danchin (2016) for more details.

[2] As a matter of fact, physical approaches and methodologies are not confined to the physical and biophysical domain and have, in part, percolated in biology and even social sciences. Such was the case, for instance, of the proposal of vital forces by some vitalists. These vital forces were conceived by analogy with Newtonian gravitation and would entail spontaneous generation as a result of this force acting on the right objects (De Klerk, 1979). Vital forces are an example of how the physico-mathematical approach typically implies an ahistorical understanding of the living, as we stress below.

are many generators of variation among which are random gene expression, instability in morphogenetic processes and randomness in biological rhythms. In particular, cellular proliferation generates variation (Soto et al., 2016). As for temporal scales, living systems undergo variation during their lives (ontogenesis), as well as during evolution (phylogenesis), and these two aspects cannot be analyzed independently (Danchin & Pocheville, 2014). In this paper, we focus on variation as a general feature of biological systems without a privileged level of analysis. This enables us to discuss general features that are proper to biology and to stress key differences with respect to physics.

The central implication of this paper is the distinction between the objects as conceived in physical theories (generic objects) and the objects as conceived in biology on the basis of biological variation (specific objects)[3]. In what follows, we discuss first shortly what generic objects are, what kind of manipulation they enable, and how their analysis grounds physical theories (section 2). Then, we contrast generic objects with the variation that biological objects exhibit. We propose that biological objects should be understood as specific (in section 3). Specific objects are, in particular, fundamentally historical, variable and contextual. Thus, the specificity of organisms encompasses biological individuation and diversity. We also discuss the interplay between specific objects and physical morphogenesis. Then, in section 4 we elaborate on the integration between the principle of variation and the principle of organization, between the notion of biological specificity for biological objects and the notion of organization and "contingent genericity" (Moreno & Mossio, 2015; Montévil & Mossio, 2015; Mossio et al., 2016). Finally, we develop the idea that biological systems are characterized by the non-identical iterations of morphogenetic processes (section 5).

2. Invariance and symmetries: physics as the domain of generic objects

The principle of variation poses novel challenges with respect to how mathematics enables us to describe the world. To better identify these challenges, we first make a detour by physics and show the role mathematics play in physical theories.

Physics is based on mathematized theories. Historically, the development of physical theories has been intertwined with the development of appropriate mathematics to frame and define their objects: they have "co-evolved".

We submit that mathematized physical theories rely on the manipulation of *generic objects* (Bailly & Longo, 2011; Longo & Montévil, 2014a). The notion of generic objects is abstract, as it lies at the core of physicomathematical reasoning. However, the intuitive idea is quite simple: generic objects are objects which are all of the same kind from the point of view of the theory (they typically obey the same equations). An apple, the Earth, an anvil, for example, are all objects with a given mass and center of gravity and, from the point of view of classical mechanics, they all obey the same equations in the vacuum. Moreover, they continue to obey the same equation during their dynamics even though they undergo some changes: this is because, in physical language, their changes are restricted to changes of state. Equations are not about specific values of the parameters or states; instead they jointly describe generic relations between parameters, states and the changes of states[4]. This is why changes of state of an object do not affect the validity of the equation which describe its behavior. For example, the mass is an element of the description of some generic objects, formalized by a generic variable m representing jointly and synthetically all the possible masses.

Physical objects, hence, are generic objects. More generally physical 'laws' are about generic objects. Consider for example the fundamental principle of dynamics: mass times acceleration equals the sum of external forces applies to the object. Here, the "external forces" are understood in a completely generic manner and any kind of forces may be involved.

Typically, a physical object is described in a mathematical space which is generated by the various quantities required to describe this object. This mathematical space is called the 'phase space'[5]. In classical mechanics, the phase space is the space of positions and momenta. This mathematical space is given in advance; it pre-exists the description of the object. The behavior of the physical object is defined as the way in which the object changes in its phase space. The space is also assumed to provide all the causes of the changes of the object, and thus it specifies the quantities that should be measured experimentally. In classical mechanics, positions and momenta, in combination with properties such as the mass, are the quantities required to understand the changes of positions and momenta over time.

A phase space, however, is not sufficient to understand the behavior of an object because the quantities it provides need to be articulated together to understand the changes of the object[6]. In physics, a theoretical framework requires equations that depend on the variables symboliz-

[3] An introduction to this distinction is given by Soto & Longo (2016).

[4] Simulations suffer from a shortcoming in this respect. While a program does describe generic relationships between the variables, a simulation run only provides one trajectory for specific values of its input. Whether this trajectory is representative of the behavior of the system for other values of the input, that is to say whether the behavior obtained is generic or not, is a very difficult mathematical issue (Stoer & Bulirsch, 2013).

[5] Some physicists restrict the notion of 'phase space' to positions and momenta. Here, phase space means in general the space of mathematical description of the object.

[6] The *a priori* diversity of possible trajectories in such a space is unfathomable in the sense that no axiomatic is sufficient to describe all their possible mathematical features.

ing the quantities describing the objects. The behavior of an object, that is to say its changes, is determined as a specific trajectory by equations that single it out in the phase space. Equations are valid for the phase space (or, at least, some regions of it) and depend on its quantities. The behavior of the object is completely determined by the quantities that define its phase space and the corresponding equations. Predicting a trajectory corresponds to making this trajectory mathematically remarkable. To this end, equations typically correspond to optimization principles (for energy, entropy, entropy production, etc.), which enable physicists to single out a trajectory, the optimal trajectory that the system follows according to the theory. Optimization principles and the ability to derive equations are essential for fundamental physical theories and special models to make predictions.

For the purposes of this paper, the key question to be asked at this point is what justifies the use of the spaces and equations in the theoretical constructions of physics. In part, these mathematical structures stem from axioms and are justified by their consequences. However, there is more to say on the nature of fundamental hypotheses of physics and the way in which they justify the use of mathematics.

Because whole classes of concrete objects are described in the same mathematical frame, they are studied as the same generic object, and all have the same behavior. As we evoked above, a piece of lead, an apple, or a planet are all the same objects from the viewpoint of classical gravitation: they all are point-wise objects with a position, a momentum, a mass, and they all are subject to the principle of inertia and gravitational forces, described by the same equations. In this respect, there is no relevant difference between them and they are described jointly and synthetically as the same generic object. At the core of this approach to natural phenomena lies the identification of non-identical objects. This identification of non-identical objects is made explicit by transformations that leave these objects invariants (i.e. symetries). Putting an emphasis on transformations is a modern approach in mathematics and physics that we build upon in this paper. In particular, invariants are best described by the transformations that preserve them and which make explicit a mathematical structure.

Generic objects are, for the most part, defined by the transformations that preserve them, and that enable us to define stable mathematical structures. We call such transformations 'symmetries'. The notion of symmetry we use is more general than the concept of geometrical symmetry in a three-dimensional space. Yet, the underlying idea is the same: geometrical symmetries are transformations which leave a geometrical figure invariant. Rotating a circle around its center, for instance, does not modify the circle: it verifies a central symmetry[7]. Similarly, symmetries (in general) are transformations which leave the relevant

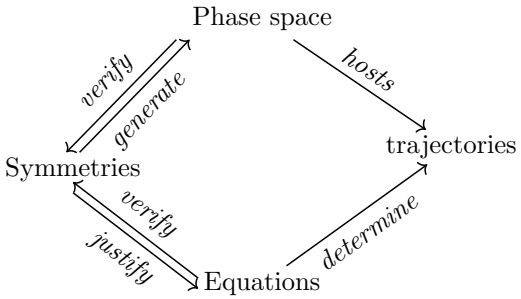

Figure 1: *Articulation of the different components defining a generic object in physics.* Equations determine the trajectory of a system, and this trajectory takes place in a mathematical space. Both the equations and the space have a structure that is described by the theoretical symmetries that frame the object and that are valid by hypothesis. There is a fundamental feedback that we do not represent here: trajectories are the endpoint which fundamentally justifies the whole theoretical construction of the generic object as experimenter can observe them.

aspects of an object invariant. For example, cutting an apple into two halves does not change the way it falls in the vacuum. Hence, an apple and its two halves are symmetric (they are the same) from the point of view of free fall in classical mechanics. Allometric relationships provide a biological example of symmetry (Longo & Montévil, 2014b). In mammals, the average period of rhythms such as heart rate or respiratory rate is found experimentally to depend on mass with the relation $\tau \propto M^{1/4}$. Measuring such relationships amounts to assuming that the basic properties of metabolism are preserved under the transformations consisting of changing sizes and species, and thus that mammals of different masses are symmetric as for their internal rhythms (West & Brown, 2005; Longo & Montévil, 2014b). Lastly, the assumption that different replicates of an experiment enable us to access the same situation also corresponds to an assumption of symmetry between the replicates: they are all supposed to behave in fundamentally the same way[8].

Symmetries are the basis of the mathematical structures in physics; that is the phase space and the relevant

[7] Another example comes from topology, a notion very useful in biology. For instance, it is possible to deform a balloon into a sphere or a rod shape without tearing and/or stitching, but it is impossible to transform it into a donut. Similarly, it is impossible to transform a cell into two cells without tearing and/or stitching the membrane, where stitching corresponds here to the fusion of the membrane by pinching, and tearing corresponds to the final separation of the cells. In all these cases, continuous deformations are considered as symmetries, insofar as they preserve topological invariants and, reciprocally, the topological invariants are the ones preserved by continuous deformations. As a result, one can define different categories of shapes on the basis of their inter-transformability. Continuous deformations fall under our concept of symmetry and are characteristic to the field of topology.

[8] Notice that such an assumption is required in order to perform statistical analyses. The most common statistical assumption is that two variables are identically distributed, that is to say that the two considered situations are symmetric as far as their probability distributions are concerned.

equations. Accordingly, they constitute fundamental physical assumptions which are less anthropomorphic than the notion of law and more meaningful than conservation principles (see for example Van Fraassen, 1989; Bailly & Longo, 2011; Longo & Montévil, 2014a).

For instance, the choice of an origin, three axes and a metric are mandatory in order to write equations and perform measurement of positions and velocities (in Galilean, special, or general relativity). Although different choices are possible, the consistency of the theory depends on the fact that the trajectories obtained in different reference systems are, in a fundamental sense, the same: in particular, they are invariant under suitable classical or relativistic transformations of the reference system. Thus, the equations of physics are symmetric under these transformations[9]. In general, the same trajectory should be obtained before and after transformations which are fundamental symmetries in the theory [10], and these symmetries enable us at the same time to formulate and justify the equations and the phase space [11].

In short, physical objects are understood as generic objects that follow specific trajectories. Theoretical symmetries ground this approach to natural phenomena. The epistemological structure of generic objects is summarized in figure 1. In the next section, we discuss the principle of variation and the major challenges that biological variation raises when one tries to frame biological objects theoretically.

3. Variation and symmetry changes: biology as the domain of specific objects

A central and pervasive property of biological systems is their ability to change their organization over time[12]. These changes are not just quantitative changes, they are also qualitative. From a physico-mathematical point of view, qualitative changes typically imply changes of the relevant mathematical structures and, accordingly, changes of symmetries. For example, changes of states of matter in phase transitions typically correspond to changes of symmetries: a liquid is symmetric by rotation while a crystal is not, because of its microscopic structure (see figure 3).

