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Universality, complexity and the praxis of biology: Two case studies

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ABSTRACT

The phenomenon of biology provides a prime example for a naturally occurring complex system. The approach to this complexity reflects the tension between a reductionist, reverse-engineering stance, and more abstract, systemic ones. Both of us are reductionists, but our observations challenge reductionism, at least the naive version of it. Here we describe the challenge, focusing on two universal characteristics of biological complexity: two-way microscopic–macroscopic degeneracy, and lack of time scale separation within and between levels of organization. These two features and their consequences for the praxis of experimental biology, reflect inherent difficulties in separating the dynamics of any given level of organization from the coupled dynamics of all other levels, including the environment within which the system is embedded. Where these difficulties are not deeply acknowledged, the impacts of fallacies that are inherent to naive reductionism are significant. In an era where technology enables experimental high-resolution access to numerous observables, the challenge faced by the mature reductionist—identification of *relevant* microscopic variables—becomes more demanding than ever. The demonstrations provided here are taken from two very different biological realizations: populations of microorganisms and populations of neurons, thus making the lesson potentially general.

It is not an everyday experience for a scientist to expose his or her ideas to analyses by a group of professional philosophers of science. While undeniably honoring, succumbing one's cogitative habits to predacious philosophers of science takes a fair amount of courage; even more so as both of us consider ourselves romantic scientists for whom words and metaphors are means to convey a message, to communicate with the understanding that things might, should and probably are misrepresented, misused, and thus become a bed for further fertilization of new ideas. In Konstanz we attempted to initiate a discussion by painting an integrated picture, where biology is described as complex natural phenomena at the population level, rather than as a complicated programmed multi-agent engineered system that is designed to accomplish pre-defined functions. We presented detailed experiments on two very different biological systems—populations of microorganisms and populations of neurons—that expose aspects of universality in biological systems. These involve universal fluctuations, emergence of statistical similarity in the temporal dynamics of, as well as in the crosstalk between levels of organization, invariance over extended ranges of time scales, and non-uniqueness of macroscopic–microscopic relations. We argued that as such, biology resists naive reductionism (or its current expression in terms of reverse engineering, discussed below) as means to achieve what is expected from a scientific discipline, that is—exposing causal relations. Of course we acknowledged that naive reductive procedures might prove efficient as practical means to advance controlling of biological phenomena, a desired biomedical outcome; this, however, is technology—not the kind of science we wanted to discuss in Konstanz.

The *microorganism system* (Stolovicki, Dror, Brenner, & Braun, 2006) is a genetically mutated population of yeast cells,

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confronted with a novel challenge they had not encountered along their history in evolution. Specifically, a strain of the yeast *Saccharomyces cerevisiae* was engineered to recruit the gene *HIS3*, encoding an essential enzyme from the histidine biosynthesis pathway, to the GAL regulatory system, responsible for galactose utilization. The GAL system is known to be strongly repressed when the cells are exposed to glucose. Therefore, upon switching to a medium containing glucose and lacking histidine, the GAL system and (with it) HIS3 are highly repressed, and the cells encounter a severe challenge. Thus we ended up with a population of yeast cells that, in order to survive, *must* find a way to develop novel modes, rewiring its complex network of interactions between different levels of organization—environment, cell physiology and genome. It turns out that such yeast systems converge to a solution by exploration; in fact, the rewired cells adapted to the challenging environment within a surprisingly short timescale, seemingly breaking an efficacy barrier that is dictated by the dimensionality of the problem (see below). Moreover, each cell in the population had the potential to find a solution and the adapted state was stably inherited along generations. We have shown that this adaptation presents an evolutionary route that is complementary to random mutations and selection. This experimental approach made it possible for us to measure long-term intracellular processes underlying the exploratory dynamics, manifested by global non-specific and non-reproducible gene expression responses of the adapting populations.[1]

