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Editorial

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The science of yesterday, today and tomorrow

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On the 150th anniversary of the original publication of NATURE magazine, Philip Ball, a former editor of and contributor to that prestigious English language publication, wondered about what the scientific contributions of humankind have been since 1869, are today and should be in the future. This is a worthy and timely subject to deal with by all those who care about our planet at large as a unique niche of life in the universe. In addition, given the precarious state of our biosphere, it behooves us to address what role we humans have played, are playing and may play in an uncertain Earth's future while still practicing this unique privilege called science.

The crux of the subject that Ball addressed is summarized on the following quote: "Some of the key questions that confront science today are about whether its methods, practices and ethos, pursued with very little real change since Maxwell's day, are fit for purpose in the light of the challenges — conceptual and practical — we now face. Can science continue to fulfil its social contract and to reach new horizons by advancing on the same footing into the future? Or does something need to shift?"[a]

Of significance in this paragraph is, of course, the "something (that) need to shift". What is that "something"? The vastness of the subject of science prevents any commentator to be exhaustive in rendering a focused and balanced analysis of the advances and the many unknowns waiting to be "discovered" by the sciences. However, this enormous task should not prevent observers and practitioners like us, at the risk of being wrong, from, first, parceling out segments within the sciences that deserve criticisms while, when warranted, offering probable candidates to "the something" that Ball refers to in his elegantly constructed analysis.

Philip Ball addresses what is wrong in his view with the way science is practiced. He claims that all along it should be "acknowledge(d) that there are assumptions embedded, often invisibly, in the way we develop models, deploy metaphors, apportion priorities, recognize and reward achievement, and recruit participants that must be questioned."

He is getting closer to identifying "the something" (at least in the biological sciences) when he states that "... It might be that the genome tells us no more about how an organism builds and sustains itself than a dictionary does about how a story unfolds".

Finally, he offers an alternative to his "glass half-full" assessment of the current state of the practice of science by suggesting that in the future "the something" might be resolved by replacing current approaches to answering basic questions. From our perspective, we identify "the something" with a variety of reductionisms underlying current research in biology. As an alternative, what about giving a chance to organicism as a productive way of answering "...what is life? What is consciousness? What makes individuals who they are? Why does our Universe seem fine-tuned for our existence? "

As wisely suggested by Ball, it will take "creative and diverse thinking" to replace the current "something". We trust that organicism will do it...

[a] Ball P. Science must move with the times. NATURE 575; 29-30, 2019

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Commentaries

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Fake news from the outer space

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Commentary on: Space the new frontier in the battle against cancer, https://www.abc.net.au/news/2019-08-27/space-cancer-frontier-uts-cells/11454430

From the press:

> Space the new frontier in the battle against cancer. An Australian space medicine researcher is preparing to launch cancer cells into space, after trials on earth show that they can be radically affected in near-zero gravity conditions.
>
> We're all very excited about where this research is heading and more importantly, the implications and impacts to potentially provide the community," Dr Joshua Chou told.
>
> The cell will be placed into a device smaller than the size of a tissue box and sent into orbit on the International Space Station. 80-90 per cent of cancer cells die without drug treatment!
>
> The idea was sparked when Dr Chou and one of his students, Anthony Kirollos, found that a micro-gravity simulator in their lab at the University of Technology Sydney (UTS) had a remarkable effect on cancer cells.
>
> "We put in four different types of cancer — ovarian, breast, nose and lung cancer," Dr Chou explained.
>
> And what we found was that in 24 hours in this micro-gravity condition, 80 to 90 per cent of the cancer cells actually die without drug treatment.
>
> This is simply in a micro-gravity environment.
>
> The simulator mimics the space environment by reducing gravity.
>
> Dr Chou thinks the reduced gravity kills the cancer cells because it stops them communicating with each other.
>
> "When we're in space, what happens to the body is that your cells start to feel this condition which we call mechanical unloading," Dr Chou said (ABC net, August 2019).

What amazing news! This info spreads by a number of "respectable" newspapers and popular magazines.

It seems that we are actually approaching a cure for cancer.

Unfortunately, nothing is true.

1. Dr Chou's statement is not substantiated by any scientific publication. Moreover, the various journalistic reports released so far do not provide any mention of specific scientific article authored by Dr Chou dealing with that matter. Instead, press reports vaguely speak about a device made by Dr Chou in order to obtain artificial microgravity. Interestingly, that tool is specifically designed to support a forthcoming study, which should to be carried out on the International Space Station (ISS). Furthermore, Dr Chou claimed having manufactured the "first Cell Biological Microgravity Device". We must remember that such a device has already been patented, it has developed (since the 90s), and it is currently in use in many laboratories (including mine)(Vassy et al., 2001; Masiello et al., 2018; Krüger et al., 2019).
2. Dr Chou is a person Dr Chou is not credited having authored any scientific publication in the field of Space Biomedicine. In fact, he is completely unknown to the international scientific community dealing with Space Biomedicine
3. The numerous studies conducted so far do not allow affirming that such a high percentage of cancer cells die (and in such a short time: 24 hours!) in the pres-

ence of microgravity. This statement is deprived of any sound evidence (Morabito et al., 2019; Grimm et al., 2014; Po et al., 2019).

In conclusion: the news published are a real shameful example of an embarrassing media fraud, as they are based on nothing. The press should be blamed for having lightly published this news, without any prior verification. It would have been better to contact any members of the Academy before disclosing such information. Thereby, spreading the news ends up discrediting both Science and the Press, irrevocably undermining their credibility. Nevertheless, it would be interesting what is the aim behind all of this. Who – and why – could be interested in divulging such fake news? Who feeds unattainable expectations in order to support the new (upcoming) space race?

Altogether, here is where science ends up and politics comes into play

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ABC net, August 2019 - https://www.abc.net.au/news/2019-08-27/space-cancer-frontier-uts-cells/11454430 ; see also: https://www.eurekalert.org/pub_releases/2019-11/uots-crt112619.ph ; https://en.azvision.az/news/116202/microgravity-seems-to-neutralise-majority-of-cancer-cells%C2%A0.html ;

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Commentaries

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The Universal Phenotype

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Commentary on: Martino, A, Giuliani, A, Todde, V, Bizzarri, M, Rizzi, A, 2019, "Metabolic Networks Classification Knowledge Discovery by Information Granulation" *Computers in Biology and Chemistry*, pp. 107187. DOI: 10.1016/j.compbiolchem.2019.107187

1. A Species-Specific Fingerprint

There is a basic (and often overlooked) difference of status between genotype and phenotype. It is now widely accepted the many-to-many relation between the two (Noble, 2011) so that the same genotype can support different phenotypes and the other way around. The complexity of phenotype/genotype relation is at the basis of many speculations and the shift from instructive to permissive character of the genotype is deeply changing our view of both physiology and evolution (Po et al. 2019, Braun E. 2015).

The difference that none (at least to our knowledge) took into consideration, is that while genotype (in its basic meaning of DNA genome sequence) is a universal feature of all the living organisms this universality does not hold for phenotypes. In other words, we cannot make a phenetic all-encompassing classification based on characters as leaves shape (animals have no leaves), brain size (only present in animals) or sensitivity to antibiotics (only pertaining to bacteria and fungi).

On the other hand, the strict state and tissue dependence of apparently low-level (and thus universal) phenotypes like gene expression, proteomic or metabolomics profiles does not allow for among species unbiased comparisons.

In order to have a universal phenotype shared at all the layers of biological organization that in turn remains sufficiently stable to be considered as a "species-specific" fingerprint, we must look at a property shared by all living organisms (with the only exception of viruses whose living organism status is in any case questionable): metabolism.

Clearly, we intend for 'metabolism' the entire set of enzyme-catalysed chemical reactions that 'can in principle' took place in an organism, while those actually taking place are strongly state dependent and thus highly unstable. Metabolism thus corresponds to the entire metabolic network having as nodes the small organic molecules present in the organism with edges between all molecule <A,B> pairs that can be transformed one into another by a single chemical reaction.

The possibility of 'going to phenotype from genotype with a single jump' offered by metabolic networks analysis, complementing phylogenetic and ecological cues, was already explored (Braun E. 2015, Borenstein et al. 2008, Lewis et al. 2012). Along similar ways the possibility to individuate the lethal mutations (Palumbo et al. 2005) by the sole analysis of metabolic network, is another fertile research avenue.

Notwithstanding this interest, all the scholars explored specific biological problems without testing the possibility of considering metabolic network wiring as a 'phenotypic barcode' of biological species exactly in the same terms ribosomal RNA 16S is a 'genotypic barcode' (Sarangi et al. 2019).

Metabolic network wiring is as stable as genotype given it stems from the enzymatic proteins encoded in

the genome of single organisms (and consequently on the kind of chemical reaction those enzymes catalyse). Notwithstanding that, the metabolic network representation does not simply equate the genotype for three main reasons:

1. The presence/absence of the enzyme A is independent of eventual changes in its sequence (many to one genotype-phenotype mapping)
2. The same chemical reaction can be catalysed by different enzymes so allowing for both multiple edges between two metabolites (simplification of phenotypes) and to the same wiring by means of different enzyme species (many to one genotype-phenotype mapping).
3. The same enzyme can be inserted in different pathways in different organisms (one to many genotype-phenotype mapping).

This paper demonstrates the mutual distances between metabolic networks wiring are able to both discriminate different species and to reconstruct the known phylogenetic relations at all levels of biological classification (Martino et al. 2019b).

This was possible by means of a very refined computational approach based on Granular Computing able to conjugate discrimination efficiency and the possibility to get biologically meaningful hints.

2. The Computational Approach

The breakthrough of the Granular Computing paradigm as a component of the vast toolbox of machine learning techniques, allowed the development of advanced pattern recognition systems able to deal with non-conventional data, such as networks (Martino et al. 2018). According to the latter, the vast majority of the information contained in structured domains (e.g. networks, sequences) can be preserved by extracting a set of meaningful "information granules" (e.g., portions of the networks) and then by describing each original network according to the number of occurrences of each information granule within the network itself. As per the paper commented, the puzzling point is: can different organisms can be discriminated according to statistically relevant chemical reactions drawn from their respective metabolic networks?

This *modus operandi* allows to solve a 'global problem' (i.e., discriminating amongst organisms having different cellular architecture, organisms belonging to different species or different kingdoms, and so on) by relying on 'atomic entities' such as individual chemical reactions in a metabolic pathway (i.e., individual edges in a metabolic network). This facet is particularly crucial if the "global problem" is hard to be analysed in its entirety in order to gather further insights, while "atomic entities" are not.

Furthermore, whether this "global problem" can be cast as an optimization problem, one gets the full benefit of the biological interpretability of the learning system, paving the way to so called Explainable Artificial Intelligent systems. In fact, one can drive the data-driven learning machine towards the selection of the smallest subset of edges which, at the same time, hold the vast majority of the information, hence endowing the highest discriminative power.

This summarizes the computational aspect in (Martino et al. 2019b), in which the authors faced four different problems located at different definition scales (discrimination between different cellular architectures – i.e., prokaryotes vs. eukaryotes, discrimination amongst different kingdoms, discrimination amongst animals, and discrimination amongst bacteria). Other than obtaining remarkable discrimination capabilities, which accounts for the reliability of the proposed metric, all four problems returned the most meaningful set of information granules (chemical reactions) which gave rise to biologically meaningful hints, fostering the use of metabolic networks as universal phenotype.

On a larger scale, this work fosters the cooperation between biologists and pattern recognition engineers, unleashing the potential of data-driven techniques towards interpretable models (Martino et al. 2018, Martino et al. 2019a).

3. Conclusion

Besides the generation of theoretically relevant hints (e.g. which specific chemical reactions happen only in eukaryotes) the practical application of the results reported in (Martino et al. 2019b) are particularly evident in ecological settings.

Each ecological space is defined by the role played by different actors (e.g., predators, preys, primary producers), the existence of a healthy environment depending upon a balanced mixture of different ecological niches occupation. In the case of microbial communities, especially in the case of internal ecologies of mucosa microbiota (Gilbert et al. 2018), the comparison between healthy and 'disease' microbial profiles is computed in terms of genotype barcode that, by definition, does not convey any biological information other than species identification.

Shifting to 'phenotype barcoding' could be much more informative because allows us to discriminate between function preserving (the same metabolic functions are carried out by different species) and function altering (a given metabolic activity is no more present) changes. This could yield a major achievement in terms of both pathology (human microbiota) and environmental sciences (microbial ecology of soil and water).

The Granular Computing approach used for solving such a very hard computational problem (needing millions of atomic comparisons as applied to metabolic networks, each having hundreds of nodes and consequently thousands of edges) allowed a dimensionality collapse and the subsequent enucleation of "discriminant edges". This is, at least in our opinion, an example of a sensible approach to Big Data that saves both the prediction efficiency and the biological interpretation, paving the way to a productive collaboration between different disciplines. Machine learning (and in particular Granular Computing Inductive Modelling) is not only a useful information processing toolset for "in silico" experiments, but represents a true paradigm revolution in science, towards an efficient and effective way to identify meaningful regularities in Big Data, for knowledge discovery and nature understanding.

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Commentaries

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Les liaisons dangereuses: genome-edited cattle, antibiotic resistance and cancer

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Commentary on: Carlson DF, Lancto CA, Zang B, Kim ES, et al, 2016, "Production of hornless dairy cattle from genome-edited cell lines", *Nat Biotechnol*; 34, 479-481.

1. The generation of life

In his epistolary novel (Choderlos de Laclos , 1782) Pierre Choderlos de Laclos depicts the alternating fortunes of the decadent French aristocracy before the Revolution of the late 18th century. Something similar happened during the history of science, marked by periods of decline followed by periods of great success in human creativity. For example, until the beginning of the nineteenth century, life was thought to emerge spontaneously from inanimate matter. In other words, it was enough to leave a dirty garment and a few ears of corn in a stable to generate a crowd of worms, insects and rodents in a few days. It took the empirical insight of scientists like Francesco Redi and Lazzaro Spallanzani to reveal the deception of the spontaneous generation of life. Today we actually know that life originates exclusively from life and that the rules that underlie the biological continuity of living beings have been written by evolution, not by modern engineers.

2. Primum non nocere

The selective breeding of plants and animals began a few millennia ago to satisfy basic human needs, and we still have a debt of gratitude to nature that allowed us to domesticate cereals, vegetables, goats and cattle for food production and others primary goods. Nowadays, the so-called genetic improvement of plants and animals obtained through technological manipulations is not designed to satisfy human needs, but to produce varieties with traits suitable for the commodities market while developing new agro-industrial patents and new commercial products. In the last half century, there have been many clear confirmations of this trend. A multitude of researchers have trafficked with organisms or parts of them (genomes, cells, tissues, and so on) based on both the illusion of being able to successfully force the deep nature of biological systems and the presumption of not making mistakes. However, lacking a "true" good reason (basic needs?), common sense suggests that invasive manipulations of the natural world should be carefully avoided, particularly when the reliability of the results and assessment of possible risks have not been clarified. The injunction "Primum non nocere" (First do no harm), which is the founding principle of Hippocratic oath and of medical practice, means to always seek solutions that cause the least possible damage, if any, in planning our actions.

3. Biotechnological failures

One of the most popular attempts to force biology (sexual reproduction) of domestic mammals was made in 1996 (the famous Dolly case), when a sheep was cloned at the Rosling Institute (Scotland) to produce "photocopied" sheep by using a controversial technique known as SCNT (Somatic Cell Nuclear Transfer). (Please note: Dolly was cloned from a cell taken from the mammary gland of a six-year-old Finn Dorset sheep and an egg cell taken from a Scottish Blackface sheep). The results of the experiments, however, proved to be incompatible with the optimistic predictions of biotechnologists. The use SCNT technique to artificially "reproduce" mammals with identical phenotypic traits failed, showing a tremendously low efficiency: indeed, most of the embryos died before they were born, while those who arrived at birth died shortly thereafter.