In the biological domain, the organization of any current organism has been shaped by permanent qualitative changes, that is, through changes of symmetries. A given biological organization is determined by an accumulation of changes of symmetries both on the evolutionary and the ontogenetic times[13]. These changes correspond to changes in the manner in which functions are performed, or even to the appearance or loss of functions.

Acknowledging that organisms can vary in this strong, functional sense, is not trivial: historically, the preformationists (as for development), and the fixists (as for evolution) have held just the opposite view. If the homunculus is already in the egg, or, in modern terms, if DNA already contains a blueprint of the organism, then development is just the unfolding of an already existing organism (with all its relevant properties and functions). Similarly, if species do not change over geological time, then obviously organisms conserve the same functions.

The idea that biological objects genuinely develop and evolve over time corresponds to the idea that the mathematical structures required to describe them also change over time. Thus, stating that development and evolution involve symmetry changes constitutes nothing more than a mathematical interpretation of the departure from the preformationist or fixist stances of development and evolution. Evolution is rarely considered as entirely determined as the unfolding of historical necessities. Similarly, development should not be seen as the unfolding of a preconstituted organization but instead as a cascade of folding leading to the setting up of an organization (figure 2 and 4).

The crucial consequence of this view is that, because of their permanent symmetry changes, biological objects should not be considered as generic objects. Organisms are not well defined as invariant under transformations. When an organism is transformed, and in particular when the flow of time operates on it, the organism may undergo unpredictable qualitative changes. As a result, biological objects are not well described by the virtuous cycle described in figure 1. Accordingly, trajectories are not entirely framed by a mathematical framework: they may escape such frameworks and require a change in the symmetries, space of description, and equations used to describe the object (figure 2).

We propose then to understand biological objects (and organisms in particular) as *specific* objects [14]. Specific objects are constituted by a particular history of relevant and

[9] Similarly, in electromagnetism the choice of assigning negative or positive charges to electrons is arbitrary; therefore, permuting the sign of charges has to leave the equations invariants (the derived trajectories remain the same).

[10] In a mathematical model, some symmetries are theoretical symmetries which cannot be violated while others are more pragmatic symmetries that correspond to a particular situation. The two things should not be conflated. For example, a theoretical symmetry is the assumption that all directions of the empty space are equivalent. However, in a particular setting, all directions may not be equivalent, for example because of the position and the gravitational field of some planets. Another theoretical symmetry is the symmetry between positive and negative charges in classical electromagnetism.

[11] Such justification of equations by symmetries is, in particular, the core of Noether's theorem, which justifies the conservation of energy (resp. momenta) on the basis of a symmetry by time (resp. space) translation of fundamental equations, among many other conserved quantities (Byers, 1999; Longo & Montévil, 2014c).

[12] While we mean here 'organization' in the technical sense discussed in Mossio et al. (2016), the reader can also interpret the notion in a more informal manner. The different parts of an organism depends on each other and form a coherent whole. This interdependence of the parts and their relation to the whole form the organization of organisms.

[13] A more detailed presentation of most of these ideas can be found in Longo & Montévil (2014a) and Longo & Montévil (2011, 2013).

[14] Our concept of specificity should not be confused with other

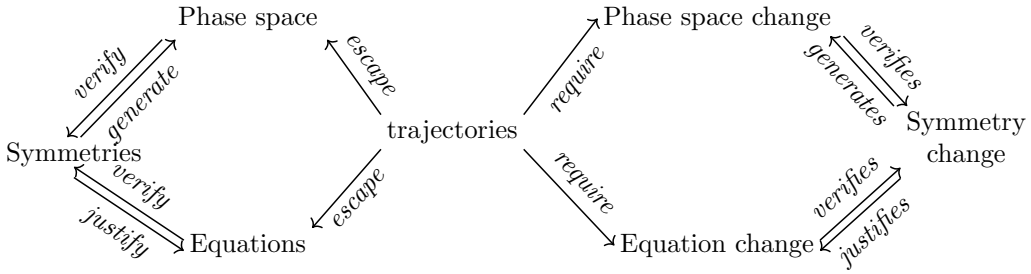

Figure 2: *Scheme of an elementary symmetry change in biology.* An initial situation, on the left, is described by analogy with physics (see figure 1). However in biology, variation can escape such a frame. Understanding the object then requires a change of symmetry and of the whole mathematical structure framing the object. Trajectories are at the center of this change, they escape the initial frame and thus require a change of the symmetries describing the object.

unpredictable symmetry changes over time, at all timescales. Specific objects can be understood as the opposite of generic objects: two instantiations of a specific object may always differ by at least one of their relevant qualitative aspects (in a given theoretical frame), while two instantiations of a generic object do not. For example, two organisms, be they clones, may always differ in one of their relevant qualitative properties, for instance because they may have undergone differences in their morphogenesis, i.e., they have been constituted by different developmental histories.

On the basis of the concept of specific objects, we can now state the principle of variation:

Principle of variation:
Biological organisms are specific objects.

The principle implies that biological organisms undergo changes of symmetry over time and that, as we discuss below, some of these changes cannot be stated in advance[15]. In other words, the mathematical structure required to describe organisms is not stable with respect to the flow of time. Qualitative changes of structures and functions occur over time and some of them are unpredictable.

We now expand on several aspects and implications of the principle of variation.

3.1. Randomness proper to specific objects

A fundamental feature of the principle of variation is that it includes an original notion of randomness: the very fact that biological objects undergo unpredictable symmetry changes. Generally speaking, the notion of randomness is often conflated with the idea that events have some probability of occurrence. However, scientific approaches to randomness are richer than the notion of (classical) probabilities (see for example Longo et al., 2011, for a discussion at the crossroads of different fields). Randomness may be defined generally as unpredictability with respect to a theory. The notion of randomness which stems from the principle of variation is not endowed with a probability measure.

Let us first characterize randomness in the case of a basic symmetry breaking, typically encountered in physical models. Let us start with a situation which is symmetric, for example a gas (figure 3, top). All directions are equivalent for this object: all macroscopic quantities (density of the gas, pressure, etc.) stay the same after rotation. When the symmetry is broken, directions are no longer equivalent; for example, there are privileged directions corresponding to a crystal structure after a phase transition (figure 3, bottom). The symmetry of the initial situation means that all directions are initially equivalent and then that it is not possible to deduce the subsequent privileged directions in the crystal. As a result, the directions of the crystal are random in this theoretical account. Moreover, since all directions are symmetric in the initial conditions, all directions have the same probability to become one of the crystal's privileged directions.

This physical situation exemplifies how symmetry breaking and randomness are associated and how the initial symmetries define and justify probabilities (see Longo & Montévil, to appear, for a general analysis of this association).

Symmetry breaking and the associated randomness are relevant for biology but we submit that they are not sufficient. Biological randomness includes a fundamentally different notion. In the above case, the possible outcomes (all the possible directions in three dimensions) are defined before the symmetry breaking, as it is the mathematical space on which symmetries act. Saying that the gas is symmetric by rotation requires us to define rotations and therefore the set of all possible directions on which rotations act. In biology, in contrast, the principle of variation poses that the list of possible outcomes and therefore the relevant symmetry changes are not pre-defined. For example, it is not possible to embed all the spaces of description of current and future organisms within the space of description of the last universal common ancestor (LUCA).

concepts of 'biological' specificity, such as chemical specificity of enzymes, or causal and informational specificity (see Griffiths et al., 2015).

[15] We would argue that even the rate of possible symmetry changes cannot be stated in advance.

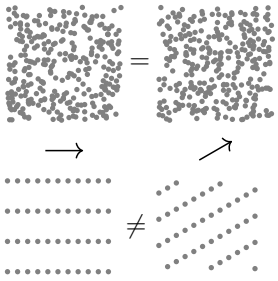

Figure 3: *Example of a symmetry breaking.* The left pictures correspond to an initial situation and the right ones to the same situations after a rotation (represented by the arrows). The above diagrams show a disordered situation such as a gas or a liquid. This situation is statistically symmetric by rotation, there are no privileged directions. By contrast, the situation below corresponds to a crystal such as graphite. It is not symmetric by rotation (except with an angle of 180°) and it thus has directions which have an intrinsic physical meaning. The transition from the situation above to the situation below implies the introduction of new relevant elements: the directions of the crystals, which are random.

A part of the relevant symmetry and symmetry changes can only be listed *a posteriori*, that is, after their realization. These changes only make sense as a result of a previous history. Not only lineages, but also individual organisms, are subject to biological randomness, as their development can sometimes take new routes which were not expected in advance (e.g. West-Eberhard, 2003).

Note that we consider symmetry changes in general and not just symmetry breaking. Symmetry breaking corresponds to symmetry changes which start from a situation that respects a given symmetry to a situation where this symmetry is no longer valid, as discussed above. Other symmetry changes are possible, for example one can go from an asymmetric situation to a symmetric one. In biology, symmetry changes include the appearance of new and unpredictable symmetries corresponding to new relevant parts and their functioning. For example, the appearance of sexual reproduction in evolution corresponds to a separation of individuals in two genders in many species, where new symmetries (or equivalence) between males on one side and females on the other become fundamental as for their role in reproduction. New associated variables become relevant, for example the sex ratio of a population.

Because of symmetry changes, the phase spaces of biological objects also change in unpredictable ways over time. Symmetry and phase space changes constitute a specific form of randomness, proper to biological systems (Longo & Montévil, 2012; Longo et al., 2012a; Kauffman, 2013; Longo & Montévil, 2013). Biological randomness typically manifests itself through the appearance of new relevant quantities, parts, functions, and behaviors over time (for example limbs, toes, toenails, all the quantities required to describe them and the various functions that they can have).

3.2. Constraints and specific objects

The principle of variation does not preclude the presence of elements of stability in biological systems. On the contrary, in order to show experimentally and describe theoretically a change of symmetry, the preceding and following situations have to be stable enough to be described. In other words, a set of symmetries has to be at least approximately valid long enough before it changes for an observer to discuss it and after the change the new set has to be met for some time too. For example a given geometry of bones is conserved during movements of the organism at short time-scales, which corresponds to the conserved symmetry of a solid (the relative positions of points in a solid do not change). However, this geometry is plastic at longer time scales and very important changes can occur especially during development (West-Eberhard, 2003). The change of two bones geometry at different times thus corresponds to a symmetry change, but the symmetries of these bones are met at short time-scales.

We call constraints the relevant stable elements at work in biological systems and their associated symmetries. Constraints are local stable elements, in the sense that they only concern a particular aspect of a given organism. In addition, constraints are contingent insofar as they, and their associated symmetries, may change over biological time (which is implied by the principle of variation).

In short, we define constraints as symmetries (i.e. stable mathematical structure) witch have a restricted range of validity and are used to describe a part of a specific object.

3.3. Constraints and randomness

In this section, we discuss the articulation between two kinds of randomness in specific objects. This discussion is more technical and may be skipped in a first reading.