The *neural experimental system* (reviewed in Marom & Shahaf, 2002; Morin, Takamura, & Tamiya, 2005) is a large scale randomly connected network of neurons, developing *ex-vivo* on top of a substrate-embedded multi-electrode array. *Ex-vivo* developing cortical networks are composed of cells obtained from cortices of embryonic or early postnatal animals, usually rats. The preference for early stage cells is due to the fact that the later in development cells are harvested, the less probable it is that they will survive and adapt to a new environment. Immediately following their extraction from the cortex, most of the neurons have no axo-dentritic extensions and are disconnected from each other. A typical cortical network, developing in a 20-mm diameter plate, may contain up to 100,000 neurons. The neurons begin to extend their axons and dendrites within hours after plating, proceeding from a population of unconnected individual cells, independent from each other structurally, to a densely connected mature phase. At this mature phase, the network is topologically complex, showing immense number of functional synapses and broadly distributed connectivity. The network contains all the types of cells that are present in the cortex at the time of extraction, including glial cells. The substrate embedded electrode array on top of which the neurons evolve, enables monitoring and stimulation of network points at high spatial and temporal resolution over a wide range of scales. This reduced set up demonstrates the wealth of possible instantiations of two primitives that characterize neural systems: (i) an extensive functional connectivity that enables a large repertoire of possible responses to stimuli; and (ii) sensitivity of the functional connectivity to activity, allowing for selection of adaptive responses. Over the past 15 years a set of tools was developed, enabling access to many fundamental issues that concern the activity of neurons in their networks. These include studies of morphological constraints, dynamics (spontaneous and evoked) of neuronal thresholds and synaptic connections at the cellular and population levels, relations between cellular and network levels of organization, representation of environmental input as a population phenomenon, adaptation and learning.[2]

The fact that the two systems—yeasts and neurons—are very different in their physics of coupling mechanisms, makes the interpretation of the foregoing universal features potentially relevant to the discussion of biology in general.

Our approach to these systems is based on the acknowledgment that both are instantiations of populations of weakly and dynamically-coupled elements (genes and neurons). Much of present-day understanding of population dynamics in general, and of microorganism and neural populations in particular, relies on sub-cellular and single cell data. The macroscopic dynamics as well as function are described as the integrated outcome of underlying, microscopic cellular complexity. Clear distinctions are made between the source of variability and the process of selection applied by the environment; in any given environment, individuals with higher functional capacity are selected. The concept of scale separation is fundamental to this picture. In the cases discussed here, time scale separation is assumed to exist between the fast microscopic dynamics and the slow macroscopic, adaptive, environmentally affected functionality. This scale separation is the major justification used for the routine practice of integration over microscopic degrees of freedom. Such coarse-graining enables to connect microscopic configurations with the macroscopic complex dynamics.

Notwithstanding the success of the above approach, it is challenged by observations that might require reconsideration of its basic assumptions: (1) Practically identical microscopic configurations may give rise to seemingly different macroscopic dynamics and function; (2) there is no time scale separation between levels of organization; and, (3) the coupling to environmental dynamics cannot be treated as a mere filtering effect. In the microorganism system, the above features are manifested in identical genomes that can exhibit quite different macroscopic phenotypes; this phenotypic variability becomes especially significant in isogenic cell populations within diverse biological contexts. Phenotypic variation is generated by a multitude of physiological mechanisms and can be maintained by epigenetic inheritance with variable degree of fidelity. Genetic and phenotypic variations generally coexist in a population, and the connection between them is complex and not one-to-one. In neural systems, the relation between cellular or network configurations to macroscopic, adaptive function, is not unique nor specific: the same neurons, networks or even the same pattern of activity, may be mapped to seemingly different functions. Moreover, the traditional allocation of slow dynamics to extended neural configurations, and the fast dynamics to the spatially microscopic configuration, does not hold. All levels

[1] For a recent review see Braun (2015).

[2] See, for instance, Branch, Wheeler, Brewer, & Leckband (2000), Chang, Brewer, & Wheeler (2001), Segev, Shapira, Benveniste, & Ben-Jacob (2001), Shahaf & Marom (2001), DeMarse, Wagenaar, Blau, & Potter (2001), Tal, Jacobson, Lyakhov, & Marom (2001), Segev et al. (2002), Marom & Shahaf (2002), Shefi, Golding, Segev, Ben-Jacob, & Ayali (2002), Xia, Gopal, & Gross (2003), Eytan, Brenner, & Marom (2003), Eytan, Minerbi, Ziv, & Marom (2004), Wagenaar, Pine, & Potter (2004), Marom & Eytan (2005), Bonifazi, Ruaro, & Torre (2005), Wagenaar, Madhavan, Pine, & Potter (2005), Tateno, Jimbo, & Robinson (2005a, 2005b), Wagenaar, Pine, & Potter (2006), Eytan & Marom (2006), Novellino et al. (2007), Baruchi & Ben-Jacob (2007), Chiappalone, Vato, Berdondini, Koudelka-Hep, & Martinoia (2007), Pasquale, Massobrio, Bologna, Chiappalone, & Martinoia (2008), Ham, Bettencourt, McDaniel, & Gross (2008), Eckmann, Jacobi, Marom, Moses, & Zbinden (2008), Shahaf et al. (2008), Wallach, Eytan, Marom, & Meir (2008), Marom et al. (2009), Minerbi et al. (2009), Zrenner, Eytan, Wallach, Thier, & Marom (2010), le Feber et al. (2010), Kermany et al. (2010), Wallach, Eytan, Gal, Zrenner, & Marom (2011), Kanter et al. (2011), Wallach & Marom (2012), Weihberger, Okujeni, Mikkonen, & Egert (2013), Reinartz, Biro, Gal, Giugliano, & Marom (2014) and Keren & Marom (2014).