Recently, the story has repeated itself by adding a new entry to the list of human failures to force the nature of complex biological systems such as mammals. Last August, the FDA (Food and Drug Administration) documented an interesting case of animal genetic manipulation that showed serious problems. The Agency found that the genetic material of animals belonging to a dairy breed modified to inhibit the growth of horns contained bacterial genes for antibiotic resistance (neomycin/kanamycin and ampicillin). The genome of these animals had been previously altered through gene-editing, a molecular protocol based on enzyme systems (nucleases) able to cut the genetic material in a precise way and increase the control over molecular changes. Furthermore, in the genome of genetically modified animals, additional genetic sequences of bacterial origin were detected, along with a duplication of manipulated DNA sequences to obtain the hornless (polled) phenotype. The work of the FDA researchers aimed to detect whole-genome sequencing data from calves that were germline genome-edited, while the screening method was able to detect unintended off-target events. The foreign DNA sequences discovered by the FDA came from the bacterial plasmid used in 2016 by the biotechnologists of Recombinetics Inc. (a Company based in Minnesota) to introduce the "polled" gene in the dairy cattle genome. It is worth noting that, being a germline molecular manipulation, every cell of the GM cattle contained antibiotic resistance genes, facilitating the transfer of antibiotic resistance to non-resistant bacteria.

4. Animal machines and biological systems

Currently there is only one GM animal species authorized (in the US) for human consumption (the AquAdvantage Salmon). Biotechnological manipulations to produce food of animal origin for human consumption should be strictly regulated to counteract products not sufficiently tested for their safety. The case in point must be taken very seriously. The presence of antibiotic resistance genes had never been detected in engineered farm animals, particularly in dairy breeds, which raises serious and legitimate concerns over the alleged safety of the so-called NBTs (New Breeding Techniques). Worldwide there is a strong pressure on public health Agencies to strengthen efforts and tools needed to prevent and reduce the spread of genes that confer resistance to antimicrobial drugs[a]. It is widely recognized that in the United States, traditionally, there is no substantial objection to the use of molecular techniques to modify plants and animals. Yet a very critical problem – leaving aside for a moment the ethical implications on the use of animals as "machines" – is that the genome-editing technique applied to dairy cattle can interfere with human food chain, leading to a number of potential risks that should not be underestimated. Indeed, the acquisition of antibiotic resistance from dairy products cannot be excluded. The genome-editing procedure has been promoted by biotechnology industry as absolutely safe, since its molecular precision would exclude the occurrence of undesired alterations. However, current biological knowledge shows that molecular manipulations of multicellular organisms fail to evade the uncertainty due to the non-linear dynamics that regulate morphogenetic and physiological processes. Furthermore, each screening approach is based on hypotheses and possible biases that could lead to not detecting many unintended alterations. Examples of unpredictable molecular events are well documented, such as the complex genomic rearrangements observed at or near the target site in many experiments involving the manipulation of mammalian genomes. It is worth noting that, in 2016, the results produced by Recombinetics researchers were published in the prestigious *Nature*

[a] https://www.who.int/antimicrobial-resistance/en/; https://www.cdc.gov/drugresistance/about.html; https://www.ecdc.europa.eu/en/antimicrobial-resistance/facts/factsheets/general-public

Biotechnology (Carlson et al., 2016). In their report, the Authors stated that the genome control for possible off-target events had given negative results: a statement that in the light of the current evidence sounds like a mockery. It should be emphasized that, among the numerous and critical health problems linked to the widespread resistance to antibiotics, bacterial infections affect anyone, particularly the elderly, young and sick, that is to say the most vulnerable individuals. The emergence of antibiotic-resistant bacteria is a real threat to these people, as antibiotics are the main line of defense when the immune system weakens. Antibiotic resistance threatens the effective prevention and treatment of an ever-increasing range of infections caused not only by bacteria but also by parasites and viruses. In 2016, around 500,000 people worldwide have developed multi-drug resistance to tuberculosis, and drug resistance is now starting to complicate the fight against HIV and malaria. The WHO believes that this is an urgent dilemma, as both resistant and multi-resistant infections are increasingly frequent and difficult to treat, as well as very costly to sustain. The health care for patients with resistant infections is much more expensive than assistance for patients with non-resistant infections, due to the longer duration of the disease, additional tests and the use of more expensive drugs. The problem foreshadows serious implications for global public health and requires action in all sectors of society and government[b]. At the state of the art, antimicrobial compounds based on new mechanisms of action are still few and, among these, most of them have not yet completed the pharmacological testing process. A further critical problem depends on findings emerging from studies on large populations of pathogenic bacteria that show a positive correlation between the ability to develop biological resistance to a drug and the ability to develop biological resistance to more drugs. This phenomenon generates simultaneously an enigma both for biomedical research and clinical treatment. In general, it is believed that the biological mechanisms that determinate the resistance to antibiotics are different in different bacterial strains. According to a hypothesis currently being tested, different strains of pathogens can reciprocally exchange molecular "tools" to develop resistance to different antimicrobial molecules, thus accumulating a shared multiple resistance (Rayamajhi et al., 2010).

[b] https://www.who.int/en/news-room/fact-sheets/detail/antimicrobial-resistance

5. Antibiotic resistance and cancer

The supporters of the absolute power of science, who in the past proclaimed that progress would have definitively made man free from infectious diseases, had naively underestimated the impressive evolutionary properties of bacteria. Thanks to their peculiarities, these microscopic creatures have done the job that perhaps no one can do better than they have, bypassing the toxicity of a large amount of antimicrobial compounds and reducing almost completely the effectiveness of their use in medical treatments. This global problem will have a great impact in particular on oncological medicine, involving over 30 million people worldwide: a huge group of patients that is destined to continue to grow. Bacterial infections are responsible for common complications among cancer patients, who often become much more sensitive for several reasons. Any type of cancer is a major cause of body stress and, as such, has the effect of lowering biological defenses. Additionally, it should be noted that white blood cell tumors, such as leukemia and lymphoma, have a great impact on cancer patients resilience by directly influencing their immune system (Pfeil et al, 2015; Leibovici et al, 2006;). After surgery, many patients require antibiotics to treat infected wounds. Moreover, conventional anti-cancer therapies performed to kill cancer cells kill also cells of our immune system. This means that patients who receive radiation or chemotherapy often develop infections that require treatment with antibiotics. Transplantations and other treatments are also impossible to perform without using effective antibiotics. Antibiotic-resistant bacteria are currently expected to make cancer treatments increasingly difficult, while the incidence of cancer cases will continue to increase in the years to come. This can result in higher mortality from cancer, more difficult and more expensive treatments and many side effects. Antibiotic resistance will have important consequences in the hospital environment, due to patient management and interactions with healthcare professionals. For example, it will be necessary to increase isolated hospitalization spaces to limit the circulation of drug-resistant infections (Teillant et al., 2015).

6. Different intelligences

The scenario reported above is not what we would have expected after the advances in well-being, biomedicine and science of the last century. Bacteria have lived on our planet for about 3.5 billion years and are the

most common life form in the biosphere. Their ancient "knowledge" of the rules necessary to survive environmental adversities seems to be much more sophisticated than the human technologies presented today as the most advanced frontier of the so-called "nature control". Although it may seem an inappropriate reflection, bacteria have developed an unquestionable intelligence, which explains why, after more than three billion years, they still have unparalleled biological success on Earth. Their extraordinary ability to renew themselves, despite the numerous survival problems they face every minute, currently puts human life to the test. Perhaps, aware of this, they are sending warning messages to our strange intelligence that, instead, is now driven almost exclusively by business and has lost the ability to place itself at the service of mankind.

Conflict of interest

The Author declares no competing interests.

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Why Basic concepts in Biology should be reframed
Is Etymology a useful tool for investigation in biology?

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Abstract

How do the words drive scientific investigation and the way we construct models? Here we want to sketch briefly how some biological concepts have changed over time, but that did not happen to the words we use to recognize them. Are we aware of the meaning of concepts we actually use as conceptual "tools" that shape our thoughts and experimental models? We want to investigate how this shift can affect the way science works, and how should etymology impact on the theoretical biology.

Keywords: etymology; biological process, biological system, organic, organic synthesis

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Introduction

One day an autistic friend, able to communicate by typing on a laptop, wrote "for me it is very difficult to put my thoughts into words. My thoughts only go into words by forcing them. My thoughts do not coincide with the meaning of words" (De Rosa, 2016).

Is there a similar problem with science? How nature's essence and laws fit inside concepts and words of science?

How do the words that we often use as crutches for our thoughts drive scientific investigation and the way we construct models?

Here we want to sketch briefly how some biological concepts have changed over time, but that did not happen to the words we use to recognize them. Are we aware of the meaning of concepts we actually use as conceptual "tools" that shape our thoughts and experimental models? We want to investigate how this shift can affect the way science works, and how should etymology impact on the theoretical biology.

Exploring basic concept in biology

The first problem we were facing was how to identify and classify basic concepts. Hence, we decided to begin the analysis from the most linear division: organic world/compounds and inorganic world/compounds.

Such partition is so deeply rooted in science that all curricula of scientific faculties contain, in the first year, an exam about organic and inorganic chemistry. Every student is told that all molecules containing carbon atoms (except for carbides, carbonates and simple oxides of carbon), are organic molecules, so that the expression *"is an organic molecule"* means that these molecules are the basic bricks of living organisms.

If we take a deeper look at the history of the words organic and inorganic, we realize that the concept underneath them has changed over the centuries. Before the XIX century, the main "paradigm" was Vitalism. Scientists believed that all the "organic matter" emerged because of special "vital forces" that characterized the living systems. For them "organic matter" meant all the compounds produced by living organisms. Their belief was that this kind of substances cannot be found in non-living organisms and that the organic compounds

were characterized by some special "vital energy" (Greenwood, 1997).

Things changed in 1828, when Friedrich Wohler produced urea, an organic compound, starting from two inorganic substances (potassium cyanate e ammonium chloride) (Wöhler, 1828). That discovery is currently recognized as the starting point of modern organic chemistry. For the first time it was demonstrated that organic compounds can be produced without requiring "vital forces", and just from inorganic compounds (Ronald, 2015).

Later on, in 1845 Adolph Wilhelm Hermann Kolbe backed this new theory of organic compounds by synthesizing the acetic acid from carbon disulfide.

Therefore, the concept of *organic* changed but the word *organic* remained the same. If the previous idea was *"something produced by life through its vital forces"* the new definition abrogated the idea of vital forces, to endorse that of *"component of living systems"* (Ramberg 2000).

Furthermore, in 1858 Friedrich August Kekulé and Archibald Scott Couper independently developed the concept of chemical structure. The main idea was that tetravalent carbon atoms could link to other atoms and/or each other in a way that all the organic matter can be structured.

Starting from that moment the concept of *organic* was definitely reframed as *"carbon-based molecules that structure all biological entities"* (Streitwieser, 2017).

Notwithstanding this definition, carbon-based molecules are not all "organic", as epitomized by some metalorganic substances or by a few different carbon structures, such as diamond and graphene, not to mention the wide array of petroleum-derived molecules.

The etymology of word "organic"

From an etymological point of view *organic* is an adjective that means *serving as a means or instrument, from or characteristic of organised living beings, forming a whole with a systematic arrangement or coordination of parts*. Intriguingly, "organic" is a word strictly bound to "organ" and "organisms" lemmas (accidentally the name of this journal).

The word *organ* comes from the ancient Greek ὄργανον, "organon", primarily means *tool, that which performs some function*. This word is connected to the root ἔργον, "ergo", which means work, action. The word *energy*, which etymologically speaking means *effective, active*, derives also from the same root.

Therefore, if we forget scientific discoveries and definitions, by keeping our attention focused on the etymology of words we can deduce the following concept: organism and energy share the same root, which means "action". In some way, we can say that, etymologically speaking, *we cannot separate the concept of structure and the concept of energy from inside the word organic/organism*. From this point of view structuralism, by abolishing the idea of vital energy and giving the whole attention to the structure has eliminated the concept of energy from the word organic.

Such point is resurfacing nowadays. Nicholson (Nicholson, 2019) presents an example where he points out the inconsistency of the sole machine-model in describing living systems. Instead, he highlights the importance of the concept of *process* at the very basis of living organisms. If we look at the word *process* from an etymological point of view, we see that it comes from the ancient latin *processus*, *procedere* which means *advancement*, *progress*, *series of organized acts*. This etymology merges the concept of action/energy with the one of organization by introducing the concept of time.

The etymological contribution of the word "synthesis" to biological models

Another biology concept we want to discuss briefly here is that of *synthesis*. Such term was introduced in biological sciences just thanks to Adolph Wilhelm Hermann Kolbe who used the word *"synthesis"* for his work about the production of acetic acid from two inorganic molecules (Kolbe, 1845).

Currently this word is commonly used for describing something "not natural" like in synthetic life, synthetic drug, synthetic fiber, etc. With use this word is slowly shifting toward a meaning close to artificial.

Yet, also nature works with synthesis, which is the case of photosynthesis, synthesis of proteins, DNA synthesis, etc. Therefore we can generally affirm that such word has been pledged to identify the process of production of a new molecule or biological entity through the re-organization of other more basic substances.

Can we use etymology to discover other meaning of that word, which we can usefully apply to biology?

Synthesis comes from the ancient Greek σύνθεσις, which means *composition*, while the original root comes from συντίθημι, which means *to put together*. The general meaning is "*composition of elements with*

the goal to form a whole", a combination of parts into a whole. That is a definition very near to the one of *organisms*. From a certain point of view, we can say that an organism is the result of a synthesis.

Moreover we have to consider that the words synthesis and system share the same root *syn-,* from the ancient Greek σὺν, which comes from the Indo-European *sem-* and means "with", "together". If we look at the etymology dictionary, we can find that they both share similar definitions. too: synthesis, as *"a combination of parts into a whole"*, system: *"organized whole, a whole compounded of parts"*. The difference lies in the desinence (ending): *hystanai* that means to *standby* and *thesis* that means *to put, to place*.

We can then say that the synthesis is the process, which puts together different parts into a system. Once a system finds a stability and acquires a property it can also be named organ because it is characterized by a special function.

It is also true that to synthesize is a function of a specific system, which opens the door to a circular or spiral mechanism that drive us toward a progressive magnification of the biological structures and their functions. Given that the development of structures requires that the system could span from lower (molecules) to higher (cells, tissues) levels, the "synthetic" process entails many different scales. Organism development is indeed a "scaling process".

Following this reasoning, we can say that synthetic processes are the way through which nature moves toward upper/bigger scales (Bizzarri, 2019).

Is it by chance that in English the verb deriving from the word synthesis is not "to synthetize" but "to synthesize" giving a crucial role to the word *size*? This advancement in size obviously happens along the time with dimension so, if we want to optimise the use of the words here analysed we can say that synthesis is the process that spatially and temporally organises the matter in a system. Unavoidably this system moves forward both in time and space, by raising size and by acquiring a history.

The simplification process and conclusions

We have then to introduce another word that having the same root as synthesis and system "*syn-*"(the same of): it is the case of *simple*. From the ancient latin word simple it is composed by *sim-/sin-* which stands for *sine*, which means *without*, and *-plico* which means *to fold*. From a first Latin etymology we can then extract the meaning of *without folds*, and *unique piece*. If we take a deeper look at the etymology of *sine*, we discover that the root *si-* comes from the Indo-European *sem-* , from which there derive both *si-*, and *syn-* which, as seen previously, means *together*. We can then say that to simplify means *to fold together (without leaving the signs of folds),* something folded in a way that appears just one thing. With a daring logical leap, we can remind the protein folding process and what it generates. The development of living structure implies that the space should be properly "structured", "organized", through subsequent and repeated "folding" organised in time. This aspect is indeed at the core of the DNA and chromatin structure.

Therefore, we can say that the words synthesis, system and simple shares the same root and, in some way, they are conceptually connected: all these terms are connected by the *goal to form a whole compounded of parts* that we can name organisms.