A constraint (or a combination of constraints) exerted on biological dynamics may lead to a situation in which symmetry changes (if any) occur in a generic manner, typically as symmetry breaking. In the case of generic symmetry changes, these 'random' changes can be stated in advance, even though their specific outcome cannot. This randomness can be derived from constraints, and it is weaker than the randomness proper to specific objects.

Let us start with morphogenesis as an example. Most (if not all) mathematical models of morphogenesis involve a symmetry change, which usually is a symmetry breaking. Consider for instance Turing's model of morphogenesis (Turing, 1952)[16]. In this model, the equations describing reactions and diffusion of chemicals remain invariant, so that their properties (rate of reactions, coefficient of diffusion, etc.) are stable constraints. In turn, these constraints lead together to a symmetry breaking, because of

[16]Turing's model is based on a basic symmetry breaking, where a situation that is initially symmetric by rotation forms a pattern of alternation of concentrations of chemicals (and new quantities are needed to describe where this pattern is located).

the sensitivity of the non-linear dynamics to initial conditions (an instability, says Turing): minor fluctuations trigger different outcomes.

Another very different example of biological symmetry breaking is the DNA recombinations in the maturation of lymphocytes (Thomas-Vaslin et al., 2013). The random process of recombination in a cell can be seen as a symmetry breaking from a situation where all the recombinations to come are equivalently possible to a situation where only one recombination is actually realized in each cell. After recombinations, the description of the system has to include which possibility each cell has "chosen". This symmetry breaking makes the diversification of the immune repertoire possible under the constraint of enzymes.

Both cases (morphogenesis and DNA recombinations) involve stable constraints, in an extremely sensitive process, which leads to a change of symmetry. These constraints are stable parts of the organization of the considered organisms. As a result, the associated changes are robust in the sense that they will occur as a consequence of these constraints. In such situations, a generic change of symmetry is established, which generates "new" relevant quantities but in a generic manner, i.e. the change belongs to a set of predefined possibilities. These new quantities are new in a weaker sense than the unpredictable new dimensions of description that specific objects can generate. For example, the recombinations in the immune system can be seen as generic, as a set of possible physico-chemical recombinations of molecules. The outcome of such recombinations is probably unique because the odds of performing the same recombinations twice are vanishingly small, but this outcome is still generic. The situation is analogous to the physical case of the positions of individual molecules in a gas which are basically unique, whereas the gas is still in a generic configuration because the gas is in a configuration of maximum entropy. However the actual immune repertoire in an adult mammal is not fully determined by the generic properties of recombinations because the recombinations are just a part of the process establishing this repertoire. The immune repertoire strongly depends on the specific history of the given organism, its environment, non-genetic inheritance (through milk and the microbiome), etc. (Thomas-Vaslin et al., 2013). The immune repertoire has a causal structure that is not determined by pre-existing regularities. The dependency on the organism's history is functional, it determines the immune response to specific pathogens and contributes to the dynamic relationship with the microbiome. The biologically relevant properties of the immune repertoire are not the generic properties of recombinations, instead they are the specific properties which stem from a history. Hence, the actual repertoire of the adult contains more meaningful novel structures than the initial probabilistic recombinations.

Now, every time we describe a symmetry change according to current physico-mathematical methodology, it takes a generic form, that is, a possible change in a pre-given space of possibilities which may be given *a priori* probabilities. Biological objects are — by hypothesis — specific, but when we describe a particular change of symmetry, it is studied *a posteriori* as a generic aspect of the object, and can be added to the past possibilities of a system. Randomness is then not correctly framed by *a priori* probabilities. Probabilities, if any, are defined *a posteriori*. A specific possibility is accommodated by the space of possibilities, but this space is obtained *a posteriori* and obviously does not include all future possibilities.

Let us unpack this idea. A physical symmetry breaking is a simple elementary process: a symmetry is met by the system, and after the symmetry breaking event, the symmetry is no longer met. The possible breakings are given by the initial set of symmetries and make mathematical sense when they can be described in a given mathematical space where the symmetry operates. However, if a situation is and always has been completely symmetric, the symmetries do not change anything and thus, cannot be properly evidenced as transformations (because the object is not changed at all). Thus the logic required to describe a new symmetry breaking has two steps. First the symmetry that will be broken has to be added to the initial definition of the system and accordingly the states that are initially symmetric have to be added to the phase space of the object. They are added because they are required to accommodate their future breaking. Then, and only then, may the symmetry be broken. Such a modeling is retrodictive: the mathematical space, needed for an equational model, can be given only after the change has been observed. In general, then, a biological dynamic must be understood as a possible path, out of many established along the biological dynamics, which consists in the composition of stepwise symmetry changes.

In a given situation, some symmetry changes can be spelled out and analyzed in a generic framework because they are stabilized by (local) constraints. Let us consider such an elementary biological symmetry change, for example in a morphogenesis model. We can describe it explicitly with generic constraints but it is also possible to leave it implicit and consider that this single symmetry change is taken into account by the specificity of the object, among many other changes. The choice depends on the perspective adopted to understand a given situation, including the scale of description and the phenomena of interest. For example, the intestine folding are usually kept implicit when studying brain morphogenesis.

Even though the boundaries of specific and generic aspects of an organism are relative and may change after a new possibility is acknowledged or as a result of a change of perspective, the accurate description of any biological organism will always involve a component of specificity. In a given representation of an organism, all changes of symmetry are then either accommodated by the specificity of the object or by generic symmetry changes. The concept of the specificity of biological objects aims to enable us to take into account theoretically all symmetry changes

without spelling out all of them explicitly.

3.4. Historicity

Historical objects are objects whose properties are acquired or lost over time, and cannot all be described ahead of time. The fact that biological organisms are specific objects straightforwardly implies that they are historical objects and, in particular, contingent objects in Gould's sense (Beatty, 1995; Gould, 1989). Historicity thus goes hand in hand with biological randomness, which corresponds to the fact that a situation after a random event cannot be stated with certainty before the event. Thus, a system showing biological randomness shows historicity: the object takes a particular path among several possible paths through time. Reciprocally, historical objects necessarily show some randomness.

Let us first consider an analogy with dynamic systems. We can see a trajectory defined by a differential equation as the sum of infinitesimal changes from the initial conditions to any time point. By analogy, it is conceivable to see biological historicity as a sum or a sequence of variations since the origin of life. However, this idea does not have a well-defined mathematical and theoretical sense, insofar as such a history is not entirely accessible. Nevertheless, it is still possible to clarify the present in the light of the past — and, as a matter of fact, this is precisley one of the aims of evolutionary theories.

As discussed in Longo et al. (2015), although historical objects exist also in physics, they are historical in a weaker sense. Self-organized physical objects, for instance, are sometimes described as historical, mostly because they depend on a symmetry breaking. For example, the appearance of convection cells in a fluid corresponds to a qualitative change in the macroscopic dynamics of the fluid. Nevertheless, self-organized objects are spontaneous: they can be obtained *de novo*. Theoretically, they can be described as the spontaneous self-organization of flows of energy and matter. Even the physical situation of the early history of the universe can be obtained experimentally "just" by tuning a parameter (by obtaining very high local densities of energy with particles accelerators)[17].

Despite these analogies though, physical self-organizing processes have no historical or evolutionary time in a strong theoretical sense; they may just have the time of a process. They entirely obey optimality principles from physics and past events have not shaped their properties, insofar as the symmetry breakings that self-organizing processes may encounter are all pre-defined within the theory. A hurricane does have, so to speak, a "birth", a "life", and it does eventually "die out"; yet, hurricanes have been the "same" kind of object for the past four billion years on Earth. Again, their time is that of a process. Their historicity is embedded within a pre-defined phase space.

The fact that we can understand such spontaneous objects on the basis of a stable generic mathematical structure is not fortuitous. Indeed, their spontaneous character corresponds to the fact that these objects can emerge from homogeneous initial conditions in the mathematical framework used to describe them. By contrast, specific objects are not framed by stable mathematical structures: they cannot be derived from homogeneous initial conditions and cannot be obtained spontaneously in practice. Even in the "origin of life" field, the aim is to produce a cell which can evolve and not a cell that is similar to all current cells as they have evolved for billions of years. Moreover the aim is certainly not to obtain a cell similar to any specific species (Pross & Pascal, 2013).

According to the principle of variation, biological objects are the result of a cascade of unpredictable symmetry changes, which implies that they do not follow optimization principles and that they are not spontaneous. To be sure, biological objects did appear spontaneously in the history of life, but should one re-run the history of the Earth, one could not expect to obtain the same biological objects. It is not even possible to state in advance the mathematical space of possible forms that could be obtained. The historicity of biological objects is not embedded within the phase space anymore (as it was in physics): rather, the principle of variation means that the phase space itself is historical (figure 4).

At first sight, though, the claim that the phase spaces in biology are historical seems too strong: aren't there some aspects of biological objects which are ahistorical? Evolutionary convergences, for instance, seem to be an example of an ahistorical aspect of the living: convergent features seem to be obtained independently of (some aspects of) the past history of the organism. Let us first point out that evolutionary convergences are not about invariant properties of a given object over time, they are about mathematical structures that are similar in different historical paths. Let us consider the case of the camera eye of the vertebrates and of the cephalopods as an example. These eyes have different evolutionary origins but they are nevertheless similar and one could argue that they would be instances of the same generic object from a physicomathematical viewpoint, when described in terms of optical geometry for example.

The principle of variation, however, implies that the convergence is very unlikely to be qualitatively exact. There would always be a relevant biological description which would distinguish them sharply by pointing to differences in their organization and in their articulation with the rest of the organism. For instance, the retina is inverted in vertebrates: the axons of photoreceptors and their connection to ganglion cells and the optic nerve are located between the receptors and the light source, creating a blind spot at the level of the optic nerve. In cephalopods, axons are behind the photoreceptor which does not create such a blind

[17] Incidentally, the idea of spontaneous generation in biology stemmed from the same kind of reasoning: (generic) biological objects would appear spontaneously by self-organization in the appropriate milieu (De Klerk, 1979).

spot. A close analysis of both the phylogenetic and the ontogenetic paths makes the difference understandable: the high modularity of the cephalopods' brain derives from an early separation of the brain's modules by an invagination of the ectoderm, in contrast to the evagination of the diencephalon, due to the late separation of the eye component of vertebrates' brains.

In short, the principle of variation implies that strict evolutionary (or developmental) convergence never occurs: symmetry changes are such that biological objects drift in a burgeoning phase space, and partial convergences always embed hidden differences which may be of importance with regard to the considered behavior of the biological object in that phase space. Reciprocally, the similarity between the organizations of different organisms stems from common descent, that is to say from a shared history.

3.5. Contextuality

Organisms are contextual objects. In our theoretical framework, the symmetries of organisms depend on its environment — both on its immediate environment and the environments encountered in its past history.

The fact that the symmetries of an organism depend on its immediate environment constitutes another similarity with self-organizing physical systems mentioned above, as the latter strongly depend on their boundary conditions. However, the principle of variation makes the contextuality of biological objects more fundamental than that of physical systems. Contrary to physics, the possible changes of symmetry due to a change of the context are not all predefined. This means that an organism in a new environment may undergo unpredictable reorganizations, which correspond to different relations between its internal constraints and the environment, as well as different relations between its internal constraints, tout court. For example, we do not know a priori the many changes that can occur when bacteria that used to live with many other species in their natural and historical environment are grown as an isolated strain in laboratory conditions. Similarly, it is always difficult to assess whether the behavior of cells cultured *in vitro* is an artifact of *in vitro* culture, or whether it is biologically relevant (meaning that it corresponds to a behavior that happens in the context of the multicellular organism from which they were taken, see Montévil et al., 2016).