show a wide spectrum of overlapping time scales (reviewed in Marom, 2010). A whole body of observations in the fields of developmental neuroscience and psychology, as well as cellular physiology shows that the non-trivial impact of the environment, similarly to the case of microorganism population, cannot be treated as a mere filter. These observations are ubiquitous, they are by no means unique to the systems presented here.

Let us focus on two (not unrelated) aspects of the above mentioned observations, generally phrased: (1) While the microscopic configuration does impose constraints on the macroscopic phenomena, many significant aspects of the latter are not uniquely determined by the former. This is a complementary image to the 'multiplicity of microscopic realizations' argument, where the same macroscopy may be realized by different microscopies. (2) There is no time scale separation between processes occurring at different levels of organization; microscopic structures operate over time scales that are traditionally attributed to macroscopic structures.

Thus the first general observation we focus on concerns causal microscopic–macroscopic relations, or lack thereof. The relations between microscopic degrees of freedom and macroscopic phenomena are often approached from the angle of multiple realizations in the sense of many (microscopies) to one (macroscopy). This pyramid-like structure of causal relations is readily observed in our systems. In the case of microorganism population, it is manifested in the variance of gene expression patterns giving rise to the same macroscopic phenomenology—adaptive growth rate—of identical 'twin' populations derived from a single mother population (Stolovicki & Braun, 2011). Likewise in a population of neurons, where the level of ensemble activity is seemingly indifferent to ongoing changes in the underlying unstable connectivity (e.g. Minerbi et al., 2009), and where different schemes of activity patterns may serve as representations of identical environmental stimuli (e.g. Kermany et al., 2010; Shahaf et al., 2008). Many years of experience, in several labs throughout the world, show that the nature of network dynamics is robust to significant variations in connectivity and other microscopic features; e.g. type of neurons—spinal, cortical, hippocampal—as well as the precise ratio of different cellular sub-populations. While this kind of many-to-one degeneracy is ubiquitous in physical systems (e.g. in the foundation of statistical mechanics), it is the other direction of one (microscopic) to many (macroscopic) degeneracy, mostly ignored, which we believe is the hallmark of biology, suggestive of the critical role of couplings between the system and its dynamic environment. In our systems, regardless of the microscopic structure, the one-to-many degeneracy reveals itself in universal broad distributions, whether one chooses to observe different realizations (organisms), different levels of organization within a given organism (proteins, cells, networks, organ, behavior), or different temporal or spatial snapshots within the same level. In the microorganism system the one-to-many degeneracy is manifested in, for instance, the collapse of protein distributions, measured under a broad range of biological realizations, to a single non-Gaussian curve, scaled by a single degree of freedom (e.g. population average, see Salman et al., 2012). This implies that protein fluctuations do not reflect any specific molecular or cellular mechanism, and suggest that some buffering process masks these details and induces universality (Sornette, 2006). In the neural system, the same feature is manifested in long-term single neuron and neuronal population excitability dynamics, which are unstable and dominated by critical fluctuations, intermittency, scale-invariant rate statistics, and long memory processes (Gal et al., 2010; Gal & Marom, 2013a, 2013b, 2014). Physics teaches us that such broad, universal distributions that emerge in different systems and scales, require either fine tuning of control parameters 'engineered' to lock the system in specific (but rare) points in phase space (this is then a critical point), or a capacity of the system to tune itself to hover around such a point, irrespective of control parameters (a process coined self-organized criticality).[3] We will come back to these two options in a little while.