This is an embryonic method of conceptual investigation and should be better and deeper investigated. Can we draw new models or reinterpret certain data by using etymology analysis? Can this method be useful in the interdisciplinary transfer of knowledge?

These are some open questions that, from our point of view, should be investigated more deeply. Here we just wanted to show the potential of this etymologically based method in doing biological investigation.

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The Blind Spot of Neuroscience

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Abstract

Neuroscientists and biologists play a trick on themselves: they turn their subject of study into an object, while pretending they are not there. Such self-induced amnesia provides a convenient approach to the study of life and mind that, paradoxically, defeats its purpose. Here I argue that the notion of pure objectivity is a pervasive and pernicious form of naïve anthropomorphism. In treating subjects as objects (including ourselves), we pretend to erect "a view from nowhere". I discuss how perception, through the lens of magic and artificial intelligence, reveals its subjective nature. We are an inextricable part of the phenomena we study. Lived experience is the very condition of scientific intelligibility.

Keywords: subjectivity, neuroscience, magic, artificial intelligence, experience

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Neuroscientists have a conflict of interests that remains undeclared most of the time: a subject (the scientist) studies another subject (a human, a fly, a mouse, a worm) but pretends that the subject of study is an object (for instance, a brain), and that the scientist is not present. Note the trick: we start with two subjects and, without noticing, as in a sleight of hand, we end with one object (Figure 1).

Such conflict of interests involves a double challenge: (i) the problem of the observer, namely, the subject that carries out the experiment, and (ii) the problem of the observed, the subject upon which the experiment is carried out.

The problem of the observer brings us to the problem of objectivity. The question is whether it is possible to say something about reality as something "out there" independently of how or who observes it. Do we see the world as it is or, according to the ancient saying, we see the world as we are?

Physicists bumped into this problem exactly one century ago. They discovered that pure objectivity is a myth. The observer cannot be left out of the equation (Bitbol, 2019). The other sciences have resisted to this conceptual revolution without virtually modifying a speck of their approach (which in turn continues to be anchored in the premises of 17th natural philosophy and 19th century physics). And yet, the role of the observer cannot be ignored indefinitely. To wear a white coat does not make us disappear from the scene. Objectivity is built across subjects.

This brings us to the second problem: the problem of the observed. It concerns (at least) the sciences of life and of mind (Thompson, 2010), which include biology, psychology and neuroscience, amongst others. Here we encounter the following paradox: most biologists study life as if it were dead (Figure 2). As a corollary, it seems, neuroscientists study the mind as if it were a mere anecdotal product of cerebral matter. But I wonder: Is my genome in a USB stick actually a copy of me? Am I really my connectome? Drowning should not be considered a swimming style (Barfield, 1988). Nor will we understand living organisms by isolating them and then reducing them to pieces. The current approach to life and mind suffers from "the Frankenstein error" (Gomez-Marin and Ghazanfar, 2019)

This habit (which is a vice) of thinking the superior in terms of the inferior is a methodological and conceptual bias we have inherited from the rhetoric of 20th

century molecular biology (Kay, 2000). That which affirms that life is nothing but biochemistry and, thus, that mind is nothing but electrochemistry. And yet, every time someone claims that "A is nothing more than B", one must remain sceptical (Noble, 2006). Love cannot be reduced to a brain scanner. You won't find the humidity of water amongst its molecules. Science is part of life, not the other way around.

Here we can invoke a marvellous idea: the *Umwelt* (Von Uexküll, 1992), which in German means world. This is not "any" world, or simply a generic world, but the world as experienced by each and every organism. Rather than the objective surroundings (for which the German have the word *Umgebung*), the *Umwelt* is the meaningful environment for the subject. Let us put an example: a stone is a stone, but a stone for a beetle has little to do with a stone for a human. For the beetle, the stone is a shelter. For the human, it may be an opportunity to hunt. To each their *Umwelt*!

There are as many worlds as living organisms, seach of them with its own particular way to look at (and act upon) the world. This observation leads us to the following discovery: all organisms share the world but not all organisms have the same world in common. Thus, when we study the behavior of a mouse in our laboratory by putting it in a small square box for a few minutes (Walsh and Cummins, 1976), it is very likely that our efforts to be objective end up being a misplaced projection.

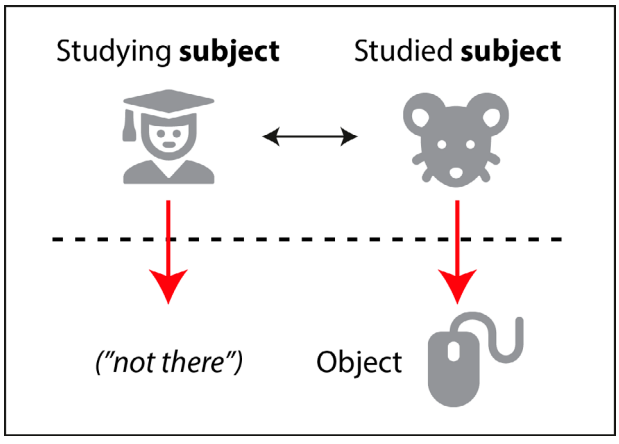

Fig. 1. A blind pursuit of objectivity removes the scientist form the scene and transforms the subject under study into an object.

We project our world, mostly in terms of abstractions or conveniences (i.e. square boxes are easier to build, pack and transport, and thus cheaper and more readily available), upon the mouse's world (which is never an *Umgebung*). Wouldn't we then fall into a subtle and sophisticated kind of anthropomorphism? There is a clash of *Umwelts* in our laboratories (Gomez-Marin, 2019).

By blindly embracing an objective frame (note the double oxymoron here), by forcing the subjective into the objective, scientists shoot themselves in the foot. It seems that, in order to tolerate the real, one must attenuate it. By means of the paradoxical structure of the double (Rosset, 1976), we make a copy and take it for the original. We thus arrive at the blind spot of neuroscience. As Morpheus put it in The Matrix, destiny is not without a sense of irony... since it seems that what science (and also often philosophy) cannot perceive is perception itself. The blind spot is thus not just an obscuration of a part of the field of perception (which we actually fill in). The spot to which we are blind is precisely what makes seeing possible. The blind spot is the neglect of lived experience (Frank et al, 2019).

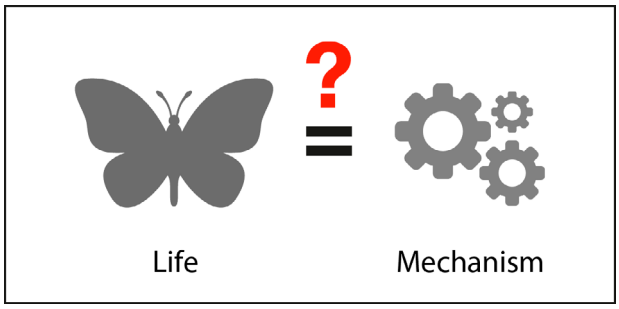

Fig. 2. Is a butterfly an undead mechanism?

To tackle this problem —and far from offering a solution—, let me devote the second part of this essay to share our efforts in this respect. In particular, I would like to discuss a proposal that uses and merges two rather unexpected elements: magic and artificial intelligence; let us call it "mAgIc" (Zaghi-Lara et al, 2019). Both are powerful mirrors to study perception and, more broadly, human cognition. At the same time, "mAgIc" represents a hybrid between the old and the new; since magic is a millenary art and AI a powerful technique undergoing a recent revival (in my opinion, both hyped and deserved). Let us start with magic.

Magic is the art to produce in the spectator the experience of the impossible. Note that one thing is to ignore how something takes place (this is actually a very common experience for the scientist in the presence of the workings of nature), while another is to be sure that what has happened cannot be (such is the experience of the spectator in the presence of the magician's workings).

It is important to remark that, for the spectator that experiences it, an illusion is arguably the "really real" (regardless of whether one wishes to call it true or false); that which one experiences concretely (Bergson, 2019), in the immediacy of one's first person lived perspective. Let us ponder the following example: the letters in Figure 3 have exactly the same intensity of grey, but only to the observer that looks at them without the grey gradient in the background (McCourt, 1982). Context is thus constitutive of content. The background is technically entangled to the foreground.

"Who are you gonna believe, me or your own eyes?" Groucho Marx's famous quote can be traced back to Goethe, who affirmed (and he did so very seriously) the reality of the optical illusion. Illusions reveal the ubiquitous presence of the mind in vision (Zajonc, 1995). Furthermore, there is no magic without spectator. Magicians are the only artists that cannot really perform their art to themselves. The experiencing subject is the kernel of the phenomenon of magic and of perception writ large.

In collaboration with a professional magician, we designed a series of simple tricks, this is, simplified but effective motor maneuvers, based on magic with coins (to make them appear, disappear, translocate, multiply, etc). First, we measured with great precision the movement of the magician during a sleight of hand (fingers, wrists, elbows, etc) in order to understand why and how his dexterity actually fools us. To that end, we used a computer vision algorithm based on artificial intelligence (Mathis et al, 2018).

To make a long story short, this is how it works: given an image, the human teaches the machine a point of interest in the image (for instance, the precise position of the nail of the right index finger of the magician, or where the coin is). This procedure is called supervised machine learning. By automatically extracting properties from the pixels in the image, and after only a few examples (this is the crucial part), the machine is, in principle, able to mark in any future image where the index finger will be. From "here, dear algorithm, is what a finger is" the machine learns and replies: "this, dear human, is where the finger is" (this actually works: https://youtu.be/KPizTPQz0tc).

But, what if rather than teaching the machine to "see" fingers or coins when they are visible, we would teach it to tell us where it "thinks" they are when not visible? Daring to track the invisible, one upgrades the machine from a mere tool to an "artificial spectator". The previous link also demonstrates that the machine can guess, as a human would, where the coin is. It is the human who infuses the machine with a kind of perception.

Next we presented the magic maneuvers to the computer. Where is the coin? A few short tricks are shown in the video, first as raw clips, and then with a red spot marking what the machine "saw". Do not blink. The hand is faster than the eye. In magic everything usually happens fast-enough so as not to leave much time to the spectator's analytic mind to discover what took place nor how.

Fig. 3. Visual illusions remind us that context is constitutive of perception, and that perception always entails perspective.

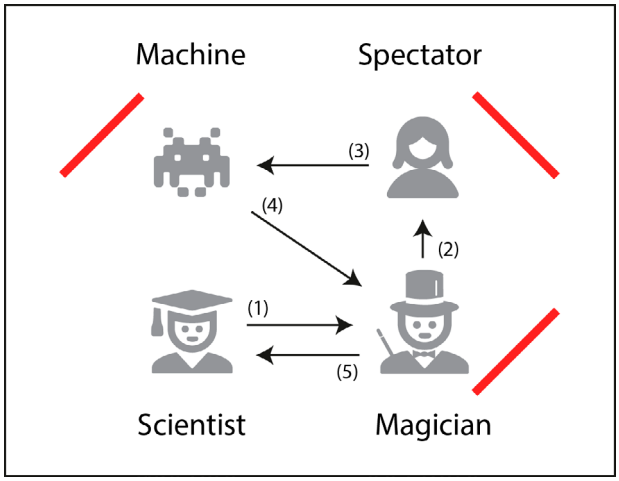

Fig. 4. Using magic and artificial intelligence as mirrors that reveal our blind spot in the act of perception.

Although magic is not meant to be served in a can, cold, suddenly, and devoid of preliminaries in a ten second video, I wonder if the magician nevertheless fooled you? Now you can watch the videos where the machine indicates where it thinks the coin should be. Do you think that the magician fooled the machine too?

We arrive at subtle realization: the point here is not about the magician telling us the trick so that we, neuroscientists, study it and then tell the magician why and

how it works. No. It is more interesting to go in the other direction: to use what magicians know in order to study something that is present in our daily life, beyond the lab and the theatre, and that is both fascinating and scary: our mind fools us virtually all the time (Stephen, 1922).

But one may still ask: what about the machine? Does it learn or not? Did we really deceive it? Here we need to be clear and cautious. Machines do not "see"; they "detect". They do not "attend" either, since they have no freedom to look at one side of the image but not at the other. Despite our colloquial use of terms, properly speaking, machines do not "think"; they "calculate" (Rosen, 2000).

Accordingly, the trick that we make to the machine is in reality a trick that we make to ourselves through what we have been able to hand over to it about our own *Umwelt*. Our approach to perception by means of "mAg-Ic" is thus a fun and fascinating mirror game (Figure 4): we ask the magician to make a trick to the spectator who then trains the machine so that, again in front of the magician, reflects and amplifies some of our own cognitive processes (Zaghi-Lara et al, 2019). A game of deforming mirrors in the line with the Greek aphorism "Know Thyself".

Magic reminds us what we know but easily forget (specially when, like Dr. Frankenstein, we are so focused in our laboratories): that mind, like life, is contingent, sloppy, inexact; it improvises, errs, learns, invents, and improvises; it deforms reality as it deforms itself. The laws of mechanics are not broken in a broken clock (Canguilhem, 1991). God is not a mathematician (Jonas, 2001). The pieces of the puzzle seldom fit, and yet life goes on.

The study of the human mind by the human mind is absolutely fascinating (Spira, 2017). We dive into a multitude of universes that, on occasions, intersect. Thus, as 21st century neuroscientists (and, for that matter, biologists, and I may even data to say physicists), we are faced with the following challenge: to cultivate a scientific mind that does not preclude its own participation (Skolimowski, 1994).

Quo vadis biology? I believe that the foundations of a new science of life and mind should be *explicitly* grounded in the felt presence of immediate experience. How will such science look like? How shall one practice it? It is early to tell. A first step, however, would be not only to acknowledge that situation matters, but also to cherish its primacy (Bitbol, 2002). Knowing is given in our experience. Human experience is actually the very condition of possibility of scientific knowledge. In a sense, science is nested in the humanities.

As scientists, when we talk about what we know (and even about what we know we do not know), we may lose sight of what we cannot see (or perhaps do not want to see). We do not see that we see. Even more, we do not see that we do not see that our seeing is never a viewless view. This is our blind spot.

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Metabolic control of muscle stem cells

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Abstract

Muscle stem cells, or satellite cells, are a population of adult stem cells involved in muscle growth and indispensable for adult skeletal muscle regeneration. As the quiescent state is perturbed, satellite cells undergo profound metabolic changes, named metabolic reprogramming, driving cellular activation, commitment and differentiation. Thus, modulation of cellular metabolism, by altered nutrient availability or with aging, can impact satellite cell stemness and fate, as well as differentiation ability. Moreover, a direct link between cellular metabolism and chromatin dynamics is emerging. Indeed, metabolic intermediates act as cofactors for epigenetic modulators, thereby regulating their activity and influencing the epigenetic landscape. Consequently, environmental cues are critical regulators of satellite cell fate, linking nutrient availability with the epigenome to impact muscle homeostasis and regeneration. Further studies are necessary to dissect the intimate connection between environmental cues, metabolic reprogramming and epigenetics, to increase satellite cell regenerative capacity in aging or diseases.

Keywords: satellite cells, metabolic reprogramming, chromatin dynamic, epigenetic modulators

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Adult stem cells (ASCs), or somatic stem cells, are primarily responsible for maintaining homeostasis in many tissues throughout post-natal life. ASCs are usually maintained in a quiescent state in their specific niche (Bardelli and Moccetti 2017; Ferraro et al. 2010; Rezza et al. 2014). The ASC niche is defined as the in vivo microenvironment, characterized by several cellular and structural components: (i) the ASCs and their progeny which provide autocrine and paracrine regulation; (ii) neighboring mesenchymal or stromal cells providing paracrine signals; (iii) extracellular matrix (ECM) or cell–cell contacts involving membrane-bound molecules; and (iv) external signals from distant sources, such as blood vessels, neurons, or immune cells (Rezza et al. 2014). Overall, the ASCs reside and receive different signals in and from the niche that determine their fate in terms of quiescence or activation (Rezza et al. 2014). When activated, ASC proliferate and differentiate to replenish damaged tissues (Ferraro et al. 2010; Jones and Wagers 2008). ASC exhaustion is prevented by their dual capacity of self-renewal and differentiation. Indeed, ASC symmetric division produces either two identical replicating cells or two committed cells, depending on surrounding signals, while the asymmetric division results in one identical and one committed stem cell (Morrison and Kimble 2006; Shahriyari and Komarova 2013). The balance between self-renewal and cell differentiation preserves resident stem cell populations as well as tissue homeostasis (Renzini et al. 2018).