The contextuality of biological objects is coupled with their historicity (Miquel & Hwang, 2016): biological organizations tend to maintain the effects of former environments and may even internalize their relationship with the environment over time. This holds at the developmental scale (think of how early plastic responses to the environment might be 'frozen' later in development, see also Gilbert & Epel (2009)), at the scale of several generations (for example through epigenetics), and at longer evolutionary scales (think, for instance, of the presence of lungs and lack of gills in marine mammals, which reflects a past terrestrial life).

Let us discuss two examples of internalization of the context on the developmental and on the evolutionary time scales, to show how it can lead to unexpected behaviors of biological objects.

On the developmental time-scale, an example of internalization of past contexts is provided by the response of cells to hormones (Soto & Sonnenschein, 2005). Basically, the response of a cell to hormones does not depend only on the specific receptor and corresponding hormone involved but, rather, on the developmental history of this cell. More precisely, precursor erythroid cells are expected to differentiate into red blood cells when their erythropoietin receptors bind with erythropoietin. However, precursor erythroid cells which have been engineered to lack erythropoietin receptors and instead have receptors for prolactin do differentiate into red blood cells when they are exposed to prolactin, a hormone associated with lactation (Socolovsky et al., 1997). Conversely, mammary epithelial cells can be engineered to have a hybrid receptor with an extracellular part of a prolactin receptor and an intracellular part of an erythropoietin receptor. These engineered cells respond like normal mammary epithelial cells to prolactin (Brisken et al., 2002). These examples show that it is not the molecular specificity of a signal binding to a receptor that determines the response of a cell to a hormone. In contact with a hormone for which it has a receptor, a cell rather responds according to the context of its cellular lineage during development, that is its trajectory in time and space (Soto & Sonnenschein, 2005).

On the evolutionary time-scale, a component of an organism, as a result of a history, may be used for different purposes in different contexts. The phenomenon of a character (be it the result of past natural selection or not) which is coopted for a current use has been named 'exaptation' by Gould & Vrba (1982). They provide many key examples, for instance: "the jaw arises from the first gill arch, while an element of the second arch becomes, in jawed fishes, the hyomandibula (suspending the upper jaw to the braincase) and later, in tetrapods, the stapes, or hearing bone" (Gould, 2002, p.1108). An ex-aptation is a re-interpretation, or re-use, of a trace of the past in a new context and, therefore, cannot be derived from the initial function of the parts involved. As a consequence, the detailed structure of the internal ear can be better understood by looking into the cumulative history of exaptations.

In light of the principle of variation, the internalization of current and past contexts provides one way (although not the only one) in which symmetry changes can occur throughout the history of an organism. As an illustration, the internalization of the context contributes to explaining the difficulty of replicating biological experiments, insofar as aspects of an experimental situation which can be relevant to the studied behavior may not be measured and can be traces of an (unknown) past (Begley & Ellis, 2012).

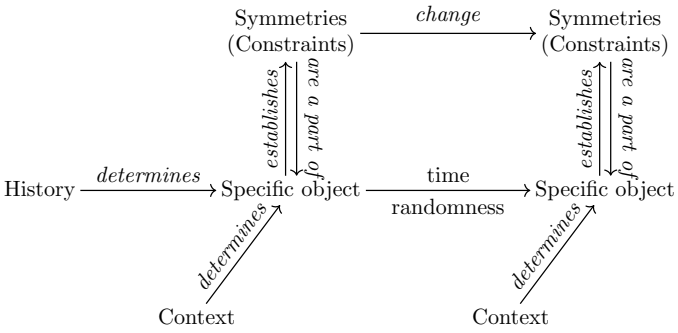

Figure 4: *Biological objects and their theoretical structure.* Specific objects are not defined by invariants and invariant preserving transformations. Instead, specific objects such as organisms undergo random variation over biological time. Their behaviors are not given by a synchronic description. Instead, they depend on a history and a context. Constraints are restricted invariants and symmetries, which may change over time and frame a part of the behavior of specific objects. Experiments and mathematical models usually investigate constraints and their changes.

3.6. Variability

The principle of variation underlies biological variability: the fact that multiple organisms or the same organism or lineage at different times exhibit differences when compared to each other.

The flow of time is the most fundamental transformation acting on biological objects: as we argued, biological symmetries and accordingly biological organizations are not preserved as time passes.

Variability tends to be stronger when considering large evolutionary time scales than for shorter time scales. When one follows the succeeding generations from the LUCA to a randomly chosen current organism, for example a rat, many relevant aspects of the description needed to understand these organisms appear and disappear through time.

Variability is also significant at physiological time scales, even at those that are much shorter than the lifespan of the considered organism. Heart rate, for example, does not obey homeostasis *stricto sensu*: the beat to beat interval is not invariant (in a healthy situation), and it does not even display fluctuations around a stable average value. Instead, the beat to beat interval fluctuates in a multiscale manner (West, 2006; Longo & Montévil, 2014b). Typically, the heart rate of a healthy subject displays patterns of accelerations and deceleration at all time scales during wake hours. Note, however, that the typical symmetries of multiscale fluctuations (scale symmetries) are not met either. Rather, many factors impact the multiscale feature of these variations of rhythms. For example, the current activity of the subject, her age, her life habits (smoking, exercising, etc.) and diseases change these multiscale features (Longo & Montévil, 2014b). These differences in the patterns of the variability of the beat to beat interval can even be used for diagnostic purposes (West, 2006; Bailly et al., 2011).

Besides the flow of time, the second set of transformations relevant to variability are the permutations of different organisms or different populations. Permutations correspond to the interchanging of different objects. They are fundamental symmetries in many physical frames: for example, it is axiomatic that all electrons follow the same equations (but they can be in many different states). In experimental biology, permutations of different animals or cells are often assumed to be symmetries: when one considers different animals of a control group, a common assumption is that they behave in the same way and that the quantitative variation observed stems from a probability distribution that would apply to all of them. This assumption, in one form or another, is required to apply theorems of statistical analysis.

According to the principle of variation, however, the permutation between these organisms cannot be taken as a symmetry. Of course, organisms are related by a shared history, which enables us to determine that they are mice, rats, etc., of a given strain. Yet, the transformation which replaces one organism by another in the same group corresponds to a comparison between the results of divergent paths stemming from a shared history. Here, divergence is taken in a strong sense and implies symmetry changes and not mere quantitative changes conserving the same symmetries. For example, qualitative behaviors differ between different strains of the same species, even in unicellular oganisms (Vogel et al., 2015). Now, we illustrate this idea with a historical example.

At the end of the 19th century, Sir Francis Galton, one of the founders of the notion of heredity, came up with a device, known as the bean box or the quincunx (see figure 5). The quincunx facilitated the simulation of a binomial distribution (the device would be used to simulate "normal variability", Galton (1894, pp.63f)). The device consisted in a vertical frame with three parts: a funnel in its upper part, rows of horizontal pins stuck squarely in its middle part, and a series of vertical compartments in its lower part. A charge of small items (say, beans or balls) would be thrown through the funnel, travel through the pins, possibly bouncing in any direction, and would be gathered by the vertical compartments at the bottom (where they would not move anymore). In the end, the distribution of the items in the bottom compartment would approximate a binomial distribution.

In our terms, the bean box works the following way. The items share a common history when they get into the funnel, and this common history leaves a trace in the result: depending on where the funnel is placed into the device (e.g. in the middle or not), the distribution of the items in the end varies. When the items exit the funnel, they take divergent paths (by bouncing on the pins) until they reach a vertical compartment. This is, however, divergence in a weak sense. For the bean box to work, all the items have to be supposed to be symmetrical, and all the realized paths have to be supposedly taken from the same underlying distribution. As a matter of fact, this

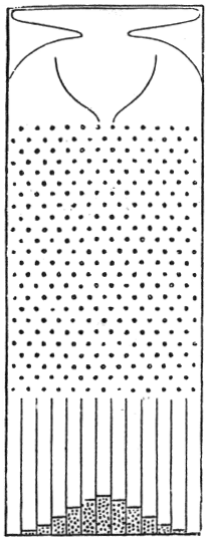

Figure 5: *Galton's quincux* (Galton, 1894, pp.63). A ball falls but obstacles lead it to move randomly to the right or to the left. The outcome is variability in the position of the balls at the bottom of the device. This device illustrates variation in a pre-defined set of possibilities. Biological variation, by contrast, sometimes involves the constitution of new possibilities, which would amount for the ball to jump outside of the quincunx.

assumption is necessary for the use of statistics in biology: when performing an experiment on — say — rats, one supposes that all rats are independent realizations of a random variable taken from the same underlying distribution and that this distribution is stemming from their most recent common ancestor. The most recent common ancestor plays the role of the funnel; and subsequent mutations, effects of the environment, spontaneous variations, etc., play the role of the pins. Variation can occur, but it will be merely quantitative and measured by the position on the horizontal axis in the bean box.

By contrast, the principle of variation posits that unexpected (and unknown) qualitative variation permanently occurs. This means that different organisms are not different realizations of a random variable taken from an underlying single distribution, as this distribution cannot even be defined. In terms of the bean box, this means that the pins unexpectedly open new dimensions (i.e. new relevant features arise), which would not be defined before the realization, and would not be reproducible after either. This is what we mean by divergence in a strong sense. Galton used his device to illustrate normal variability where variability would be quantitative, in a pre-defined space. By contrast, the principle of variation implies that variation can be qualitative (i.e. symmetry changes) and that the space of variation is not pre-defined. This, to reiterate, applies both at ontogenetic and phylogenetic scales[18].

[18]In experimental biology, organisms are often kept as historically close as possible, they may be siblings for example. The aim is then to keep the divergence in their organization limited. We call this experimental methodology, which aims at selecting biological objects in such a way as to reduce variability "symmetrization". A more detailed analysis of biological experiments will be the object of a specific paper.

3.7. Modelization and specific objects

Current mathematical modeling practices in biology borrow mostly the epistemology of physics and are based on generic objects following specific trajectories. So far, we have argued that the theorizing of physical phenomena is based on stable mathematical structures and on the corresponding analysis of generic objects. We advocate, by contrast, that biological organisms are specific objects moving along possible phylo-ontogenetic trajectories. Organisms have a historical and contextual nature and change their organization and functions over time.