The second general observation mentioned above concerns lack of time scale separation. There seems to be no time scale separation between processes occurring at different levels of organization; microscopic structures operate over time scales that are traditionally attributed to macroscopic structures and *vice versa*. Time scale separation is a practical and most basic tool used in analysis of physical systems. It enables coarse graining, lumping of many microscopic degrees of freedom to a small number of effective system variables. Where such separation does not exist, the path towards complexity is wide open. In the microorganism case, gene expression—an intracellular, microscopic process—is dominated by slow collective modes that are usually attributed to the population level (Stolovicki & Braun, 2011). In the neural system, regardless of the observed level of organization (protein, cell, network or behavior), above lower boundaries that reflect fairly well understood physical constraints, observed and reported timescales are practically continuous, ranging from milliseconds to years (Marom, 2010). Under such conditions, in both systems, reported time scales often reflect circumstances that are imposed by the observer through the measuring procedure. One consequence of the multitude of time scales within each level, relates to the inability to conveniently assume that fast effects are due to processes that take place at the small spatial scales, and slow effects are due to spatially extended systems. The fallacy of such a 'default' assumption is readily exposed when one considers, for example, the speed of observed 'learning and memory' in macroscopic populations, a process that is expected to be sluggish, given the combinatorial large space of possible configurations. For instance, it turns out that the microorganism system can adapt to unforeseen challenges within a few generations, practically instantaneous in evolution terms; moreover, the rapidly emerging adapted state is stably inherited (Braun & David, 2011; David, Stolovicki, Haziz, & Braun, 2010). This is analogous to the facts of fast learning and memory in humans and other organisms, attributed to whole brain mechanisms. Such rapid adaptations, learning and memory that seemingly break efficacy limits imposed by the dimensionality of the problems, are also observed in our large-scale neuronal networks (Shahaf & Marom, 2001; le Feber, Stegenga, & Rutten, 2010).

The above examples of microscopic–macroscopic degeneracy of observables led us, in Konstanz, to reflect on the possible indeterminacy entailed by the combination of many-to-one and one-to-many relations among levels of organization: Regardless of the level one chooses to analyze, the extent to which observables from that analyzed level determine the phenomenology at other levels, seems limited. We suspect that one possible origin of the above microscopic–macroscopic degeneracy is related to our (experimentalists) habitual isolation of parts from the whole in standard experimental praxis. Biological systems under natural conditions are embedded in environments, which are in themselves dynamical entities that mould the—and are coupled to—many levels of system organization, from the single cell to the whole organism and population of organisms. Disconnecting the system's dynamics from the dynamics or statistics of the environment, might lead to erroneous classification of system's phenomena. A demonstration of the latter point involves the interpretation of neuronal response variability under different environmental statistics (Gal & Marom, 2013a).

[3] See, for instance, Dickman, Muñoz, Vespignani, & Zapperi (2000).

Universality of the kind exemplified above presents a challenge to reductionism in biology. In physics it enables to classify and compare phenomena that in spite of their apparent dissimilarities, belong to the same class; this, one might argue, is the goal of physics. While the exposure of universals is a most significant aspect of biological research (e.g. DNA, cell structure, energy production and consumption, etc.), much of biology is about specificity, telling the origins of *differences* between species, phenomena, capacities. Viewed from this angle, when something is identified as universal across biology, its explanatory power in regard to the origin of differences is limited. The above reported universality might imply that the observables we chose to characterize are not the relevant ones—should the differences between species, phenomena and capacities are sought for. In other words, the fact that the distribution of protein content in cell populations is invariant suggests that protein content by itself cannot be the cause of the observed macroscopic phenomenological differences. More generally, it implies that the search for the relevant system variables should become the most important and urgent for the advancement of biology. Without identification of the relevant variables, the practice of experimental biology becomes a fishing expedition, dictated by fashion and technological barriers. As demonstrated below, reverse engineering of biological systems constitutes an example for going amiss upon lack of well defined *relevant* system variables.