Several signaling events influence specification and maintenance of stem cell lineages in tissues. For instance, the cooperation between the Wnt, beta-catenin, and BMP/Notch signaling is essential to control stem cell self-renewal in the intestinal stem cell niche (Clarke 2006). Besides, Wnt3a has been implicated in self-renewal and proliferation in the hematopoietic (HSCs) and neuronal stem (NSCs) cells (Wexler et al. 2009). Another important pathway influencing the maintenance and differentiation of ASC is the TGF-beta signaling, including bone morphogenetic proteins (BMPs), Nodal, and activins (Watabe and Miyazono 2009). TGF-β1 modulates the proliferation of mesenchymal stem cells (MSC) by inducing Smad3-dependent nuclear accumulation of β-catenin in MSC, which is required for the sti-

mulation of MSC proliferation (Jian et al. 2006; Watabe and Miyazono 2009). Further, TGF-β and activin promote chondroblast differentiation at early stages, while TGF-β inhibits osteoblast maturation at late stages during MSC differentiation (Maeda et al. 2004; Roelen and Dijke 2003). Finally, the Notch pathway is known to support the maintenance of tissue homeostasis during adult life. Indeed, cell-cell interactions activate notch signaling, thereby generating cell diversity from initially equivalent cell populations (Lowry and Richter 2007). For instance, specific Notch activity levels dictate progressive restrictions during adult hematopoiesis and in the adult brain (Bertrand et al. 2002; Demehri et al. 2008; Shimojo et al. 2011).

2. Cellular metabolism influences ASC

Energy metabolism is emerging as a key regulator in maintaining stemness and in determining cell identity (A. Harvey et al. 2019). Besides providing energy, metabolism and derived metabolites influence stem cell life-cycle, in addition to allowing cell adaptation to the systemic environment (Rossi et al. 2008; Shyh-Chang, Daley, et al. 2013).

Different metabolic pathways, including glycolysis, the pentose phosphate pathway, the tricarboxylic acid (TCA) cycle and oxidative phosphorylation (OXPHOS), allow addressing cell energy requirement in specific cell state (Folmes et al. 2012). Nutrient resources actively modulate ASC survival, proliferation, commitment and differentiation (Oburoglu et al. 2014; Renzini et al. 2018; Scicchitano et al. 2016) and increasing evidence suggests that metabolic remodeling bring forward the cell fate establishment, from maintenance and acquisition of stemness to lineage commitment and specification.

The increased energy demand during ASC activation and differentiation requires higher ATP and ROS levels. Coherently, ASC metabolic profile shifts from glycolysis to mitochondrial OXPHOS, supported by a dynamic change in mitochondrial morphology and activity (Ochocki and Simon 2013; Yu et al. 2013) (Fig. 1). This rapid metabolic transition is finely regulated by the protein tyrosine phosphatase mitochondrial 1 (PTPMT1), as reported in HSCs. Ptpmt1-depleted HSCs failed to differentiate both in vitro and in vivo due to alterations of mitochondrial metabolism (Yu et al. 2013). Similarly, the glycolytic rate of NSCs declines significantly during differentiation. Indeed, NSCs display a general decrease in glycolysis genes and glucose transporters (Candelario et al. 2013; Zheng et al. 2016).

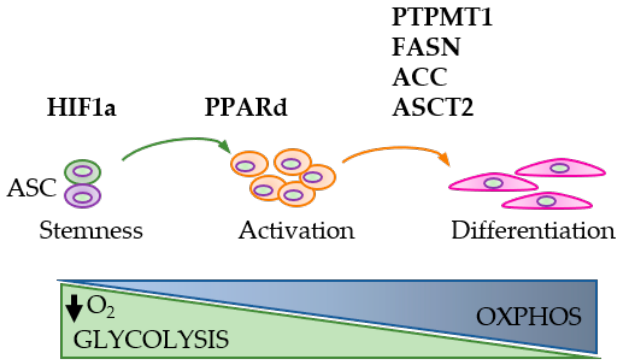


Figure 1. Metabolic and genetic control of adult stem cells. Adult stem cells (ASC) undergo a well-defined metabolic road map during their activation and differentiation, which is finely regulated by different genes.

Moreover, lipogenesis, mediated by fatty acid synthase (FASN) and acetyl-CoA carboxylase (ACC), is required for lipid membrane synthesis during mouse adult neurogenesis

Other findings uncover a role for amino acids in the regulation of ASC commitment and differentiation. For instance, the erythroid specification of HSCs is strictly dependent on glutamine metabolism. Indeed, by blocking the ASCT2 glutamine transporter or by inhibiting glutaminolysis, HSCs were diverted to a myelomonocytic fate (Oburoglu et al. 2014).

In addition to nutrients, numerous ASCs, including HSCs, MSCs, and NSCs, reside in a hypoxic niche (Chen et al. 2008; Parmar et al. 2007; Renault et al. 2009) (Fig. 1). Low levels of oxygen positively influence the maintenance of an undifferentiated state, affecting proliferation and cell-fate commitment (Mohyeldin et al. 2010). The hypoxic environment is associated with a glycolytic metabolism, which allows reducing ROS production from mitochondria. The pro-glycolytic metabolism is intrinsically established in quiescent ASCs through the upregulation of many glycolytic enzymes and the concomitant downregulation of oxidative phosphorylation proteins (Simsek et al. 2010; Takubo et al. 2013). The hypoxia-inducible transcription factors (HIF), which are stabilized and activated under low oxygen conditions, underpin the oxygen effect on stem cell fate, linking cell metabolism and stemness. For instance, HIF1α is a key transcriptional regulator of metabolism, in addition to directly regulating the wnt/β-catenin pathway to ensure the maintenance of ASCs. Indeed, HIF1α was shown to enhance the expression

of pyruvate dehydrogenase kinase (PDK) 2 and PDK4, which prevent pyruvate from entering the TCA cycle, leading to mitochondrial respiration inhibition (Takubo et al. 2013). Further, Hif1α gene deletion in adult NSCs results in their gradual loss, due to impaired integrity of the vascular niche; similarly, in HIF1α-deficient mice, HSC quiescence state is lost, and HSC number decreased (Hu et al. 2016).

In addition to the glycolytic phenotype, the contribution of lipid catabolism to the maintenance of ASC quiescence has been partially elucidated. The PPAR-δ–Fatty Acid Oxidation (FAO) pathway has been reported in the control of HSC asymmetric division and maintenance. Indeed, pharmacological inhibition of mitochondrial FAO, or genetic deletion of Ppard, resulted in altered HCS asymmetric division and increased symmetric commitment, leading to decreased HSC function and exhaustion (Ito et al. 2012). Further, lipid oxidation via the eicosanoid pathway might generate molecules able to affect HSCs fate, such as prostaglandin E2, that enhance HSC proliferation by activating Wnt signaling (Goessling et al. 2009). Similarly, NSCs depend on FAO for their proliferation, while quiescent muscle satellite cells rely on FAO and pyruvate oxidation once they become activated (Ryall, Dell'Orso, et al. 2015).

Therefore, beyond the well-known role in the energetic support, increasing evidence implicates that metabolism drives stem cell fate. Further metabolome characterizations will provide an opportunity to map stem cell metabolism aiming to suggest potential targets for improving tissue homeostasis and regeneration, also during aging and disease.

3. Muscle stem cells

Muscle stem cells, named satellite cells (SCs), are responsible for muscle homeostasis, growth and repair throughout life. Upon stimuli, as ASCs, SCs are activated and can either divide symmetrically to generate two stem cells, favoring stem cell expansion, or divide asymmetrically, to generate a stem cell and a committed one (Kuang et al. 2007). SC self-renewal capacity is a prerequisite to maintain muscle stem cell number under physiological conditions, to ensure repetitive muscle repair and to ensure the life-long preservation of contractile tissue. Importantly, a perturbed balance between symmetric and asymmetric divisions contributes to muscle diseases, such as Duchenne Muscular Dystrophy (Dumont et al. 2015), or aging (Madaro and Latella 2015; Price et al. 2014).

SCs were initially identified for their unique position, between the basal lamina and the sarcolemma, by using electron microscopy (MAURO 1961). All SCs do express the paired box transcription factor Pax7, whereas only a sub-population of SCs co-expresses Pax3. Both these paired box transcription factors are genetically located upstream of the myogenic regulatory factors (MRFs), basic helix-loop-helix (bHLH) factors, which include Myod1, Myf5, MRF4 and myogenin (Buckingham and Relaix 2007; Yin et al. 2013). Upon activation, satellite cells need to sequentially express MRFs to permit their commitment and differentiation towards myogenic lineage (Weintraub et al. 1991). Myogenic commitment is ensured by the sequential expression of Myf5 and MyoD (Rudnicki et al. 1993), while myogenin triggers myocyte terminal differentiation (Venuti et al. 1995). Ensuing expression of MRFs is guaranteed by numerous transcriptional and post-transcriptional regulatory mechanisms, including reciprocal inhibition between Pax7 and Myod1 and myogenin expression (Olguin et al. 2007), or epigenetic control of Myf5 expression in mRNP granules (Crist et al. 2012).

Despite being initially considered a homogeneous population of committed muscle progenitor cells (Bischoff and Heintz 1994), accumulating evidence supports that SCs are a heterogeneous population regarding gene expression, engraftment efficiency and muscle regeneration potential. Single-cell analyses revealed the presence of a subset of satellite cells expressing high levels of Pax7 and low levels of Myf5, at both RNA and protein levels, within satellite cell pool (Cho and Doles 2017). Moreover, a subset of satellite cells never expressed Myf5 (Kuang et al. 2007), highlighting the considerable heterogeneity within the satellite cell population. The differential expression of MRFs leads to distinctive abilities in self-renewal and niche engraftment (Kuang et al. 2007).

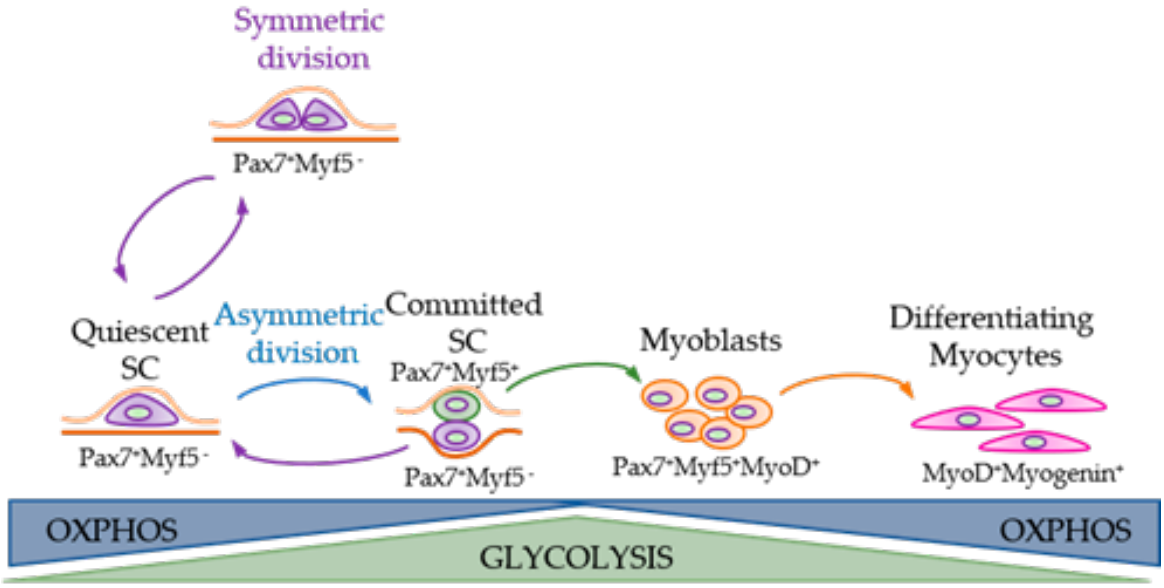
3.1. Metabolic control of SCs

Several pathways, including those of cell metabolism, have been identified as potential contributors to SC heterogeneity (Cho and Doles 2017). Indeed, metabolism is no longer a functional endpoint of signaling pathways. Rather, it is an active player in modulating enzyme activity and SC biology. For instance, two satellite cell subpopulations have been identified in rats with distinct metabolic profiles: Low Proliferative Clones and High Proliferative Clones. These are more characterized by glycolytic and stemness-like characteristics than the

Low Proliferative Clones, which result already committed (Repele et al. 2013).

Under physiological conditions, quiescent SCs possess reduced metabolic activity (Pala et al. 2018), characterized by fatty-acid oxidation metabolism (Fig. 2). This has been reported in freshly isolated SCs, without or after an in vivo fixation that prevents isolation artifacts (A. J. Harvey et al. 2016; Machado et al. 2017; Ryall, Cliff, et al. 2015). By inhibiting fatty-acid oxidation, SCs undergo commitment, without modifying their proliferation rate (Gatta et al. 2017). Similarly, pharmacological inhibition of fatty acid oxidation leads to altered SC differentiation, proving that SC physiology relies on peroxisomal, rather than mitochondrial fatty-acid oxidation (Pala et al. 2018).

Quiescent SCs actively and reversibly transit between a G0 and a GAlert phase in response to injury, becoming primed for cell cycle entry and possessing enhanced tissue regenerative function. Such transition, from quiescent to activated state, is accompanied by a metabolic reprogramming, from fatty acid and pyruvate oxidation in quiescent SCs to glycolysis and glutaminolysis in activated SCs, with a concomitant decrease of NAD+/NADH levels and increase in mitochondriogenesis (Pala et al. 2018; Rodgers et al. 2014; Ryall, Cliff, et al. 2015) without a an increase in oxygen consumption (Ryall, Cliff, et al. 2015; Ryall, Dell'Orso, et al. 2015) (Fig. 2).



Several studies identified the intracellular signaling autophagy and the molecular players Sirtuin 1 (SIRT1) and 5′ adenosine monophosphate-activated protein kinase (AMPK) as pivotal regulators of such metabolic reprogramming (Cantó et al. 2009, 2010; Cerletti et al. 2012; Ryall, Dell'Orso, et al. 2015; Tang and Rando 2014), providing experimental tools to push satellite cells towards stemness or differentiation processes. In particular, the nutrient sensor SIRT1, through AMPK, triggers the autophagic flux, thereby promoting SC activation (Tang and Rando 2014) (Fig. 3). Similarly to what observed when autophagy is inhibited, the deletion of SIRT1 in SCs compromises autophagic flux, deregulates the activation of the myogenic program and compromised muscle regeneration in response to cardiotoxin-induced muscle injury (Ryall, Dell'Orso, et al. 2015). After activation, SCs can either undergo self-renewal or commit to skeletal muscle lineage (Kuang et al. 2007). Self-renewal is tightly controlled by cellular metabolism: deletion of AMPK in SCs provokes a decrease in oxidative capacity and correlates with an increase in self-renewal, delaying SC differentiation and compromising muscle regeneration (Theret et al. 2017). Instead, SC differentiation correlates with an increase in the OXPHOS state (Pala et al. 2018). Besides, skeletal muscle differentiation is accompanied by decreased NAD+/NADH levels, which, in turn, reduce SIRT1 activity (Fulco et al.2003; Sartorelli and Caretti 2005).