This physicomathematical modeling practice in biology leads to many technical and epistemological problems. For example Boolean networks (see Kauffman, 1993) are used to model gene networks and are defined as random networks where the existence of an edge between two nodes follows a given probability distribution. Such an assumption is a way to model protein or gene networks in an ahistorical manner (and for example to generate them *de novo* in simulations). This disregards the fact that the actual phenomena are the result of evolution, and thus that actual biological networks depend on the historical interplay between living beings and their environment, even at the molecular level (Yamada & Bork, 2009). Hence, they are not a sample of a random network following a given probability distribution. This is also true for cell networks: in a tissue, cell to cell interactions or the production of proteins are largely a context- and history-dependent phenomenon. For instance, the "normalization" of a cell transferred from a cancer tissue to a healthy one can be understood as the effect of tissue context (and its history) controlling individual cellular activities (Soto et al., 2008; Sonnenschein & Soto, 2016). These examples show that the standard modeling strategies of a biological system struggle against the historicity and contextuality of biological organisms.

We interpret the "big data" approach, that aims at taking into account a massive amount of data in a model, as an attempt to address the consequences of the historical nature of biological objects while keeping the physical methodology of establishing intelligibility on the basis of generic objects. Such an approach, however, raises the question of the intelligibility of their object, because the complicated mathematical structures of models based on big data make only computer simulations possible. Other more physical approaches focus on generic features that even these historical systems would meet. For example, scaling laws in networks have been extensively investigated, but their validity is criticized (Fox Keller, 2005). Globally speaking, however, the methodological emphasis on generic features implies that the biological meaning of specific variations, and their role in a given organism, is lost. The issue is that without stable generic features, the

question of the objectivity of these models is open, insofar as their description and behavior will have a high degree of arbitrariness: the models will miss the consequences of the principle of variation and, thereby, display invariants which are not valid.

Most mathematical models do not aim at capturing features of whole organisms but, rather, at singling out some constraints, corresponding to specific parts of an organism. Typically, they focus on the morphogenesis of an organ or a tissue, for example the formation of leaf disposition (phylotaxis) (Douady & Couder, 1996), the organization of the cytoskeleton (Karsenti, 2008), the morphogenesis of vascular networks (Lorthois & Cassot, 2010), etc. Even though this approach has obvious merits and has provided remarkable insights, it does not take into account that these organs or tissues are parts of the whole organism and that the possible reorganizations of these parts are essential to variability, development, and evolution. From a mathematical viewpoint, one aspect of this weakness can be expressed as the fact that models miss some degrees of freedom corresponding to the changes of phase space that follow from changes of symmetry, in accordance with the principle of variation.

Although mathematical models are more and more used in biology, we think that the key challenges raised by biological organisms, in particular variability, historicity and contextuality, have not yet found a proper methodological and epistemological treatment. We hope that the principles discussed in this issue will contribute to better identify and address these challenges.

3.8. Conclusive remarks on the principle of variation

The principle of variation leads to a change of perspective with respect to physics. Historicity, contextuality, and variability are fundamental every time an organism is under scientific scrutiny. Rather than trying to avoid the intrinsic difficulties in mathematizing these features, our theoretical frame aims at building on them. To be sure, the randomness of symmetry changes limits the actual knowledge we can obtain on a given organism. At the same time, however, this new kind of randomness can be studied as such, and opens up new avenues of investigation.

Last, underpinning our discussion above is the fact that the principle of variation involves two kinds of changes: changes of the biological object itself (philosophers would say this is an ontological change) and changes in the question asked about this object (philosophers would say this is an epistemological change). For example, developmental biology studies features that appeared with multi-cellularity: the field is thus a result of biological variation. Reciprocally, growing cells in lab conditions comes with modifications of their behaviors which in turns affects the questions at stake and possibly their future culture conditions. Thus, in our view, the instability of biological objects goes hand in hand with the instability of biological questions: they co-constitute each other.

4. Bringing organization into the picture

Let us begin with a methodological remark on the articulation of the principles of variation and organization. The theoretical definition of a biological organization at a given time is closely related to how it may change, and that for two related reasons. First, the organization of every current organism is the result of a cascade of changes over ontogenetic and evolutionary time scales. Second, the appropriate theoretical definitions and representations of scientific objects are, generally speaking, those that enable us to understand the changes of these objects. For example, positions, momenta, and the mass are both necessary and sufficient to understand the changes of position of the planets of the solar system in classical mechanics. This justifies the theoretical representation of the planets on the basis of these quantities. In this respect, an appropriate framework for organisms requires the articulation of organization with the changes that it may undergo. To some extent, this question has been neglected in the past insofar as biological organization has been mostly approached as a mathematical fixed point, which leads to the concept of organizations as maintaining themselves identically over time.

4.1. Organization grounds constraints in specific objects

Even though organisms should be understood as specific objects, as the principle of variation posits, we would argue that some of their parts exhibit generic features in a restricted sense. As mentioned in section 3.2 above, we refer to these parts as constraints. More precisely, constraints are characterized as entities that control biological dynamics (processes, reactions, etc.) because of some symmetrical (conserved) aspect which they possess at the relevant time scales. For example enzymes are not consumed in a chemical reaction that they nevertheless change completely. Similarly, the geometry of the vascular system is conserved at the time scale of blood transport, and this transport is constrained by the vascular system.

The stability of constraints, however, has to be explained by a sound theory of biological organisms, especially in the long run. Indeed, beyond the time scale at which a constraint operates, constraints undergo degradation. A constraint may be further stabilized by a process being under the control of another constraint, which is itself stabilized by another constrained process, and so on: if the chain of dependencies folds up and the constraints can be said to be mutually dependent, the system of constraints is organized. The constraints that constitute an organism are the organized ones, which (i) act on a process stabilizing a constraint of the organism and (ii) depend on at least another constraint of the organism. The key aspect in this framework is that constraints are stable at a given time-scale, while being stabilized by processes taking place at other time scales, so that constraints behave as local invariants with respect to the processes they constrain.

However, while the time scales of constraints in the principle of organization are the intrinsic time scales of the processes and constraints under study, they do not preclude a change at these or other time scales for reasons extrinsic to these objects. Changes of organization stemming from the principle of variation can alter a constraint at any time scale. In this event, the former constraint may lose its status of constraint or may operate differently.

The cohesion of organisms is one of their fundamental features, and this cohesion has been the object of many theoretical investigations, for example as autopoiesis (Varela et al., 1974) or as work-constraints cycles (Kauffman, 2002). Following this line, we argue that biological organisms realize closure of constraints (Montévil & Mossio, 2015; Mossio et al., 2016): functional parts of organisms act as constraints on each other, and they realize a mutual dependence. Closure is basically the circularity in the relation of dependence between constraints. The principle of organization that we propose states that the constraints of organisms realize closure.

We postulate that the stability of functional constraints hinges on their mutual dependence (Montévil & Mossio, 2015; Mossio et al., 2016), so that the overall stability of biological organisms is justified by the closure of constraints. When we consider the principle of organization and the principle of variation together, constraints are contingent in two complementary ways. They are contingent because of their historical nature and because their existence depends on the circularity of closure instead of being grounded on other stable first principles.

4.2. The epistemological status of closure under variation

By relying on the principle of organization, it is theoretically meaningful to work on sets of constraints that verify closure. Following the principle of variation, however, constraints are not necessarily conserved over time and may undergo changes which cannot be stated in advance. As a result, the validity of closure must extend beyond a given configuration of constraints. The validity of the principle of organization should not be understood as based on a given set of constraints (or invariants) which would happen to realize closure. Accordingly, the principle of organization is not deducible from a set of invariants and symmetries (as in "physical laws"), rather, it is the condition of possibility for the existence and persistence of biological constraints (i.e. local invariants and symmetries). For this reason, we suggest that closure constitutes the *principle of organization* that, alongside variation and other principles, frames the biological domain as a whole (see Montévil & Mossio, 2015; Mossio et al., 2016).

In epistemological terms, stating that the principle of organization is a fundamental principle implies that it cannot be deduced from underlying stable symmetries and becomes an (irreducible) theoretical principle for biological organisms. Closure becomes an *a priori* that replaces the *a priori* of space and time in physics, or, more precisely, of the phase spaces of physical theories.

In a theoretical sense, the generality of biological analysis is made possible by the permanent relevance of organization as closure, despite the continual symmetry and phase space changes. To a certain extent, the situation of closure is similar to that of the energy of a physical system being conserved despite its permanent changes of state. In the case of a change of constraints, an organized object goes from one closed regime to another, unless the organism does not succeed in establishing a new regime and dies.

4.3. Relevant variation with respect to closure

The principle of organization understood as the closure of constraints leads to the idea that the relevant changes of the organism involve constraints subject to closure. The changes of constraints that do *not* impact the constraints subject to closure fall in two categories: those that affect the environment and those that affect the organism (in other aspects than the constraints subject to closure). If a change of constraint affects the environment, it may be biologically relevant, for example if it affects other organisms. If the change affect the organism, but not its organized constraints, then it is not significant for the organism in the light of the principle of organization: these constraints do not play a role in the biological system (although they may be involved in an unpredictable organizational change).

As for the changes that may affect the organization, a general distinction can be made between irrelevant and functional variations. On the one hand, processes and constraints may undergo irrelevant variations, for example small quantitative variations, i.e. quantitative fluctuations that neither undermine nor modify the overall organization. This is variation that, in a word, the organism does not need to control in order to ensure its stability, by hypothesis. On the other hand, variation can be functional, in the sense of resulting in a change of one of more constraints, of their relations, and hence of the very organization. Examples of quantitative variation are for example moderate differences in the weight of some organs like the liver, or in enzyme concentrations; examples of functional variations are the reshaping of bones and musculature to perform a new function or to perform differently an old function (West-Eberhard, 2003). Of course, the quantitative variation of a given constraint can also be *potentially* functional, in the sense of enabling the possible further emergence of functional variation, including pathological ones.

Another example of functional variation is random gene expression, which has been studied both in unicellular (Eldar & Elowitz, 2010) and multicellular organisms (Dueck et al., 2016). In this literature however, functional variation is mostly understood in an evolutionary sense, while closure provides a systemic interpretation of functions (Mossio et al., 2009). As a result, closure enables us to conceive functional variation that is not necessar-

ily inherited, provided that the constraint resulting from variation is still subject to closure.

4.4. Closure remains closure under variation

As discussed in Mossio et al. (2016), closure contributes to making both internal and external variations possible. The circularity of closure weakens the coupling between what is going on inside a system and its boundary conditions (Barandiaran & Moreno, 2008). Such a decoupling enables variation beyond what would be permissible if the system were completely determined by its boundary conditions (such as in physical self-organization). Reciprocally, an organism can stand a relatively unstable ecosystem because of its autonomous stability due to the closure of constraints.

Under our principles, functional variation cannot lead to a violation of the organization principle — except in the case of death. This means that any change affecting the constitutive constraints are changes from one organized situation to another. In our frame, closure is always met, even though the constraints relevant to closure may and do change. The continuous alteration, loss or acquisition of functions result in the realization of new organizational regimes; each regime, in turn, achieves a form of stability determined by closure as a mutual stabilization of constraints. Being subject to both the organization and variation principles, biological organisms realize a succession of different instances of organized regimes over individual and evolutionary times. Then, the stability achieved by the organism is not conservative, but it is for a part cumulative, insofar as it sustains many functional innovations, and makes their preservation over time possible.