Unlike the over-loaded concept of reductionism, reverse engineering is a relatively well-defined procedure that exposes itself to critical analysis. It is common to think of reverse engineering in clandestine contexts (military or industrial), yet the concept has a broader meaning in the language of technology, denoting the process of detailed examination of a functional system, in the face of limited *a-priori* knowledge of its design principles. In this sense we all do reverse engineering, trying to figure out 'mechanisms' underlying the observed. The above described biological universal features, namely: two-way degeneracy and lack of scale separation, lead to serious difficulties in pointing at a relevant level of organization at which a 'mechanism' is to be sought for. The multitude of possible mappings exposes the inherent difficulty of reverse engineering—that is, its indeterminacy. Universality makes the naive idea of using reverse engineering (and naive reductionism in general) to uncover unique underlying principles of operation practically hopeless. Congruent with this logic, it has been repeatedly demonstrated that application of reverse engineering to the study of functional biological 'toy' systems with *known* (but concealed) design principles, may result in a theory that mimics the phenomena, successfully predicts the behavior of the system, yet is *wrong* with respect to the actual, underlying principles. Viewed from the other pole—inferring the macroscopic function of a system from its known microscopic structure and activity is likewise non-trivial.[4] Such demonstrations of indeterminacy offer us, scientists, an exercise in modesty. Experienced biologists committed to reverse engineering sometime respond to the above thoughts, saying: "Do you have an alternative? Otherwise, your claims are destructive!". Well, it is not in our (scientists) mandate to find reasons to do wrong things when the right things to do are unclear. Of course we do not completely negate the use of reverse engineering in biological sciences. Rather, we remind ourselves that reverse engineering is a practical process; if it succeeds in extracting a predictor that works, irrespective of its relation to the actual design principle, the process is considered successful and applicable. However, unlike technology, the business of biology *as a basic science* is not to uncover a plausible mechanism but rather to discover the actual design principles underlying the natural phenomenon; this is where the naive version of reverse engineering in particular, and naive reductionism in general, epistemically fails.

The puzzle of how order emerges given the above universality characteristics constitutes a serious challenge. Of the three different physical routes from disorder to order (thermal equilibrium, physical constraints and dynamic self-organization) the first two can immediately be rejected: living systems are out of thermal equilibrium, and constraining universal systems of the huge dimensions presented by biology entails carefully designed configurations of combinatorial large space. Self-organization is currently the only remaining option, and versions of it are explored in many different contexts, including the experimental systems described here (see, for instance, Mora & Bialek, 2011, and references therein). Regardless of the physical machinery underlying the universal characteristics, the emerging space of configurations provides a substrate for selection upon which functionality evolves. The picture arising is akin to exploration in phase space, dictated by the nature of interaction between the different levels of organization and the dynamic environment. Insistence on isolating pieces and processes from the unity of the whole in general, and decoupling them from the environmental dynamics in particular, might lead us astray. Whichever path biology—as a fundamental discipline—chooses to take, a first step must involve sincere acknowledgment of bio-complexity, acknowledgment reflected both in designing experiments and construction of theoretical frameworks.

In Konstanz we reflected on the above universality, complexity and the praxis of biology; we provided concrete examples taken from several of our own experimental studies, and left the stage for the philosophers that analyzed our conceptions and (more important) our misconceptions. The aura surrounding most (but not all) responses of the philosophers represented adherence to conservative reductionism. This reception of our critical views by the philosophers surprised us, and we contemplated its provenance: Maybe these responses of the philosophers reflected a natural tendency to hold on to simplicity in face of the overwhelming, unfathomable richness of phenomena reported in the biological literature? Or, maybe the philosophers' support of mainstream views stems from the details of complicated experimental procedures, measures, analyses, jargon and (too-often) contrasting 'conclusions'? We did experience the respect payed by the philosophers to scientific knowledge. But we missed daring criticism and the joy of the free-spirited when faced with a call for re-thinking.

Thus we left Konstanz; frustrated, sent back to our benches with instructions to look closer, increase the resolution of our observations, cut the systems to ever smaller pieces, go downwards along the straightest of paths. We know our trade—biology—and for instructions of this kind we need not approach philosophers for advice. We know that there is more in philosophy than cheerleading present-day biological hubris. Hence for us Konstanz was an interesting, thought provoking, but an admittedly distancing experience.

[4] Examples for such 'hands-on' criticism on naive reverse engineering include: Hopfield et al. (1986), Lazebnik (2004), Krishnan, Giuliani, & Tomita (2007), Marom et al. (2009), Kumar, Vlachos, Aertsen, & Boucsein (2013), Vlachos, Zaytsev, Spreizer, Aertsen, & Kumar (2013); for an extensive analysis of similar difficulties encountered in the context of small, well-studied neural networks of behaving animals see Marder, O'Leary, & Shruti (2014).

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