Coherently, an increase in NAD+/NADH levels inhibits muscle cell differentiation (Fulco et al. 2003). Similarly, glucose restriction inhibited muscle cell differentiation by activating AMPK and the transcription of the NAD+ biosynthetic enzyme Nampt, which increases the NAD+ intracellular levels, thereby activating SIRT1 (Fulco et al. 2008) (Fig. 3). All these studies highlight the importance of metabolism and autophagy-mediated generation of ATP for SC activation and differentiation. It will be interesting to assess whether changes in SC autophagy are associated with pathological conditions charac-

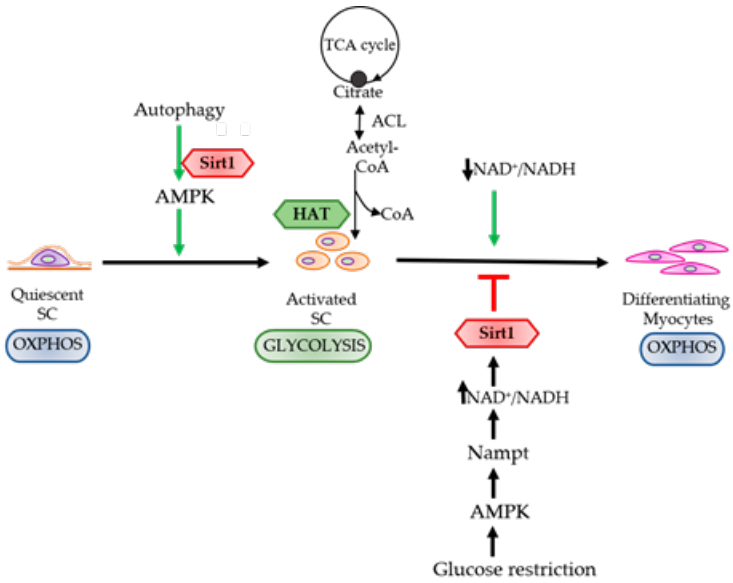


Figure 3. Interplay between metabolism and epigenetics in satellite cells. Epigenetic regulators tightly depend on nutrient availability and control satellite cell (SC) quiescence, activation and differentiation.

Aging is accompanied by a decline in adult SC function, termed SC senescence, which leads to the loss of tissue homeostasis and regenerative capacity (Kuilman et al. 2010; López-Otín et al. 2013). In aging, muscle stem cell dysfunction may be caused by both extrinsic (Chakkalakal et al. 2012; Conboy et al. 2005) and intrinsic cellular signaling (Sousa-Victor et al. 2014). Altered metabolism has been well documented in senescent SCs, which present a reduction in most of the metabolic pathways, except for glycolysis. Indeed, senescent SCs rely on glycolysis rather than OXPHOS for ATP production (Abreu 2018; Baraibar et al. 2016; Pala et al. 2018). Concerning the signaling, senescent SCs show compromised autophagy and reduced AMPK activation (García-Prat et al. 2016). Importantly, AMPK activation by an AMP analog triggers autophagy and modulates numerous cellular pathways by promoting SC proliferation and improving in vivo transplantation efficiency, overall reverting the aged phenotype in muscle stem cells (White et al. 2018). Moreover, nutritional intervention, e.g., providing NAD+ or subjecting SCs to caloric restriction, improve SC function (Cerletti et al. 2012; Zhang et al. 2016).

Thus, metabolic reprogramming may be considered as a potential tool to manipulate muscle stem cells in sarcopenia and potentially in disease states. Further studies on SC metabolic rate are needed and will likely lead to the identification of novel cellular targets able to regulate muscle stem cell biology.

3.2 Metabolic control of epigenetics in SCs

In addition to providing cellular energy and to directly influence stem cell behavior, metabolism regulates

Figure 2. Metabolic control of satellite cell biology. Satellite cells (SC) division, commitment, and differentiation is defined by specific gene markers and controlled by different metabolic states.

epigenetic mechanisms by modulating nutrient and metabolite availability (Kaelin and McKnight 2013). Indeed, most of the epigenetics writers or erasers, i.e., the enzymes able to modify chromatin structure, use metabolites as co-factors (Berger and Sassone-Corsi 2016).

A clear example in SC biology is represented by NAD+, a co-substrate for Sirtuin deacetylases (Verdin 2015). During the metabolic reprogramming from the quiescent to the proliferative state, the increased glycolysis induces a decrease in cellular NAD+ levels. As a result of reduced SIRT1 deacetylases activity, global acetylation of histone 4 (H4K16) occurs, contributing to SC activation (Ryall, Cliff, et al. 2015; Ryall, Dell'Orso, et al. 2015) (Fig. 3). Interestingly, NAD+ cellular levels decline with age (Imai and Guarente 2014), but the consequent epigenome change has not been defined yet.

Acetyl-CoA is another metabolite that directly affects cellular epigenome, being used as the acetyl donor for histone acetylation (Everts et al. 2013; Wellen et al. 2009). Acetyl-CoA derives from carbohydrates through glycolysis, from fatty acids through β-oxidation and from threonine metabolism. Acetyl-CoA can also be produced by the conversion of citrate, derived from the TCA cycle, via the enzyme ATP-citrate lyase (ACL). Modulation of ACL expression in SCs directly affects the net amount of acetyl groups available, thus altering the acetylation status of H3(K9/14) and H3(K27) at several differentiation gene loci, including Myod1 and fast myosin heavy chains, thereby regulating their expression (Das et al. 2017; Moussaieff et al. 2015). Overexpression of ACL enhances Myod1 expression, promoting SC differentiation in vitro and muscle regeneration following injury in vivo (Moussaieff et al. 2015) (Fig. 3).

Another epigenetic mechanism, acting on both DNA and histones, is the methylation, which finely tunes gene expression in SCs (Dilworth and Blais 2011; Laker and Ryall 2016). The addition of the methyl group is mediated by different methyltransferases, specific for DNA or histone proteins. However, the methyl group resource is S-adenosyl-methionine (SAM) in either case, derived from the one-carbon cycle (Etchegaray and Mostoslavsky 2016; Mentch et al. 2015).

SC proliferation is characterized by the enrichment in permissive H3K4me3 marks in genes involved in cell-cycle progression (Laker and Ryall 2016; Segalés et al. 2015), while repressive H3K27me3 mark mediated by Ezh2 is required on the Pax7 gene when SC exit the cell cycle to terminally differentiate (Palacios et al. 2010). Although a role for one-carbon cycle has not yet been reported in SCs, several studies highlighted that amino acids are crucial for determining mouse and human embryonic stem cell self-renewal (Shiraki et al. 2014; Shyh-Chang, Locasale, et al. 2013; Wang et al. 2009) or differentiation (Comes et al. 2013) via modulation of the epigenetic landscape.

While SAM is the methyl group donor for both DNA and histone methylation, α-ketoglutarate (αKG) is a necessary cofactor for both histone and DNA demethylation, by interacting with Jumonji domain-containing histone demethylases, or ten-eleven translocation methylcytosine dioxygenases (Laker and Ryall 2016). Although no data are yet available regarding αKG levels in SCs, is has been reported that αKG can either promote self-renewal or induce differentiation of the embryonic stem cells (depending on the pluripotent state) by affecting both DNA and histone methylation levels in the regulatory regions of pivotal transcription factors (Carey et al. 2015; Hwang et al. 2016), thus confirming that αKG can be used to manipulate stem cell fate.

Stem cell biology is therefore tightly and dynamically modulated by the interplay between metabolism and epigenetics, implying that changes in metabolism may have global consequences on SC epigenome and, consequently, on their function. It is of interest to better define the intimate connection between metabolism and epigenome in SCs, both in physiological and pathological conditions, in order to consider nutrient availability as a potent tool to manipulate SC functions.

Our growing comprehension about the link between cell metabolism and the epigenome raises significant questions particularly relevant for the efficacy and safety of cell transplantation and disease models: 1) does *in vitro* manipulation of nutrients alter downstream cell function? 2) does/how the in vivo metabolic environment delay cell integration following transplantation? Plausibly, low nutrients may lead to poor stem cell transplantation retention and integration. Furthermore, metabolic and epigenetic factors may have developmental, stage- and tissue-specific functions. Whether modulating the culture environment is, therefore, capable of improving SC expansion and/or engraftment remains to be explored. This information will be useful for the development of more physiological media formulations and culture conditions to support long-term SC viability.

Further studies focusing on how metabolites or nutrients directly affect SC epigenome, linking these epigenetic changes to different SC destinies, are demanded.

4. Conclusions

SCs constitute a promising tool for regenerative medicine approaches. Maintaining their number and function is of particular relevance to skeletal muscle pathologies, including aging or genetic diseases. Furthering our understanding of the underlying molecular mechanisms and fundamental aspects of stem cell heterogeneity will be relevant to clinical applications exploiting somatic stem cell populations, either through cell replacement or pharmacological manipulation.

Accumulating evidence indicates a metabolic roadmap during SC transition from the quiescent to the activated, committed, or differentiating state, a process known as metabolic reprogramming. The influence of metabolic reprogramming on SC self-renewal, commitment, or differentiation, as well as the use of pharmacological inhibitors of the intracellular pathways involved in these processes, provide a proof-of-concept for developing effective therapeutic interventions for SC therapies, improving muscle regeneration or augmenting the SC pool in degenerative muscle disorders.

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Cancer biomarker discovery without assumptions about cancer biology: The double dip design

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Abstract

The biomarker pipeline to improve cancer screening begins with the discovery and validation of a cancer prediction model involving markers for the early detection of cancer in asymptomatic persons. Unfortunately, this biomarker pipeline has led to few markers for clinical use. An unappreciated reason for this lack of success is that standard discovery uses a convenience sample of specimens from persons with symptomatic cancer and no cancer. Standard discovery in a convenience sample implicitly makes a questionable assumption about cancer biology, namely, that highly predictive biomarkers in asymptomatic persons persist until symptomatic cancer arises when they outperform markers associated with symptomatic cancer. If cancer arises from a sequence of driver mutations and biomarkers are associated with driver mutations, this assumption may be plausible. However, if cancer arises primarily from changes in the microenvironment, the assumption is questionable. To circumvent the need for this assumption, I propose the double dip design. The double dip design starts with standard discovery in a convenience sample (as this is standard practice) followed by the usual validation sample of stored specimens from asymptomatic persons. If validation fails, it re-uses the original validation sample of stored specimens for more relevant biomarker discovery, followed by a second validation sample of stored specimens from asymptomatic persons. Recently developed statistical methods to reduce validation sample size make the double dip design feasible.

Keywords: biomarker, cancer screening, early detection, sample size, sensitivity, specificity, validation

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1. Introduction

There is a great need to develop new cancer screening modalities that decrease false positive screens, lessen overdiagnosis, and reduce cancer mortality. In general terms, a cancer screening modality is a cancer prediction model based on markers and risk factors. Markers are measurable indicators of biological state influenced by early-stage carcinogenesis. Examples include genomic markers in the blood, cyst fluid markers, antibody arrays, metabolites, protein markers in the urine, exosomes, circulating tumor cells, mutations in various genes, imaging results from mammography or ultrasound, and prostate specific antigen (Lippman et al, 2018, Sauter, 2017, Young et al. 2018). Markers can be collected at multiple times in each participant. Risk factors are measures of increased susceptibility to cancer, such as age, family history of cancer, and germline mutations.

The biomarker pipeline to develop a better cancer prediction model for use with screening has two phases: discovery and validation. In the discovery phase, investigators formulate a cancer prediction model, which involves both selecting (discovering) markers and fitting a model. In the validation phase, investigators use an independent sample to evaluate the performance of the cancer prediction model. A cancer prediction model is validated if it has good prediction performance (discussed more precisely later).

Discovery under the standard design involves specimens from persons with symptomatic cancer and controls without cancer. I call the discovery sample a convenience sample, because it is relatively easy for investigators to obtain specimens. Although the purpose of discovery in a convenience sample is to fit a cancer prediction model, the convenience sample provides no direct information for prediction.

Validation under the standard design involves stored specimens from asymptomatic persons. Investigators follow asymptomatic persons several years and measure markers in stored specimens from all participants

who developed cancer (cases) and a random sample of participants who did not develop cancer (controls) (Baker, Kramer, and Srivastava, 2002).

This standard design for biomarker discovery and validation has led to few clinical markers for early detection of cancer. Most markers for cancer early detection in widespread use were discovered between the mid-1960's and mid-1980's. These include carcinoembryonic antigen (CEA), prostate specific antigen (PSA), and carbohydrate antigen 125 (CA125).

Various researchers point to poor statistical methodology in study design as a likely explanation for the lack of success in finding and validating new biomarkers for the cancer early detection (Ransohoff, 2004, Pepe et al 2008, Ransohoff and Gourlay, 2010).

I discuss a more fundamental reason explaining the lack of success, namely that the standard design for cancer biomarker discovery requires a questionable assumption about cancer biology. In addition, I propose the double dip design which allows for cancer biomarker discovery with any assumptions about cancer biology.

2. Drawbacks of standard discovery

The standard discovery with a convenience sample implicitly assumes that highly predictive biomarkers in asymptomatic persons persist until symptomatic cancer arises when they outperform markers associated with symptomatic cancer. This assumption is consistent with the somatic mutation theory of cancer, that successive driver mutations lead to cancer, and the implication that biomarkers are associated with driver mutations. If the somatic mutation theory does not hold, standard biomarker discovery in a convenience sample would miss promising marker in asymptomatic persons in the following two ways.

First, a convenience sample would fail to discover a transient marker associated with preclinical cancer and not symptomatic cancer. Such a transient marker might signal the start of irreversible changes that lead to cancer. Possible examples of transient markers are markers related to stem cell signaling (Lipman et al, 2018) or intercellular signaling between stromal and epithelial tissue (Sonnenschein and Soto, 2016; Soto and Sonnenschein, 2011; Baker 2015, Baker 2018)

Second, a convenience sample would fail to discover a persistent biomarker of preclinical cancer that is masked by a better performing biomarker associated only with symptomatic cancer. For example, carcinoembryonic antigen (CEA) almost perfectly classifies the presence of colorectal cancer in a convenience sample involving specimens from persons with symptomatic colorectal cancer (Thomson et al, 1969). However, CEA poorly predicts the development of colorectal cancer in stored samples from asymptomatic persons (Thomas et al, 2015). Consider a biomarker M in asymptomatic persons that, unlike CEA, performs well for cancer prediction in asymptomatic persons. However, in a convenience sample, biomarker M does not perform as well CEA. A candidate cancer prediction model in the convenience sample that included both CEA and biomarker M would incorrectly indicate that biomarker M makes little, if any, contribution to cancer prediction in asymptomatic persons, simply because there is little room for improvement in cancer prediction with CEA in the model.

3. The double dip design

Baker (2009) proposed a discovery phase using stored specimens from asymptomatic persons. Although this design would avoid the assumptions with standard discovery in a convenience sample, it is unacceptable to most investigators. Many investigators are reluctant to perform discovery using stored specimens from asymptomatic persons, thinking it wastes precious specimens (ignoring the downside of wasting stored specimens to validate unpredictive markers discovered in a convenience sample).

The double dip design circumvents the limitations of the standard design in a practical manner (Figure 1). The double dip design starts like a standard design with discovery in a convenience sample and validation using stored specimens from asymptomatic persons.

The key to the double dip design is the next step. If the validation sample indicates poor performance of the prediction model formulated in the convenience sample, the double dip design re-uses the prospective validation sample as a second-chance discovery sample -- a procedure which I call the double dip. The double dip yields a cancer prediction model based on markers in stored specimens from asymptomatic persons –which is what is needed for relevance to early detection. To evaluate the second-chance cancer prediction model, the double dip design requires a second prospective validation sample using stored specimens from asymptomatic persons.