Changes of the organization may correspond to several situations depending on the constraints involved. They may be more or less local with respect to the rest of the organization. We propose a typology on this basis:

- A first situation consists of a local change of a constraint, such that it does not induce a change in the relationship between constraints. For example, a supplementary branching event in a network or tree structure (such as vascular networks or mammary glands) does not correspond to a major reorganization of the constraints of a system. Let us remark, still, that this situation corresponds to a basic symmetry breaking involving the appearance of new relevant quantities of preexisting kinds (for example the angle between the new branches). Therefore, such a change is generic (a branching among many possible branchings). In section 3.1 and 3.3 above, we discussed such examples of generic symmetry changes in the context of specific objects. In turn, the new constraints can be stabilized by generic constraints (insofar as the new branch is stabilized in the same manner than the preexisting ones). In the context of closure, a simple example of a generic stabilization is the inhibition of the proliferation of estrogen-target cells by albumin: after a cell division (which is a symmetry change[19]), the same albumin maintains its inhibitory effect on the new cells.

- Another situation corresponds to a change involving a modification of the relationship between preexisting constraints as they come together to generate a new biologically structure or dynamics. Such a change is fundamentally non-local with respect to the graph of interacting constraints. In this kind of situation, some constraints act on processes which they did not constrain before the change. This corresponds typically to the notion of exaptation. In general, such a change implies the alteration or the appearance of specific constraints that establish the new behavior: the important difference with respect to the case described in the previous paragraph is that various other constraints are also mandatory to enable the emergence of the new behavior.

- Finally, a change in organization might result from the appearance of new constraints. In order for a new constraint to be included in the closed system, the organization must be reshaped so that the new constraint be integrated to the organization (Montévil & Mossio, 2015; Mossio et al., 2016). There are two aspects to this: the new constraint must be maintained by other constraints (I) and maintain another constraint (II). Whether (I) or (II) occurs first corresponds to different scenarios. It is fairly easy to picture a constraint being maintained (criterion I) starting to play a role in an organization after some time (criterion II). For instance, in mammalian development, lungs are first formed and maintained (I) and they acquire a functional role only after birth (II). However, the opposite may also happen, for example, thanks to generic physiological responses discussed above: a change of behavior leading to mechanical friction (II) leads to the strengthening of the skin by keratinization (I). Lastly, the two aspects can be coupled. For instance, some structures (such as muscles, bones, etc.) which are not used (II) may atrophy (I), and reciprocally their use (II) may lead to their further development and strengthening (I).

The key issue about changes of organization is the inscription of the change in a new organization. After a change of constraints takes place at relatively short time scales, the altered constraints involved may be stabilized by other constraints, at longer time scales. These stabilizing constraints are then typically solicited differently than before the change: they maintain, for example, the

[19] Cell division corresponds to the disappearance of an object and the appearance of two new non-identical objects, see Sonnenschein & Soto (1999; Longo et al. (2015; Soto et al. (2016; Montévil et al. (2016).

same tissues but in a different macroscopic shape or configuration. This can happen through generic physiological responses (e.g. keratinization of oral mucosa subjected to friction, resorption of bones under compressive stress, etc.). These changes do not happen only in the interaction with the environment, they happen in essential developmental, metabolic and regulatory processes (as in the developmental processes mentioned above). Another example is given in David et al. (2013): the authors show that "jamming" the regulation of key metabolic genes of yeast cells did not lead to their death but, instead, to new dynamic behaviors which enabled them to thrive after a transition period.

A change of constraint, or the appearance of a new constraint does not necessarily lead to a stabilization of the new situation. In particular, organized constraints might tend to restore the initial situation because constraints subject to closure are maintained by another constrained process. For example, a mutation in mRNA is not going to be sustained because the production of new mRNA will not carry the same variation. One might refer to such a tendency as a form of organizational "inertia". In such a case, the new constraint may vanish at a relatively short time scale. The diametrically opposite situation (among others) is also possible. It corresponds to an *amplification* of a change affecting a constraint, which in turn destabilizes other constraints in the longer run. It is typically the case in carcinoma where, as stated by the Tissue Field Organization Theory of carcinogenesis, the lack of sufficient constraints on the epithelium can lead to a progressive disorganization of the tissue and, sometimes, disrupt the organization of the whole organism leading to death (Sonnenschein & Soto, 2016).

Overall, the principle of variation complements the principle of organization, which should not be conceived as a "fixed point" that iterates itself always identically. Rather, *organisms change while staying organized*. Variation participates in the robustness of closure in changing environments. Changes of organization actually enabled the maintenance of organisms over very long time scales (during evolution). Last, but not least, current organisms are the product of such variations. Current biological organizations are determined by their (partially) cumulative variations, and this process enables organisms to explore more and more complex organization (Gould, 1997; Bailly & Longo, 2009; Longo & Montévil, 2012; Soto et al., 2016).

5. Non-identical iteration of morphogenetic processes

As a last step, we discuss in this section the connection between the organization and variation principles and the "framing principle" proposed in Longo et al. (2015), according to which biological phenomena should be understood as "non-identical iterations of morphogenetic processes". As mentioned in Mossio et al. (2016), we submit that organization and variation, taken together, constitute a "organismal specification" of the framing principle. The latter is an informal overarching principle that can be further specified by the two principles of organization and variation.

The framing principle applies to morphogenesis understood in a general sense, that is, both to organogenesis and to proliferation with variation at the cellular level. In other words, both in organ generation (for example, lungs, vascular systems, plants' organs etc.) and in reproduction, a form is iteratively (and hereditarily) produced, yet never identically. Let us now develop what it means for biological phenomena to be "non-identical iterations of morphogenetic processes".

By *non-identical*, we mean (as discussed above) not just quantitative changes but rather unpredictable changes of symmetry, thus unpredictable qualitative changes in the behavior of the object. In the context of the organism, the relevant changes are the ones impacting the organization, that is to say, the ones changing the constraints subject to closure.

The *iterations* are those of organized objects, subject to closure. However, they should be understood in several ways depending on the particular kind of objects they refer to.

First, closure is by definition about circular causal architectures. For instance, consider a simple closed system, where C_1 generates C_2 (at time-scale τ_1), C_2 generates C_3 (at time-scale τ_2), and C_3 generates C_1 (at time-scale τ_3, say this is the fastest of the three). To discuss iterations, let us consider a perturbation on C_1 at t_0. This perturbation impacts C_2 significantly at time $t_0 + \tau_2$. Then, C_2 impacts C_3 at time $t_0 + \tau_2 + \tau_3$. Finally C_3 impacts C_1 at time $t_0+\tau_2+\tau_3+\tau_1$, and this closes the loop.[20] Thus, with the flow time, the circularities of closure lead to iterations of closed patterns. More generally, in a loop described by closure, the duration of the loop as a whole corresponds to the scale of the slowest process. At this time scale, the iterations are the whole set of constrained processes which stabilize and maintain the organization of the organism. Following the principle of variation, these iterations are associated with unpredictable changes of symmetry.

Second, the organizations themselves are iterated. This adds to the principle of organization the notion of *reproduction*. By reproduction we mean the process of going from one organized object to two (or more) organized objects[21]. Reproduction pertains to the notion that the default state of cells is proliferation (with variation and motility) (Soto et al., 2016; Longo et al., 2015) which complements the principles of organization and variation.

Reproduction is also essential in that organizations which undergo variations may undergo deleterious variations. As

[20] Note that the iterations of these loops are not just about successive operations. Instead, all constraints are active simultaneously. Incidentally, this is why a perturbation approach is better suited to show the iterative structure underlying closure.

[21] Note that some situations can be fairly complex. Indeed, some organizations include constraints which act across generations.

a thought experiment, a cell which would never proliferate but would undergo variation should have a finite life expectancy because at some point a deleterious variation would occur. As a result, varying organizations require reproduction to be sustained in an open-ended manner. Reproduction enables a balance between the exploration of possibly morbid variations and the maintenance of a strain of organized systems.

Finally, the framing principle applies also to organ formation. Iteration is a very common morphogenetic process which takes place for example in branching morphogenesis of glands such as the mammary glands, the lungs, etc[22]. Iteration processes explain the abundance of fractal-like structures in biology (Longo & Montévil, 2014b). Such multi-scale structures play a particular role because they link different scales, coupling macroscopic and microscopic entities. As such they constitute spatio-temporal coherence structures, which we propose elsewhere to interpret as biological levels of organization (Longo et al., 2012b).

6. Conclusions: back to theoretical principles

Biological variation is relevant at all levels of organization, and, for example, it is manifested in the default state of cells (proliferation with variation and motility). The principle of variation that we propose states that biological organisms are specific objects and, thereby, fundamentally different from the objects defined in physical theories. The principle, which draws directly on Darwin's insights on biological variation, embeds a specific notion of randomness, which corresponds to unpredictable changes of the mathematical structure required to describe biological objects. In this framework, biological objects are inherently variable, historical and contextual. A specific object such as an organism is fundamentally defined by its history and context. Its constraints which may be described by mathematical structures are the result of a history and may change over time.

Our approach to variation contrasts with a relevant part of the theoretical literature on biological organization which aims at investigating the origin of life by the means of minimal or physical models. The strong point of these models is that they lead to tractable mathematics (see for example Luisi, 2003; Ruiz-Mirazo & Moreno, 2004). Here, we aim instead at combining organization and variation in a framework that focuses on current organisms, with the massive amount of history that they carry. This difference between the two methodologies corresponds to distinct but complementary aims, and, crucially, to the fact that the concept of organization has been traditionally approached without stressing the importance of variation, its pervasiveness and its conceptual consequences. This has led modeling attempts to focus on generic objects, which are, we think, unable to adequately represent current biological objects.

In order to understand current biological objects, articulating the principle of variation with the principle of organization is necessary. In our framework, organization grounds the relative stability of a set of constraints by the circularity of closure. It controls and counters (a part of the) variation that would be deleterious and would undermine the very existence of the organism. At the same time, organisms undergo quantitative and functional variations, both of them being crucial requirements for their increase in complexity, their adaptability, and, in the end, the sustainability of organization itself as suggested in Ruiz-Mirazo et al. (2004). One of the central challenges of a full-fledged theory of organisms consists in providing a coherent account of how they manage simultaneously to restrict *and* undergo variation.

The epistemological structure of our framework is distinct from the one of physical theories. In physical theories, assumptions on the validity of stable mathematical structures (symmetries) come first, and they may lead to randomness in a given mathematical space. In our framework, variability comes first and closure justifies the validity of constraints.

The notion of constraint is central to our framework. Constraints are the building blocks of mathematical modeling in biology and are the main objects of experimental investigation. The theoretical notion of constraints that we propose should lead to a reinterpretation of mathematical models that are based on them. In our framework constraints depend on the rest of the organism and the rest of the organism depends on them (principle of organization). Moreover, constraints may undergo unpredictable variations (principle of variation).

The principles of variation and organization do not aim at providing a complete framework to understand biological objects (the default state, for instance, is also required), but they elaborate on both the Darwinian and organicist traditions and lead to a significant departure from the physical methodology, which opens the way to original research directions.

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[22]Note that iterations in organ formation are not just iterations of a shape (such as iterations of branching): they involve the whole set of constraints which enable the maintenance of shape. In the case of epithelial branching structures for instance, this includes the basement membrane and the activity of stromal cell which maintains this membrane and the collagen of the tissue around a new branch.

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Toward a theory of organisms: Three founding principles in search of a useful integration

Ana M. Soto[a,b,*], Giuseppe Longo[a,b], Paul-Antoine Miquel[c], Maël Montévil[d,e], Matteo Mossio[e], Nicole Perret[a], Arnaud Pocheville[f], Carlos Sonnenschein[a,g,b]

[a] *Centre Cavaillès, République des Savoirs, CNRS USR3608, Collège de France et École Normale Supérieure, Paris, France*
[b] *Department of Integrative Physiology and Pathobiology, Tufts University School of Medicine, Boston, MA USA*
[c] *Université de Toulouse 2, 5 Allée Antonio Machado 31058 TOULOUSE Cedex 9*
[d] *Laboratoire "Matière et Systèmes Complexes" (MSC), UMR 7057 CNRS, Université Paris 7 Diderot, 75205 Paris Cedex 13, France*
[e] *Institut d'Histoire et de Philosophie des Sciences et des Techniques (IHPST) - UMR 8590, 13, rue du Four, 75006 Paris, France*
[f] *Department of Philosophy and Charles Perkins Centre, University of Sydney, Sydney, Australia*
[g] *Institut d'Etudes Avancées de Nantes, France.*

Abstract

Organisms, be they uni- or multi-cellular, are agents capable of creating their own norms; they are continuously harmonizing their ability to create novelty and stability, that is, they combine plasticity with robustness. Here we articulate the three principles for a theory of organisms proposed in this issue, namely: the default state of proliferation with variation and motility, the principle of variation and the principle of organization. These principles profoundly change both biological observables and their determination with respect to the theoretical framework of physical theories. This radical change opens up the possibility of anchoring mathematical modeling in biologically proper principles.

Keywords: Default state, biological organization, organizational closure, variation, individuation

> All evolutionary biologists know that variation itself is nature's only irreducible essence...
>
> S.J. Gould, 1985.

> In the Origin of species (1859), he [Darwin] made it quite clear that variation alone was not enough to account for species transformation: one had also to assume that such variations were passed on to the following generations.
>
> S. Müller-Wille, 2010.

> In all in-depth analysis of a physiological phenomenon, one always arrives at the same point, the same elementary irreducible agent, the organized element, the cell.
>
> C. Bernard, 1874.

1. Introduction

The first decade of the new millennium was dubbed as the beginning of "the post-genomic era." Its arrival was greeted by the biological sciences establishment and the pharmaceutical industry with the exceedingly optimistic view that new technology and the usual reductionist approaches that characterized the last half of the 20th century will (again) cure cancer, bring about personalized and precision medicine, and more. Indeed, the rhetoric and promises have not changed from the time President Nixon declared the War on Cancer, in spite of the meager returns of this extremely expensive undertaking. The latest "moon-shot" aimed at curing cancer "once and for all" proposed by President Obama has generated a significant wave of public criticism regarding the costs of the project, its likely minimal impact on prevention and public health policy, the inequalities of access it would engender due to high cost of the "personalized' therapies and, finally and most important, the dubious probability of success (Interlandi 2016; Breivik 2016; Bayer and Galea 2015; Joyner et al. 2016). However, critiques of the philosophical stance at the coreof the biological research fueling this program have yet to propose a cogent theoretical alternative to the one that has dominated biomedical research for the last 70 years. Although the genesis of this special issue is mostly unrelated to this type of gigantic projects, we believe that this issue's content provides a critical analysis and addresses the limitations posed by the hegemonic,

*Corresponding author
Email addresses: ana.soto@tufts.edu (Ana M. Soto), longo@ens.fr (Giuseppe Longo), pamiquel@univ-tlse2.fr (Paul-Antoine Miquel), mael.montevil@gmail.com (Maël Montévil), matteo.mossio@univ-paris1.fr (Matteo Mossio), arnaud.pocheville@sydney.edu.au (Arnaud Pocheville), carlos.sonnenschein@tufts.edu (Carlos Sonnenschein)
URL: http://www.di.ens.fr/users/longo/ (Giuseppe Longo), http://montevil.theobio.org (Maël Montévil)

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reductionist, dominant world view which is metaphor rich and theory poor. This issue's content also analyses the role of scientific theories not only in their ability to provide intelligibility but also as the most practical tools for framing research and for constructing objectivity. Most importantly, the articles in this issue of PBMB put forward some basic principles that help in constructing a comprehensive theory of organisms.

Since Aristotle the idea of goal-directedness, i.e., teleology, provided a useful framework for understanding a main characteristic of organisms, namely, the "goal" of keeping themselves alive. A salient example of this phenomenon is provided by a goat studied by Slijper (Slijper 1942a; Slijper 1942b). This animal was born with paralysis of its front legs and soon learned to move around by hopping on its hind legs. This behavioral accommodation resulted in dramatic morphological changes in the bones of the hind legs and the pelvis, as well as changes in the morphology of the pelvic muscles (West-Eberhard 2005).Two millennia later another great philosopher, Immanuel Kant, worked on the distinctions between the ways of acquiring knowledge regarding the living and the inert. Regarding teleological thinking, he stressed the interrelatedness of the organism and its parts and the circular causality implied by this relationship. Teleological judgement was described as an epistemic organizing principle which allows for the explanation of the biological object through its unity (this object being the cause and effect of itself), before giving a discrete description of its parts. Following Kant's ideas teleology was adopted as a heuristic by the teleomechanists (Lenoir 1982); for Blumenbach, *Bildungstrieb* (vital force) was a teleological agent the cause of which, like Newton's gravity, was beyond the power of reason. However, the consequences of this organizing principle, like of those of gravity, were still amenable to scientific inquiry (Lenoir 1980). Thus, teleology was an extremely useful concept for the development of several biological disciplines in the late 18th and the 19$^{\text{th}}$ centuries.

Several historians, philosophers and biologists addressed the overall changes in the practice and conceptualization of biological phenomena that took place in the 20th century (Mayr 1996; Gilbert and Sarkar 2000). One of them, Lenny Moss, described a turning point, "the phylogenetic turn"; which changed the perception of the organism. In Moss' own words, "the theater of adaptation changed from that of individual life histories, that is, ontogenies, to that of populations over multiple generations, that is, phylogenies." Moss' phylogenetic turn imposes a choice "... between a theory of life which locates the agency for the acquisition of adapted form in ontogeny—that is, in some theory of epigenesis versus a view that expels all manner of adaptive agency from within the organism and relocates it in an external force—or as Daniel Dennett (Dennett 1995) prefers to say, an algorithm called 'natural selection'" (Moss 2003). Because of this change, agency, normativity and individuation, hitherto considered the main characteristics of the living, almost disappeared from biological language. Since then, cells and organisms became passive recipients of a program. As a consequence, it is not surprising that biology has a theory of evolution but not a theory of organisms.

In spite of the strong impact of the teleomechanists, their perspective was not universally accepted; in fact, two competing currents emerged regarding biological thinking. Their main difference was whether or not there were singularities of the living that required a different outlook than that used in mechanics. The 200 year old dispute between these two stances continued well into the 20$^{\text{th}}$ century as a polarization between reductionists and organicists, although the latter moved from the mechanical worldview to one inspired by the mathematical theories of information (Longo et al. 2012). Indeed, the introduction of the notion of "program" [see Perret et al, this issue, and (Longo et al. 2012)] was greeted as a sound theoretical way to get rid of the concept of "teleology" (Mayr 1996). However, the adoption of the metaphors and the powerful tools conceived and used by the reductionists blurred the distance between the two currents (see Perret et al, this issue, and Longo et al. 2012). The current state of affairs is that even those that consider themselves organicists are for the most part using the pervasive language of molecular biology, a language that forces causative power to molecules, and in particular, to genes. Nowadays, the main difference between reductionists and organicists is that the latter are keenly aware that, when they practice analytical reductionism, they may be destroying the very phenomena that they are trying to understand.

In addition to the conceptual problems generated by the phylogenetic turn and the molecular biology revolution, the availability of immensely large databases has been greeted by the declaration that the scientific method is obsolete (Anderson 2008). To the contrary, the perspective proposed throughout this issue buttresses rather than opposes the scientific method. Thus, the objective of this issue is to propose theoretical principles for the construction of a theory of organisms which could overcome both the hindrances arising from the reductionist and informational stances of the 20$^{\text{th}}$ century, and circumvent the choice imposed by the new synthesis between phylogenesis and an organismal approach.

Based on the organicist tradition, three principles are proposed to postulate a theory of organisms, namely: 1) the default state of proliferation with variation and motility, which is rooted in the cell theory, 2) the principle of organization, and 3) the principle of variation which applies to morphogenesis and inheritance. Additionally, examples are given of how these principles can guide biological research on morphogenesis and cancer (see Montévil, Speroni Sonnenschein and Soto, Sonnenschein and Soto, this issue). The aim of this concluding article is to articulate the ideas that have been expounded in the preceding articles of this issue into a coherent body.

2. Philosophical stances

In contrast to evolutionary biology, organismal biology still lacks a widely accepted global theory. For this reason it would be very helpful if practitioners would make explicit which are the principles, the postulates, and the concepts that frame their research; in short, their philosophical stances. From the organicist perspective developed in this issue, biological systems are characterized by the simultaneous co-existence of opposites as exemplified by change and stability, the incomplete separation between internal and external (topology), and before and after (time) the notions of extended present, memory and anticipation [See Miquel and Hwang, this issue, and (Longo and Montévil 2011b)]. Organisms are open systems that handle flows of matter and energy by means of and for the maintenance of their metabolism. The internal constraints defining such a system are always disturbed by external ones; thus, in order to understand what is happening in the system, we must simultaneously access the multiple levels on which this system is integrated (Stengers 1997). For instance, the cell as a whole is integrated into a more complex system, the tissue, the organism, in which it will not act similarly as to when it is placed in a conventional in vitro culture. For example, in a cardiomyocyte the proteins that channel the ions, (calcium, potassium) carry currents that change the cell voltage. In turn the cell voltage changes the ion channels (Noble 2006). Thus the components alter the behavior of the heart and the heart alters the behavior of the components, yet both components and the heart are integrated into a higher multicellular structure, the organism. This means that the working of such a system is never defined by initial constraints. Additionally, the system is historical and in relentless change from fertilization to death, being built and remodeled throughout life.

In sum, the historical way by which a system of natural events operates is not a consequence of its initial description. Instead, it acts and it produces novelty (novel qualities and novel structures) in the real world. Thus, emergence, understood here as the appearance of new observables through time, is not a simple epistemic property. It has ontological and theoretical meaning (Soto et al. 2008).

3. From the inert to the alive

Physical theories are grounded on stable mathematical structures, based on regularities such as theoretical symmetries. The physical object is both defined and understood by its mathematical transformations. These operations permit a stable description of space; this space is objectivized as the space providing theoretical determination and specifying the trajectory of the object (usually done by optimization principles). In sum, physical objects are generic and their trajectories are specific (see Longo & Soto, and Montévil et al, this issue).