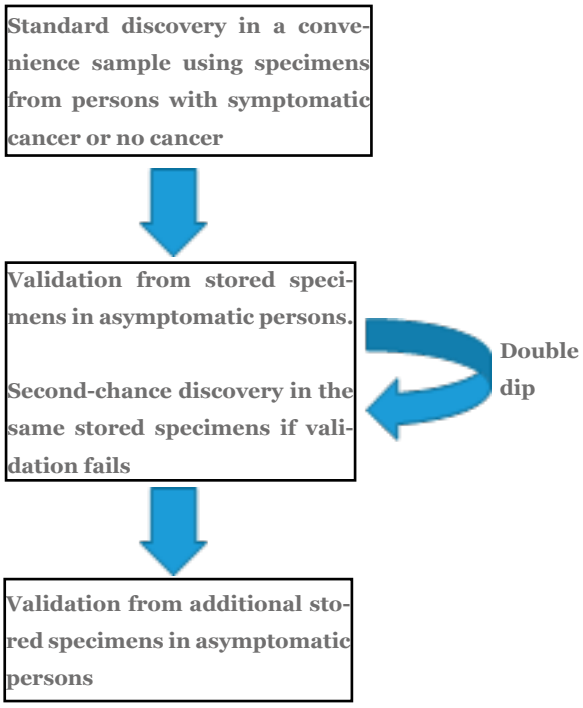

Figure 1. Double dip design

5. Sample size

The main drawback to the double dip design is the need for two validation samples of stored specimens. Fortunately, a recently developed statistical method yields reasonable sample sizes for validation (Baker, 2019). The key is to estimate sensitivity (probability of a positive cancer prediction given develop cancer) imprecisely and to target 100% specificity (probability of a negative cancer prediction given no cancer arises in the study). The high specificity ensures a high positive predictive value (probability cancer arises in the study given a positive prediction) regardless of the sensitivity.

Under Scenario 1, the target values are 80% sensitivity with lower bound of 50% and 100% specificity with lower bound of 99.5%. Under Scenario 2 (which is easier to achieve), the target values are 50% sensitivity with lower bound of 20% and 100% specificity with lower bound of 99.5%. Both scenarios require specimens from 12 cases (persons who develop cancer) and 740 controls (persons who did not develop cancer). Under Scenario 1, the cancer prediction model is validated (achieving target performance) if at least 9 out of 12 case specimens are positive when 0 control specimens are positive. Under Scenario 2, validation requires at least 5 out of 12 case specimens to be positive when 0 control specimens are positive. See the online supplementary appendix for sample size calculations. Table 1 (which applies to both scenarios) shows the sample sizes for the total number of persons contributing specimens in each validation sample.

Probability of developing cancer during the study	Validation sample size
1.0%	2000
1.5%	1300
2.0%	1000
2.5%	800

Table 1. Validation sample sizes. All designs are based on 12 cases and 740 controls to yield target performance.

4. Discussion

A potential limitation of the double dip design is that the second-chance discovery sample (which is the original, re-used, validation sample) may involve too few cases for adequate discovery. If this is a concern, investigators could double its size and still have a reasonable sample size in many scenarios.

An important determinant of sample size is the probability of developing symptomatic cancer in the study. As shown in Table 1, higher probabilities of developing symptomatic cancer in the study translate into smaller sample sizes. Therefore, investigators should collect specimens from populations at high risk of developing symptomatic cancer with the understanding that results strictly only apply to high risk persons.

The double dip design can increase the efficient the use of stored specimens in trials where biomarker discovery and validation are not the main goals. Investigators collected stored specimens in two large prevention trials with lung cancer incidence as the primary endpoint, the Alpha-Tocopherol Beta Carotene Lung Cancer Prevention Trial (ATBC) (ATBC Cancer Prevention Study Group, 1994) and the Beta-Carotene and Retinol Efficacy Trial (CARET). (Omen et al 1996). In using these stored specimens to predict prostate cancer, Baker (2000) essentially performed a double dip design with discovery in ATBC stored specimens and validation in CARET stored specimens.

The formulation of the cancer prediction model can involve the “discovery” of markers from high-dimensional data such as might arise from microarrays or other -omics approaches. It can also involve reverse time models to better accommodate varying numbers

of markers collected over time in each person (Baker and Tockman, 2002).

There is a growing appreciation of the advantage to using stored specimens for discovery. Ransohoff (2017) wrote, "As stated by one observer 'We need to turn conventional wisdom on its head' and use precious specimens far earlier than we currently do (Z Feng personal communication)." Until now there has been no acceptable path to the ideal discovery using stored specimens. The double dip design provides such a path.

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Marxism and the Crisis in Modern Biology

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Abstract

Modern biology, especially biomedical research, is currently embroiled in crisis. This crisis is not unsurprising considering the bourgeois culture and philosophy that has guided scientific research since the Molecular Biology revolution and aided by the increasing public-private partnerships. The resolution of this crisis can only be achieved through a radical shift in how we understand and practice science, and the Marxist philosophy of dialectical materialism can provide us with the necessary tools to do so. In this paper, I provide a brief overview of the development of dialectical materialism and its application over the years to understanding the natural world. I also show that biologists have also independently adopted similar views as research has progressed over the years. Lastly, I argue that the epistemological crisis and the subsequent crisis observed in the practice in science are two sides of the same coin, and that Marxist philosophy can help break out of this vicious cycle.

Keywords: dialectics, Marxism, biology, biomedical research, materialism

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1. Introduction

> ...science, in all its senses, is a social process that causes and is caused by social organization. (Levins and Lewontin, 1985)

In 2014, the prominent cancer researcher Robert Weinberg published a paper admitting that the current paradigm of cancer research, while uncovering many technical details, has ultimately failed to unravel the complexity of this set of diseases (Weinberg, 2014). This is not an isolated incident - earlier this year, the biotech company Biogen halted two Phase 3 trials for it's heralded Alzheimer's drug (Feuerstein, 2019), bringing up the debate of whether beta-amyloid, the hallmark of the disease, was actually a proper drug target; consequently, other theories of Alzheimer's disease progression (Begley, 2018) are currently gaining more traction. Cancer immunotherapy, widely acknowledged as a revolutionary breakthrough, is currently grappling with inexplicable phenomena such as diabetes in patients receiving IO therapy (Dolgin, 2019) and the low rate of response by patients (Haslam and Prasad, 2019). Most recently, off-target toxicity was found to be widespread among cancer drugs in ongoing clinical trials (Lin *et al.*, 2019).

These few instances are just the symptoms of a much larger crisis embroiling biomedical research and modern biology in general. The reproducibility crisis has been well documented in the past decade (Baker, 2016) and scientists are struggling to resolve that issue (Oransky and Marcus, 2017); a paper in 2011 showed that the higher the impact factor, the more number of retractions occur in a journal (Fang and Casadevall, 2011). The Precision Medicine initiatives undertaken both publicly and privately are failing to live up to their hype (Aelion *et al.*, 2016; Hasan, 2016a). Sham conferences and predatory journals have spawned at an alarming rate; current experimental strategies are guided by confirmation bias, an obsession with mechanistic details, genetic determinism, and investor relations rather than a proper theoretical framework (McHenry, 2008; Pagano, 2017; Maxmen and Warren, 2019). The absence of a proper theoretical framework has also resulted in abuse of statistical tests such as "p-hacking" (Head *et al.*, 2015) and the conflation of statistically "not significant" results with no biological effect or importance (Amrhein, Greenland and McShane, 2019; Montévil, 2019; Rubin, Schaeberle and Soto, 2019). The consequences of this epistemological crisis in biology are not limited to academia and are manifested in the econom-

ic, political and social landscapes as observed through the cases of Theranos (Fiala and Diamandis, 2019), the liquid biopsy company that promised the moon without any data, the bitter CRISPR patent battle (Ledford, 2017), the rise of eugenics under the guise of hyper-rationalism and the subsequent co-option of genetics by white supremacists (Hasan, 2019), the MIT-Epstein scandal (Farrow, 2019) and other high-profile incidences of conflict-of-interest between scientists and their work (Wadman, 2012; Glanz and Armendariz, 2017; Thomas and Ornstein, 2018), the exorbitant prices of drugs in the US that benefit only the pharma companies (Paton, 2019), the large turnover of startup biotech companies and a reduction of investment in R&D by big pharmas (Mazzucato, 2018), their increasing reliance on blockbuster drugs (Kresge and Lauerman, 2019) and insidious marketing strategies to create demand as exemplified by the Opioid Crisis (Keefe, 2017), an inflation of translational claims based on experiments with faulty pre-clinical models (Kaelin, 2017) and clinical trial endpoints that serve profit rather than patients (Kemp and Prasad, 2017), and much more.

The litany of what is currently wrong with biomedical research, or modern biology in general, is long but should not come as a surprise. After all, Levins and Lewontin (1985) had already pointed out

> "Modern science is a product of capitalism. The economic foundation of modern science is the need for capitalists not only to expand horizontally into new regions, but to transform production, create new products, make production methods more profitable, and to do all this ahead of others who are doing the same. Its ideological underpinnings are congruent with these needs and also with the political philosophy of bourgeois revolution... The commoditization of science, then, is not a unique transformation but a natural part of capitalist development."

The crisis in modern biology is then two sides of the same coin - the epistemological one, where reductionism reigns supreme with ad-hoc corollaries that fail to properly explain complex phenomena, is inherently tied to the practice of science under capitalism.

The entrenchment of capital and private interests in scientific research, a public good (Roy and Edwards, 2017), has been steadily increasing since the 1980s, as a result of a series of legislations that allowed for the patenting of "anything under the sun made by man", followed by waves of privatization to boost translational research and public-private partnerships in the 90s, and further programs undertaken by state agencies in the 2000s such as FDA's Critical Path Initiative and NIH's small business grants (Bouchard and Lemmens, 2008). However, more money has not resulted in better research - when NIH's budget doubled between 1997-2003, the growth was observed mainly in ancillary markets such as reagent companies, expansion of universities, and number of NIH contractors (Pagano, 2017); ironically, a greater push for more private-public collaborations is offered as an answer to the money-product disconnect (Bertagnolli, Canetta and Nass, 2014).

What then is to be done? The solution to this crisis, both epistemological and practical, requires a radical shift in how biologists approach their work. In this essay, I will argue that theoretical practices grounded in the Marxist philosophy of dialectical materialism is poised to do so. Marxist philosophy, often restricted to the realms of political economy and sociology, nonetheless has a long history of enriching the natural sciences, not to mention that both Marx and Engels were influenced by Darwin's theory of change in nature that they latter applied to describe changes in the social order. While a superficial understanding of Marxism can be dangerous, as seen in the case of Lysenkoism, a deeper understanding of dialectical materialism, especially the dialectics of nature, can solve the current epistemological crisis. This wouldn't be the first time that Marxism would have rescued biology from a crisis: in 1931, at the Science at the Crossroads conference, BM Zavadovsky noted that the path to resolving the vitalism vs mechanism and reductionism vs mysticism debates lay in dialectical materialism, which went beyond the "attempts to embrace all the complexity and multiformity of the world through either a single mathematical formula of the mechanical movement of molecules or through the vitalist idea of a single 'principle of perfection,'"(Sheehan, 2018). Similarly, on the question of the inheritance of acquired characteristics, as geneticists grouped themselves into Lamarckists vs Morganists, it was a dialectical understanding of genetics that Zavadovsky argued pointed towards Mendel and Morgan's ideas (Ibid).

In the following sections, I will present a historical primer on the development of dialectical materialism as it pertains to natural sciences (dialectics of nature), present evidence from both historical and contemporary science that a dialectical framework is at the very least necessary starting point for unraveling modern biology's present epistemological crisis in all its dimensions and, I argue, provides the tools for resolving that crisis.

2. A Primer on Dialectical Materialism

2.1 Hegel, Marx & Engels

The 19th century German philosopher G.W.F. Hegel revived the logical mechanism of 'dialectics' to understand the process of development, historical or metaphysical; for Hegel, dialectics was 'the only true method' of scientific and scholarly exposition, a method that 'is in no way different from its object and content - for it is the content in itself, *the dialectic which it has in itself*, that moves out' (Singer, 2001). The three-step Hegelian dialectic process (thesis, anti-thesis, synthesis) describes the opposing forces working against each other to produce a novel object or phenomenon can be used to understand the course of history or the development of thought. In *Philosophy of History*, Hegel used this logical mechanism to describe the political transformations that European societies underwent over centuries - from ancient Greek democracy as the thesis to the Reformation and the French Revolution as the anti-thesis, and lastly contemporary German society as the synthesis (Singer, 2001).

Hegelian dialectics, however, was too idealistic to describe the material world and man's relation to it. This realization by Karl Marx and Friedrich Engels led them to re-work Hegelian dialectics to place matter in the center rather than the Idea. For Marx, "the idea is nothing else than the material world reflected by the human mind and translated into forms of thought.[1]" This key difference changed dialectics from an idealist to a materialist philosophy and where Marx broke with Hegel. Marx's materialism stemmed from his study of Epicurus and Feuerbach, both of whose materialism he criticized to be too "contemplative"; for Marx, "we transform our relation to the world and transcend our alienation from it - creating our own distinctly human-natural relations- by acting, that is, through our material praxis" (Foster, 2000). However, Marx did internalize parts of Epicurean philosophy, which proposed that the movement of atoms was not entirely pre-determined but rather, some atoms "swerved", which created the element of chance and indeterminacy (Ibid). Additionally, Epicurus also proposed a "principle of conservation" and rejected teleology and reductionism, both features which are key to understanding Marx's dialectical materialism (Ibid).

Marx's dialectical materialism was also inspired by his readings of Darwin's theory of natural selection; in a letter to Friedrich Engels, he wrote "it is remarkable how Darwin recognizes among beasts and plants his English society with its division of labour, competition, opening up of new markets, 'invention', and the Malthusian 'struggle for existence'" (Ibid). While Marx had realized that man's relation to nature is dialectical, it was Engels who wrote down the three laws of dialectics of nature (Engels, 2012):

1. The law of the transformation of quantity into quality and vice versa.
2. The law of the interpenetration of the opposites.
3. The law of the negation of the negation.

Using these three laws to describe natural phenomena, Engels concluded that "in nature nothing takes place in isolation. Everything affects and is affected by every other thing" (Ibid). Engels' natural worldview consisted of one in constant motion, where equilibrium existed due to contradictions and not as a steady state. What Engels was arguing for, as Sheehan (2018) describes, was "a developmental and integrative way of thinking grounded in a developmental and integrative ontology." She also pointed out, however, that Engels' use of Hegelian terminology created an array of "conceptual confusions" that affect Marxist discourse to this day, questioning the validity of the dialectics of nature.

2.2 The Dialectics of Nature Debate

Sheehan's realization above had come from a series of debates on the nature of dialectical materialism that arose from Engels's adoption of Hegelian terminology. In the early days of the Soviet Union, the line between scientists and philosophers were drawn along *a priori* philosophy versus experimental science line. The experimentalists, or mechanists, accused the dialecticians of forcing Engels's laws onto natural processes, whereas the dialecticians claimed that mechanists were unable to understand the reciprocity between theory and praxis. Nikolai Bukharin, in an effort to erase any residual Hegelianism from dialectical materialism, inadvertently adopted a mechanistic form of materialism while sacrificing the Epicurean materialism that left room for chance and indeterminacy. A version of this mechanistic materialism, as espoused by Lysenko, was later codified as the orthodox Marxist philosophy of nature by Stalin during his regime.