In biology, we posit instead the instability of theoretical symmetries, which are likely to change when the object is transformed along the flow of time, such as when a zygote develops into an adult animal. Biological objects, i.e., organisms, are specific and hence they are not interchangeable. Their trajectories are generic; they are not specified by the phase space (Longo and Montévil 2014). These biological objects are the result of a history that represents a cascade of changes of their regularities, they exhibit variability and show contextuality; unlike inert objects they are agents. Moreover, organisms not only are able to create their own rules, they also have the capacity to change them [see Miquel and Hwang, this issue, and (Canguilhem 1991)].

4. The cell theory: a starting point towards a theory of organisms.

Canguilhem traces the history of the cell theory back to the 18^{th} century, and finds two main components, each addressing a fundamental question, namely, i) the composition of organisms, this is the cell as the element "bearing all the characteristics of life" and ii) the genesis of organisms. Canguilhem attributes to Virchow the priority of putting these two components together (Canguilhem 2008). The second element of the theory, that is, the genesis of organisms applies, of course, to both unicellular and multicellular organisms. Moreover, from the inception of the cell theory, it was stated that the egg from which sexed organisms are born is a cell whose development can be explained by the division of said cell into daughter cells by cell proliferation. In this regard, the cell was in the view of Claude Bernard "a vital atom". Bernard stated "In all in-depth analysis of a physiological phenomenon, one always arrives at the same point, the same elementary irreducible agent, the organized element, the cell" (Claude Bernard Revue Scientifique, Sept 26, 1874-cited by (Canguilhem 2008)). From this dominant position at the end of the 19^{th} century, the theory endured and survived criticism about whether anatomical or functional syncytia negated the cellular structure of multicellular organisms. Another problem that has been debated since Virchow's time is whether or not the cells are individuals. In the case of unicellular organisms there is no problem in stating that cell and organism are the same and that they are individuals. However, attributing individuality to both the cells in multicellular organisms as well as to the organism that contains them posed problems that led some to reject the cell theory. From our perspective, it is the concept of the level entanglement that provides a useful perspective of the relationship between organism and cells: the zygote is both a cell and an organism, and with each cell division, these two levels of individuation become more obvious. In other words, we may adopt Simondon's philosophy and look at individuation as a process rather than a thing (see Miquel and Hwang, this issue).

Back then and today, the cell theory plays a unifying role between evolutionary and organismal biology; it provided a link between the individual and its progeny in which the cell itself is a vehicle of inheritance. Within this theoretical perspective, the cell is the irreducible locus of agency.

5. The founding principles: from entanglement to integration?

5.1. Genealogy of the three proposed principles: the default state, the principle of organization and the principle of variation.

Each of these principles has its own history prior to the inception of the ORGANISM group. The *default state* was initially proposed by Soto and Sonnenschein (Soto and Sonnenschein 1991); it was based on experimental work done starting in the early 1970s while studying the role of estrogens on the proliferation of their target cells and is rooted in the cell theory and in the strict materiality of life. The default state is further anchored on the notion that *the cell* is an organism and is the origin of all organisms. The joint work of Longo, Montévil, Sonnenschein and Soto resulted in the integration of variation into the default state of proliferation and motility: at each cell division variation is generated. In addition to the default state, a supracellular source of variation was identified. This is the "framing principle of non-identical iterations of morphogenetic processes in organogenesis," which accounts for the generation of globally regular patterns of non-identical structures, typically observed in organogenesis (Longo et al. 2015b). The work of Miquel, Soto and Sonnenschein also addressed the generation of new observables while examining the concepts of emergence, downward causation and level entanglement (Soto et al. 2008). The principle of variation can be traced to Bailly and Longo's analysis of the differences between physical objects and biological objects, the concept of extended criticality [(Longo and Montévil 2011a) and Longo and Soto, this issue], and of course, the Darwinian idea of descent with modification.[1] The relentless change addressed by the principle of variation points to the major difference between the theories of the inert and those of the living. The other side of the coin, namely, stability, needed to be addressed as a main component of biological organization.

[1] The concept of extended criticality is based on the physics of phase transitions, which deals with the emergence of a new object, as exemplified by the transition between water vapor and snow crystals. Phase transitions occur at a point, the "critical temperature". This point marks the passage from one symmetry to another, and from one macroscopic object or structure to another. Extended critical transitions, instead, span a non-trivial interval such as an organism's lifetime. In this context, an organism continually undergoes critical transitions, whereby both the objects and the symmetries change. The organism and its components are permanently reconstructed with variations.

The history of the *principle of organization* can be traced back to the concept of autopoiesis (Varela et al. 1974), closure (Rosen 1991) and work-constraints cycles (Kauffman 2002), which has been further elaborated by Montévil and Mossio (Montévil and Mossio 2015 and Mossio et al this issue).The principle of organization is the fundamental source of biological stability. The notion of closure of constraints as the means to achieve and maintain stability was traditionally applied to intracellular processes. Mossio et al explored the concept of constraints being conserved at the time-scale of the process being constrained (see Mossio et al, this issue); this concept opens a point of entry for the mathematization of biology. We exploited this notion by modeling mammary gland morphogenesis using the default state and its constraints (Montévil et al, this issue).

5.2. How to organize these principles into a coherent set?

Our theoretical work addresses both unicellular and multicellular organisms. Following Darwin's strategy regarding phylogenesis, it seems prudent not to delve into the transition from the prebiotic to the biotic world, but to anchor our principles in the biotic world. By this we mean that we are agnostic about whether or not the principles that we propose for to study organisms are relevant to the abiotic world, since even a hypothetical biochemical structure able to instantiate closure is not an organism, and a self-replicating molecule is not an organism undergoing multiplication.

The three principles we propose are irreducible to one another and none of them could be construed as the "condition of possibility" for the other two, at least in this our first analysis about how they are related.

5.2.1. The role of the default state

The biological default state (proliferation with variation and motility), expresses agency and modifies the causal structure with respect to the theories of the inert. Our proposal on the default state has straight-forward consequences on what requires an explanation in the sense of a theoretical cause. The default state does not require such a cause. To the contrary, what would require an explanation is a departure from the default state (quiescence, restrained variation, lack of motility). This theoretical cause should be distinguished from the notion of differential cause, whereby a difference introduced in the system, like a carcinogen, leads to a difference in the system's behavior. In order to conceptually move from a differential cause to a theoretical cause, it is necessary to understand how the differential cause alters the constraints on the system (Longo and Soto, this issue). In addition to physical constraints, there are also chemical constraints that affect morphogenesis. For example, those imposed by collagen, phospholipids or DNA. The ability of an organism to generate new constraints at each new iteration produces diversity given that iterations are not identical.

5.2.2. The role of constraints

Biological constraints and their actions are key objects for biological investigations in the framework of a theory of organisms. All the principles proposed in this issue relate closely to the notion of constraint, which is shaped by the proposed founding principles.

The default state is rooted in the cell theory and the notion of the cell as an agent. Constraints are objects which are much simpler than cells, and the action of constraints on cells require a specific principle: constraints act by forcing cells out of the default state. The positing of a default state for cells leads us to discuss the action of constraints on cells that reduce, hinder or canalize their ability to proliferate and to move. This approach overcomes the metaphoric and anthropocentric use of the notion of signal, since it acknowledges the agency of cells. Cells are no longer passive things like rocks that have to be acted upon to make them do something (proliferate or move).

The principle of organization leads to the inclusion of specific constraints in an organism, and thus to assess whether a given constraint is functional, that is, it participates in closure. Constraints of an organism are constraints that are both maintained by other constraints and in turn they maintain other constraints. Given the interdependence of the organism and its parts, it is never sufficient to analyze a given constraint or a given set of constraints in isolation. However, as discussed in this issue (Montévil et al), an analysis of constraints on the default state resulted in an insightful explanation of glandular morphogenesis in a 3D model of the breast. As mentioned in that article, additional constraints at the tissue level and organismal regulation via hormones are obvious incremental additions needed for a biological analysis. In sum, additional constraints will need to be taken into consideration to understand the global biological organization in which the phenomenon studied, mammary gland morphogenesis in our case, is rooted.

The principle of variation manifests itself in the default state, since each cell division generates two similar but slightly different cells, and by virtue of this default state, into the Darwinian notion of descent with modification. The principle of variation also applies at supra-cellular levels as in the framing principle of non-identical iterations of morphogenic processes (Longo et al. 2015a). The principle of variation establishes that constraints should not be considered as phylogenetic invariants. Instead, constraints are subject to variation. For instance, a morphogenetic process which is described as a set of constraints is not necessarily conserved in a lineage. Instead, it will be typically altered both for some individuals and at the level of groups of individuals, for example in a particular species. Changes of constraints are thus intrinsic to the notion of biological constraints.

6. Conclusions

Scientific theories provide organizing principles and construct objectivity by framing models, observations and experiments. Numerous mathematical concepts and structures originated from the analysis of physical phenomena; these mathematical innovations, in turn, helped to organize physical concepts in a novel way. A classic example is Newton's invention of infinitesimal calculus which was motivated by an analysis of velocity and acceleration. Calculus made these concepts mathematically intelligible, and thus, the movements of planets became intelligible. In the 19^{th} century, Riemann's geometry was invented as an attempt to understand Newton's gravitation in relation to the curvature of space, and it was later used by Einstein in the physics and mathematics of Relativity. In the 20^{th} century, Dirac's delta, Feynman's integral and other brand new theories, such as Gauge Theory, were entirely motivated by investigations in quantum physics. As in the earlier examples, these mathematical inventions shed new light on the physical phenomena. These are just a few examples of a creative synergy between these disciplines. Why has this not been so in biology?

Symmetries and conservation laws are strictly linked and are fundamental both in mathematics and physics. In biology on the contrary, variation is at the core of both the theory of evolution and the theory of organisms that we have sketched and intend to develop. The existence of a principle of variation explains why biology has not yet inspired mathematicians to create structures that would open the possibility of formalizing biological concepts. However, pointing out to the differences between inert and live objects opens the way to better understand what would it take to arrive at this distant objective: the development of a "mathematical biology" that will play the same role that mathematics has played in physics, and which is very different from the applied mathematics transplanted directly from physics that is routinely used to model biological phenomena (Longo 2015).

Biological objects are agents capable of creating their own norms; they are continuously harmonizing their ability to create novelty and stability. Positing the three principles enunciated herein has also opened the way to explain morphogenesis and carcinogenesis (Montévil et al, Sonnenschein and Soto, this issue). These principles profoundly change both biological observables and their determination with respect to the theoretical frames of physical theories. This radical change opens up the possibility of anchoring mathematical modeling on properly biological principles. Turing showed that there is an epistemological gap between modelization and imitation (Turing 1950; Turing 1952). While the former is based on a theory about the object being modeled, the latter is not. Thus, biological principles are needed to move beyond imitation. For example, the model of ductal morphogenesis presented in this issue is based on the default state and the intrinsic constraints generated by the epithelial cells. By identifying

constraints to the default state, multilevel biomechanical explanations become as legitimate as the molecular ones. Finally, analysis of the differences between the physics of inanimate and living matter led us to propose three principles that provide a reliable perspective for the construction of a much needed theory of organisms. In addition to this theoretical purpose, these founding principles have been useful for framing experiments and mathematical modeling.

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