The core of the problem, as Sheehan argued, lay in the relationship between the natural sciences and

[1] Capital. Vol I -1873 Afterword

philosophy and how the history of the two were related in Marxist thought. In fact, it appears that the rejection of dialectics of nature by Western Marxists was partially inspired by the state of contemporary knowledge on natural processes. In *History and Class Consciousness*, György Lukács wrote on dialectical materialism that

> It is of the first importance to realize that the method is limited here to the realms of history and society. The misunderstandings that arise from Engel's account of dialectics can be put down to the fact that Engels - following Hegel's mistaken lead - extended the method to also apply to nature. However, the crucial determinants of dialectics - the interaction of subject and object, the unity of theory and practice, the historical changes in the reality underlying the categories as the root causes of changes in thought, etc. - are absent from our knowledge of nature. (Lukács, 1972)

Going a step further, Alfred Schmidt distinguished between Marx and Engels' philosophies of nature on the basis of the question whether extra-human nature was also dictated by the laws of dialectics (Schmidt, 2013). Schmidt argued that "the concept of nature cannot be separated, in either philosophy or natural science, from the degree of power exercised by social practice over nature at any given time", thus echoing Lukács that dialectics are only applicable to nature through man's labour in relation to nature. In Schmidt's view, "it is only the process of knowing nature which can be dialectical, not nature itself." Schmidt also argued that by analyzing the findings of contemporary natural science using dialectical categories, Engels' dialectics of nature "remained external to its subject-matter". Schmidt thought that the dialectical process was incompatible with the scientific method since he considered the latter to be oriented towards formal logic and did not reflect the historical processes behind the objects. Consequently, the critical theorists and latter Western Marxists took on the neo-Hegelian position that dialectical materialism is only applicable for understanding social and historical changes and not natural phenomena.

2.3 Eco-Marxism and Man's Relation to Nature

More recently, drawing on Marx's early works such as the Paris Manuscripts and *Theses on Feuerbach*, eco-Marxists such as Foster and Burkett have pushed back against the critical theorists' rejection of dialectics of nature (Cassegård, 2017). In *Marx's Ecology* (2000), John Bellamy Foster argues that Marx had refused to distinguish dialectical materialism from natural sciences and that Marxist philosophy was "predicated on the ultimate unity between nature and society". A similar assertion can also be found in Schmidt's writings (Schmidt, 2013), where he argues that both the early and the later Marx recognized that natural history was inseparable from social history, but precisely because of man's relation to nature through labor (admittedly, Foster shares similar views on human labour existing in a *metabolic* relation with nature).

Foster's arguments rely on the Epicurean materialism aspect of Marx's philosophy, and this is where he sees a break occurring between Engels' dialectics, which he deemed to be too mechanistic and deterministic. In his view, Marx's materialism extends beyond just "social praxis" to a "natural praxis" which incorporates an ecological perspective where the "biosphere constitutive of our own existence even as we transform it through our actions" (Foster, Clark and York, 2010).

At the kernel of the debate between eco-Marxists and the Frankfurt school Western Marxists then lies the relation between man and nature. Schmidt had outlined how the later Marx had concluded that man will never transcend their antagonistic relationship with the environment (an assertion that has later been used for a Baconian interpretation of Marxist philosophy) whereas Foster and eco-Marxists argue that the relation between man and nature is one of harmony. .

In a critique of Foster's "natural praxis", Cassegård (2017) argues that nature is viewed as dialectical by Foster because it is the object of praxis. According to Cassegård, then, what is considered as a dialectics *of* nature actually is a dialectics of praxis *in relation* to nature. He criticizes Foster's dialectical analysis of evolutionary biology in support of 'natural praxis' as being still within the 'contemplative' realm, even when dialectics is used as a heuristic device. In an effort to reconcile the positions held by Schmidt and Foster et al, Cassegård invokes other critical theorists (Adorno, Marcuse, Horkheimer, etc.) to show that while "nature must remain a realm of necessity, does not mean that [human beings'] relation to nature must be one of perennial antagonism or domination."

The fact that science is a human enterprise then becomes a crucial point to assert the validity of Marxist dialectical materialism as a way to interpret our relation to the material world. The following sections outline the historical application of dialectics to biology and more recent developments that prove the validity of such application.

3. Dialectics & Biology

> What is amazing is the similarity in the thinking of naturalists and dialectical materialists. The so-called dialectical world view is by and large also the world view of the naturalists, as opposed to that of the physicalists. (Mayr, 1997)

If Marx is considered to be the primary author of the new chapter on dialectics in Western philosophy, it can be said that Darwin fulfilled a similar role for materialism and natural science (Foster, 2000). Changes in natural phenomena, or natural history, upto the Enlightenment period was firmly in the hands of natural theologists, who considered nature to be teleological and governed according to laws set by a Supreme Being. It was Darwin's theory of natural selection, deeply rooted in philosophical materialism, that presented a radical break away from theological explanations of natural processes and moved the study of natural phenomena into the materialist realm.

Engels' laws of dialectics, influenced by Darwin's work, was taken up by Soviet scientists in various forms; a detailed analysis of such work can be found in Loren Graham's book *Science and Philosophy in the Soviet Union* (1972). But Soviet scientists were not the only ones adopting a dialectical framework to make sense of their findings - in the West, biologists such as JD Bernal, JBS Haldane, Joseph Needham, Marcel Prennant to name a few, were also applying the dialectical framework to understand biological phenomena and the practice of science to varying degrees. Bernal considered Marxist philosophy to be an extension of the scientific method and believed that "Marxism transforms science and gives it greater scope and significance" (Sheehan, 2018); according to Haldane, Marxism could be applied to understand the process of development of science and the history of science as a human activity. Needham, while unconvinced of the value of Marxism in ethics and politics, still believed dialectical materialism to be "the quintessence of scientific method," as "the natural methodology of science itself"(Ibid). Both Bernal and Needham insisted that dialectical materialism would be of great service to biologists by pointing the way towards the most promising hypotheses and by indicating which questions were meaningful and answerable.

How does Engels' laws of dialectics translate to a framework for biology? Ernst Mayr, in his essay *Roots of Dialectical Materialism* (1997), attempted to provide an answer - the first law is a principle of non-reductionism, the second is an explanation for the presence of energy in nature that removes any sort of divine or external requirement and the third describes continuous changes in nature, i.e., evolution (Mayr, 1997). Therefore, dialectical materialism provides a theoretical bulwark against reductionism in biology as well as a framework to understand the changes underlying natural phenomena. However, the adoption of the Soviet interpretation by Western biologists presented a unique problem: the Soviet interpretation placed mechanistic materialism at the core of the dialectical framework and mechanistic materialism is inherently reductionist while under the dialectical framework, "biological phenomena, although historically connected with physicochemical phenomena, were not reducible to physicochemical laws" (Zavadovsky as quoted in Sheehan, 2018). In an effort to resolve this internal contradiction, Richard Levins and Richard Lewontin presented a variant of of dialectical materialism that they believed was a "simultaneous negation of both mechanistic materialism and dialectical idealism" (Levins and Lewontin, 1985).

3.1 Dialectical Biology

Levins and Lewontin applied dialectical materialism to biology, especially ecology and evolutionary biology, in an attempt to break away from the grip of Cartesianism which they deemed, along with contemporary dominant Western philosophy, inadequate to explain the complexities underlying large scale biological phenomena such as population ecology, evolutionary genetics, etc. They argued that the reductionism inherent in such philosophies undercut the importance of interactions between parts that made up the whole, ignored emergent properties, and forced science to choose separate causes for the same phenomenon. In their words, "where simple behaviors emerge out of complex interactions, reductionism takes that simplicity to deny the complexity; where the behaviour is bewilderingly complex, it reifies its own confusion into a denial of regularity" (Levins and Lewontin, 1985).

Using the dialectical framework and a host of evidence drawn from ecology and genetics, Levins and Lewontin proceeded to describe the interactions between genes, environment and the organism that results in the development of the organism, without ascribing causality to any single level of biological organization, and that these interactions, also termed "norms of reaction", should be the proper object of scientific investigation. Under this framework, development then

becomes a context-dependent open-ended process, similar to Alessandro Minelli's "disparity view" of development, which goes beyond the life cycle of the organism and extends to post-reproductive events like aging and pathological changes such as carcinogenesis; additionally, in Minelli's opinion, development may be reversible, not easily distinguishable from metabolism, not limited to adaptive traits and describe both permanent and temporal morphological changes (Minelli, 2014).

For Levins & Lewontin, the organism constitutes both the subject and the object of evolution, since the organism actively constructs its environment that in turn actively affects the development of the organism:

> ... an organism does not compute itself from its DNA. The organism is the consequence of a historical process that goes on from the moment of conception until the moment of death; at every moment gene, environment, chance, and the organism as a whole are all participating... Natural selection is not a consequence of how well the organism solves a set of fixed problems posed by the environment; on the contrary, the environment and the organism actively codetermine each other.

They further argued that Darwin's theory of natural selection did not explain the origin of variation or that if selection resulted in differential reproduction of variants, then eventually there would not be any more variation for further evolution as a population would achieve uniform fitness. To resolve this contradiction, Levins & Lewontin proposed that Darwin's ideas can only reach full maturity when the organism is integrated with the "inner" and "outer" forces, as in the genotype and the environment, and viewed as both the subject and the object of evolution, as it is under dialectical materialism.

Lewontin went on to further solidify the necessity of using a dialectical approach to studying evolution and development of an organism. In his book *The Triple Helix* (2001), he writes "that the ontogeny [development] of an organism is the consequence of a unique interaction between the genes it carries, the temporal sequence of external environments through which it passes during its life, and random events of molecular interactions within individual cells. It is these interactions that must be incorporated into any proper account of how an organism is formed", thus establishing the organism as a site of interaction between the environment and genes (Lewontin, 2001). Therefore, under dialectical materialism, the long-running Nature vs. Nurture debate is replaced by how Nature AND Nurture contribute to the development of an organism.

3.2 The Organism as the Holobiont

While Levins & Lewontin had largely applied the dialectical framework to biology above the individual organism level, Gilbert & Tauber (2016) did the same but at the individual organismal level to question what constituted biological individuality. Historically, an individual organism has been delineated by anatomical borders, functional integration through division of labour and communication between its parts, and a hierarchical system of control (Nyhart and Lidgard, 2011). However, using a host of scientific evidence that proves the ubiquity of symbiosis, Gilbert and Tauber argue that modern biology negates this notion of the individual organism; rather, organisms are "holobionts" - multi-genomic, composite organisms "whose physiology is a co-metabolism between the host and its microbiome, whose development is predicated upon signals derived from these commensal microorganisms, whose phenotype is predicated on microbial as well as host genes, and whose immune system recognizes these particular microbes as part of its "self" (Gilbert, Sapp and Tauber, 2012; McFall-Ngai *et al.*, 2013)." Gilbert & Tauber went on to show how dialectics exist at all levels of development of the holobiont - from fertilization (two cells fuse to become one) to organogenesis (stromal-epithelial interactions), the development of the immune system, symbiotic interactions between microbial and host cells, the construction of the ecological niche for the holobiont, and even down to the molecular level where stereo-specificity is determined by a set of interactions (induced fit model) rather than the deterministic "lock and key" model. Taking all these together, Gilbert & Tauber questioned the current conception of immunity as a defense mechanism and argued that immunology should be brought under the larger umbrella of ecology and proposed the field of "eco-immunology", since immunology has long been used to delineate the organism as a biological individual (Pradeu, 2010).

Eco-immunology, a complement to the "Eco-Evo-Devo" discipline (Gilbert, Bosch and Ledón-Rettig, 2015), is then used to understand the role of the immune system in the physiological and functional integration of the organism with its environment and dispels the binary notion of immunity being a defense mechanism. This is exemplified in the need for specific microbes for proper development of the brain, gut and reproductive

tissues across a host of animals (Hadfield, 2011; Sampson and Mazmanian, 2015). This idea, similar to Ilya Metchnikoff's idea that biological individuality was a result of the dynamic interactions among eukaryotic cells and between eukaryotic and symbiotic microbes then posits that the organism "was not a given, but rather a "work-in-progress" that underwent lifelong development in dialectical exchange with other potentially competing intra-organismal elements" (Gilbert and Tauber, 2016).

The organism as a holobiont is therefore the fruition of the application of a dialectical materialist framework to modern biology, and provides a novel way forward to continue doing so to unravel the complexities of natural phenomena. However, Western Marxists have long criticized such an application of the dialectics of nature - that it cannot be "arbitrarily foisted upon the world of nature from outside; that the dialectics of nature is an anthropomorphic projection of human concepts onto nature". But in *Dialectics of Nature*, Engels had clearly emphasized that there was no question that the laws of dialectics were **abstracted from** the history of nature and human society. In fact, he had already foreseen how biology was to be the fore-runner of a dialectical world-view in the sciences and that biologists would benefit from acquainting themselves with dialectical materialism. The main argument against the idea the dialectics is forced upon nature comes from Ernst Mayr's realization (quote at the beginning of the section) that naturalists and dialecticians share the same world-view. The two major developments in modern biology, as presented in the next sections, provides concrete evidence to Mayr's statement and validates Engels.

3.3 Neo-Lamarckism

With the rise of observations in developmental plasticity, it would appear that Lamarckian concepts of transmission of heritability are quickly gaining traction in Western science. While fetishism around the gene as the central identity has been the key ideology of the neo-Darwinians such as Richard Dawkins, and has propagated the DNA as the blueprint of life idea, neo-Lamarckian systems of transmission of inheritance as proposed by Eva Jablonka and Marion Lamb (1995) push back against this reductionist view of evolution. Jablonka and Lamb argue that short term evolution does not depend on new mutations in the DNA, but rather on epigenetic modifications that uncover genetic variants already present in the population. Additionally, genes undergo "shuffling" through recombination during cell division, thus giving rise to further variation within the population. They also argue that the structure of the chromatin affects changes in the DNA sequence and therefore "highlights the complexity of the role of the environment in evolutionary change, the environment is not just the agent of selection. Through its effects on genes phenotype, it also biases the direction, rate and type of DNA changes at the locus", echoing Levins & Lewontin (1985). Jablonka and Lamb also propose group selection rather than individual selection, and counters the neo-Darwinian idea of the gene as the unit of selection by proposing groups of cells as units of selection instead (similar to Gilbert's holobiont concept) (Jablonka and Lamb, 1995). Cognizant of the fact that inheritance at the social and behavioral level are different compared to genetic and epigenetic level, Jablonka and Lamb (2014) describe four properties of Behavioral Inheritance Systems (BIS) that are founded on a fusion of collective-individual activity devoid of genetic hierarchy. They argue that

> With variation transmitted by the symbolic system, there is a quantum leap in social complexity with families, professional groups, communities, states, and other groupings all influencing what is produced in art, commerce, religion and so on. Construction plays an enormous role in the production of variants, yet because symbolic systems are self-referential, the rules of the systems are powerful filters. The ability to use symbols also gives humans the important and unique ability to construct and transmit variants with the future in mind (Jablonka and Lamb, 2014)

In his analysis of evolutionary theory using dialectics, Julio Munõz-Rubio (2018) argues that this mechanism of inheritance is essentially a dialectical one since Jablonka and Lamb's work implies the evolutionary process to be a synthesis between the genetic information and the environmental influences, which Levins & Lewontin (1985) had described to be conceived as "two opposed, active, and mutually selective elements", thus forming "a dialectical *Aufhebung* of the organism-environment"(Munõz-Rubio, 2018).

3.4 Principles for a Theory of Organisms

Since the Molecular Biology revolution in the 1950s with Watson & Crick's discovery of the structure of DNA and the consequent establishment of Central Dogma of Molecular Biology, experimental biology has been steadily alienated from its theoretical counterpart. This is not to say that biological theories didn't

exist, but was rather abandoned as a storage of ideas from which to generate hypotheses. Increasingly, in the frenzy of "hypotheses-driven" science, aided by a genetic deterministic outlook and advanced sequencing techniques, there has appeared a reductionist science which fails to recognize the nascent contradictions between experiment and theory. A simpler version of this can be found in large genetic screen studies for complex diseases with the follow-up occurring only with a handful of genes, while at the same time the experiment is already biased by establishing a hypotheses *a priori* without a proper theoretical framework.

The scarcity of a proper biological theory of the organism, one which would be a complement to evolutionary theory but would describe the life cycle of the organism from conception to death, was recognized by the ORGANISM group (Soto, Longo and Noble, 2016). In an attempt to fulfill that absence, the group established three major principles that would serve as the basis for a theory of the organisms that would refute the dominant reductionist understanding of phenomena at multiple levels of biological organization. These principles were established on the basis of two important realizations - 1) there exists differences between inert and living that require separate theoretical development and 2) in biology, "ontogenesis and evolution are about relentless changes of symmetries, and the phase-space is being created along rather than set *a priori*" as compared to physics (Ibid). These realizations are also attempts to dispel the borrowing of theories from other fields, mainly physics, to explain biological phenomena, which has also resulted in the adoption of vernacular from information theory to describe biological interactions, such as "program" and "signaling", with the implicit understanding that organisms are machines (Nicholson, 2013).

The principles for a theory of organisms are as follows (Soto, Longo, Miquel, *et al.*, 2016) -

1. A principle of biological inertia: the 'default state' of proliferation with variation and motility.
2. A principle of variation that accounts for the emergence of novelty through development and evolution
3. A principle of organization that accounts for the stability of organisms.

These principles present a radical transformation for experimental biology - attributing the organism with the ability to create their own "norms" (Ibid) shifts the view from the organism from being a passive agent of change, as articulated by findings from *in vitro* tissue culture studies over the decades, to one where the organism's default state is constrained by the environment; in fact, as both theoretical and experimental studies show, organisms act on their environments to create constraints on their own mobility and proliferation and therefore results in organization (Barnes *et al.*, 2014; Montévil *et al.*, 2016). In fact, these principles are able to resolve long-standing confusions within the cancer research field - the Tissue Organization Field Theory (TOFT) that posits the default state of cell as proliferation with variation and motility and that cancer is a tissue-based disease, along with the principle of organization, shows that carcinogenesis arises from the disruption of interactions between the stromal and epithelial compartments of the tissue (Sonnenschein and Soto, 2016). TOFT also provides explanation for emergent properties observed within carcinogenesis, which the dominant reductionist Somatic Mutation Theory (SMT) is unable to (recall Weinberg's admittance in the Introduction section) (Soto and Sonnenschein, 2005).

Although the derivation of these three principles are separate, it is abundantly clear that the laws of dialectics can be abstracted from these principles and their use. At first glance, it is obvious that these principles and dialectics both share the anti-reductionist nature, and stress on the importance of interactions between the organism and its environment, and among the multiple levels of biological organization. Both Hegelian dialectics (thesis, anti-thesis and synthesis) and Engels' dialectics of nature are in concordance with these principles - the "incessant breaking of symmetries" (Longo and Soto, 2016) by organisms can be viewed as a constant flow of thesis, anti-thesis and synthesis; Engels' dialectics of nature, a la Levins & Lewontin and Gilbert & Tauber, is also observed within the applications of these principles to biological phenomena - the first law is exemplified by phase-space changes and symmetry-breaking, the second law is manifested in TOFT and the third law in the negative control of cell proliferation that is based on the default state (Soto, Longo, Montévil, *et al.*, 2016).

4. Towards a Radical Science

> It is important to emphasize that the way science is is not how it has to be, that its present structure is not imposed by nature but by capitalism, and that it is not necessary to emulate this system of doing science. (Levins and Lewontin, 1985)

The above evidence presented from biologists make it clear that contrary to forcing dialectics on nature, it appears that biologists have developed similar fra-

meworks (systems biology, the pluralistic extension of evolutionary theory, and the principles for a theory of organisms) to understand complex phenomena. This realization might raise the question of whether Marxist philosophy is actually needed to resolve the crisis in modern biology. The answer to that, in my opinion, is a resounding yes, precisely because a key tenet of Marxism is missing from science. While parallel developments have been made in the epistemological arena, the practice of science severely lacks any understanding of labour and the process of production. Science is still very much in the grips of the capitalist mode of production, and the bourgeois philosophy that guides the research paradigms cannot be separated from the bourgeois practice of science.

4.1 Lysenkoism and Marxist Biology

A discourse in Marxist biology is incomplete without any reference to Lysenkoism, a particular set of agricultural practices and scientific ideas of heredity based on Trofim Lysenko's understanding of dialectics of nature. However, while Lysenko's science may have been dubious (Gordin, 2012), it should be noted that Lysenkoism represents the confluence of political, economic and scientific factors that led to the controversial ideas about genetics and subsequent applications in agriculture during Stalin's regime in the Soviet Union (Levins and Lewontin, 1985; Clark and York, 2005; Gordin, 2012; Sheehan, 2018). The political aspects of Lysenko's meteoric rise to power in Stalin's government is described elsewhere and not the focus of this section.

Lysenko's proposed theory of heredity ignored the existence of genes (but did acknowledge the existence of chromosomes), and posited that heredity was based solely on the interaction between environment and the organism, and therefore intentional changes to the environment can direct organismal growth. In this formulation, however, the organism becomes the passive object of change rather than an active agent. Moreover, the codification of Engels' dialectics of nature, as viewed by Lysenko and his followers, removed any possibility of chance as an ontological property (Levins and Lewontin, 1985). But as explained above, Marx's dialectical materialism based on Epicurean materialism, included chance as an ontological property. Therefore, Lysenkoism did not fully represent Marxist philosophy, and became the "vulgar Marxism" that it had sought to abolish in the natural sciences. It is then quite unfortunate that Lysenkoism continues to be held up by the West as Marxist science (Kean, 2017) when it actually went against the tenets of Marxist philosophy which advocates for unity of structure and process, and the wholeness of things based on interactions of its parts. It should be noted that the resurgence of Lysenkoism in Russia in the last two decades have been not because of a better understanding of Marxist philosophy, but rather a confluence of geopolitics, anti-science sentiments and scientists who, with the advent of epigenetics, trying to rehabilitate Lysenko (Kolchinsky *et al.*, 2017). However, as pointed out above and also by Kolchinsky et al (2017), the problem with Lysenkoism lies at the ideological level, but not due to the incorporation of ideology in the sciences.

Considering the evidence presented above, it can be concluded that biologists have arrived at a very similar view of organism, environment and natural history as dialectical materialists had proposed. In some way Lukács was right - the contemporary knowledge was not sufficient to validate Engels' dialectics of nature; but he was also wrong in concluding that therefore Engels' laws are unusable for understanding our natural world. The resolution of the crisis in modern biology cannot be achieved just through introduction of theories. As Bernal concluded after his analysis of scientific practice under both capitalism and socialism, the crisis in science is an "inescapable feature of the capitalist mode of production" (Sheehan, 2018). Similar sentiments have been echoed by later scientists, whether they identified as Marxist or not. The common theme between them was the realization that scientific practice is not ideologically neutral, and the analysis of science under capitalism has shown a widespread "abuse" of science historically (Rose and Rose, 1972).

It's not only scientists that realized the heart of the problem lay in the bourgeois practice of science. The British Marxist Christopher Caudwell (born St. John Spriggs), argued that the conflicts in biology was due to the dualistic nature of bourgeois culture itself and the resolution of the conflicts lay in breaking out of it.

4.2 A Science for the People

Current science is considered to be apolitical and rational, and free of value judgement. This illusion, created by decades of entrenchment of bourgeois philosophy, especially after the disastrous effects of Lysenkoism, has quietly transformed scientists and trainees into the "biomedical workforce", a proletarianization of scientists to speak (Levins and Lewontin, 1985; Lazeb-

nik, 2015). In contemporary society, scientists occupy what has been termed as the professional-managerial class (PMC; Press, 2019) that exists between the working and the ruling class. Historically, while the PMC has understood the necessities of the working class, their allegiance has unfortunately been with the ruling class (Winant, 2019). Considering that biomedical researchers are increasingly encouraged to become entrepreneurs (one only needs to look at the number of startups on university research campuses), it is understandable how the ruling class stands to benefit from maintaining the distinction between the PMC and the working class (trainees, staff, custodial workers, etc). A quick look at the state of academics in the US universities reveal that while some academics may enjoy a greater status and income in this current capitalist order, the majority of the biomedical and scientific research workforce are getting squeezed harder and harder. Tenured and adjunct faculty (Hasan, 2016b; Birmingham, 2017), graduate students (Academics Anonymous, 2018) and postdocs (Nature Editorial, 2018), and even undergraduate students (in the form of skyrocketing tuition costs; Maldonado, 2018) are all exploited for their labor as they face more and more restrictions on their rights as workers as universities relentlessly pursuit capital accumulation. This exploitation has resulted in a mental health crisis (Flaherty, 2018) among graduate students, and threatens the productivity or the state of research altogether. At the same time, the US universities are experiencing an administrative bloat (Tufts Daily Editorial, 2017) with increasing salaries for university presidents (Bauman, Davis and O'Leary, 2019).

The steady neoliberalization of universities (Seal, 2018) also coincide with the alienation of theory and practice within science, in an effort to remove any ideological influence. Unsurprisingly, considering the sociopolitical history of capitalism, science has historically been used to uphold the *status quo* of the bourgeoisie, regardless of the outright racist, sexist, oppressive and other discriminatory consequences, and will continue to do so unless the grip of bourgeois culture has been broken in scientific research. While liberal critique of science sees instances such as the Tuskegee syphilis experiment and sociobiology as isolated incidents of abuse of science, this reductionist interpretation fails to incorporate a historical analysis, which again points to the need for a Marxist philosophy in the practice of science. Early attempts to create a proletarian science, such as Bogdanov's Proletkult in the Soviet Union or collectivization of scientific workers in the US in the form of workers unions, did not survive the changes in the political landscape for various reasons.

In writing for the revitalized *Science for the People* magazine, Helen Zhao (2019) discusses how science, both theory and praxis, can be radicalized and what the movement's goals should be. Reviewing the comments from a host of scientists-activists, she asks "where do the 'experts' and 'expertise' belong - if anywhere - in a science emancipated, a science for the people (Zhao, 2019)?" The answer can be found in Caudwell's formulation of proletarian science, as described by Sheehan (2018),

> For Caudwell, proletarian science was the integration of sciences (...) within an integrated world view. Caudwell said quite firmly that it was not a matter of imposing the dictatorship of the proletariat on science. It was not a matter of the honest worker telling the scientist what was what in his laboratory or in his theory. Nothing was to be imposed on science. Nothing was to be imposed on the scientist, not even by himself. It as a matter of assimilation of the scientist to the cause of the proletariat, to the construction of a new society in which he played his full part within the process and as a scientist. Science was to be developed by scientists, but a new type of scientist, with his feet more firmly on the ground, with his mind more opened to the whole, with his life and work more organically connected to the society of which he formed a part.

5. Conclusion

The evidence presented above supports the proposal that Marxist philosophy of dialectical materialism, is poised to provide a resolution to this crisis. The application of dialectics has been observed at key stages of theoretical development in modern biology, and can be further used a heuristic device alongside the ORGANISM group's principles for a theory of organisms, Jablonka and Lamb's evolutionary theory, Gilbert and Tauber's concept of the holobiont as the biological individual, and Minelli's theory of development. However, science does not exist in isolation and only Marxist philosophy can guide the transformation required in the practice of science that is required to break out of the clutches of bourgeois culture and philosophy. Therefore, the crisis cannot be resolved unless there is unity between theory and praxis, as Marx proclaimed "philosophers have hitherto only interpreted the world in various ways; the point is to change it."[2]

[2] Eleventh thesis on Feuerbach (1845)

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Aristotle and the search of a rational framework for biology

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Abstract

Chance and necessity are mainstays of explanation in current biology, dominated by the neo-Darwinian outlook, a blend of the theory of evolution by natural selection with the basic tenets of population genetics. In such a framework the form of living organisms is somehow a side effect of highly contingent, historical accidents. Thus, at a difference of other sciences, biology apparently lacks theoretical principles that in a law-like fashion may explain the emergence and persistence of the characteristic forms of living organisms that paradoxically, given the current importance attributed to chance, can be grouped into organized structural typologies. Nevertheless, the present essay shows that since its origins in Aristotelian natural history, biology aimed at achieving rational, non-accidental, explanations for the wide variety of living forms endowed with characteristic behaviors that constitute the landscape of biological species.

Keywords: attractor, biological form, hypothetical necessity, neo-Darwinism, scientific explanation, teleology

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1. Introduction

> ...when any one of the parts or structures, be it, which it may, is under discussion, it must not be supposed that it is its material composition to which attention is being directed or which is the object of the discussion, but the relation of such part to the total form. Similarly, the true object of architecture is not bricks, mortar, or timber, but the house; and so the principal object of natural philosophy is not the material elements, but their composition, and the totality of the form, independently of which they have no existence. (PA, I. 5, 645a 30-35)

The explanation of natural phenomena is a central goal of science. However, there is a need for criteria able to discriminate between scientific explanations and other sorts of explanations such as mythological, religious, traditional, etc. Scientific explanations are based on rules or principles considered as natural laws. Thus scientific explanations are attempts for establishing nomological (according to law) connections between phenomena (Kim, 1964). Every explanation constitutes and argument or set of arguments but true explanation is achieved when we become aware that the phenomenon explained has been fitted within a system of rationally justified beliefs present in our mind (Ponce, 1987). An important aspect of scientific explanation is the absent or minimal appeal to historical accidents, as the explanation is supported on principles that determine the course or organization of natural systems. However, the current standing of neo-Darwinism as the central guiding paradigm in contemporary biology, implies that historical explanations are the mainstay in biology. This situation derives from Darwin's assumption that living organisms continuously undergo small but random hereditary changes on which natural selection impinges, thus selecting the variants that are better adapted to the standing environment. Therefore, evolutionary change depends on the continuous random variation among the individuals that constitute a given species. Yet behind this assumption reasonably supported by evidence, there is another one rather questionable but sponsored implicitly or explicitly by most neo-Darwinists: any sort of life form may result from such piecemeal variations provided that it survives (Denton, 1988). Thus, it is assumed that living systems may display any set of phenotypical traits if such traits happen to be adapted to the current environment.

According to this view there are no laws or principles of structural and functional organization proper to biology and so, biology becomes a collection of historical narratives; for example: which species derives from

which ancestors and under which historical circumstances, since the only necessary constraint is survival. Thus at a difference of other sciences, such as physics and chemistry, in which basic, law-like principles of organization allow to understand the observed structures in terms of regularities, biology is not intelligible in a law-like fashion but only in terms of survival, given that natural selection is currently posed as the only true explanatory principle in biology (Dobzhansky, 1973). Therefore, neo-Darwinian biology lacks principles able to explain why it appeared such a robust structure as the tetrapod limb and so, it should be acknowledged that the theoretical framework of current biology is rather limited when compared to the intellectual milieu of the rational morphologists from the XVIII and XIX centuries, such as Richard Owen, Geoffroy Saint-Hilaire or Georges Cuvier.

Modern genetics sustained by molecular biology provides reasonable mechanistic explanations for a vast number of phenotypical traits that distinguish the members of a given species from those members of another species. Such piecemeal variations are the fuel of speciation, understood as the superficial diversification of a basic morphological type, given that such a process do not lead to the emergence of new organs or structures or in other words, such piecemeal variation explains microevolution but it cannot explain macroevolution. Moreover, genetics cannot explain the origin of the whole form typical of a given species. These limitations pose the question of whether the explanations based on historical accidents are truly the mainstay for biology and so this scientific discipline must renounce to achieve rational explanations supported on law-like principles. However, a survey of the biological treatises of Aristotle, acknowledged in the Western tradition as the founder of biology, suggests that since its origins biology aimed at finding general principles, supported on both observation and reason, so that the explanations based on chance or historical accidents would perform only a marginal role in biology.

2. The biological treatises of Aristotle

The term biology appeared for the first time in Lamarck's *Hydrogeologia*, published in 1802, and there is no equivalent term for it in the works of Aristotle and yet, it is an accepted fact that the collected works of Aristotle contain a large number of observations and theoretical reflections concerning issues that in current terms fall within the domains of zoology, comparative anatomy, physiology, embryology, botany and ecology, disciplines that conform a large portion of the contemporary landscape of biology. For Aristotle the study of living beings was a fundamental aspect of the study of nature. Indeed, a survey of the classical Becker, reference edition of Aristotle collected works, indicates that from a total of 1,462 pages, 426 (~ 30%) deal on biological subjects. The main Aristotelian works dealing on biological matters are History of Animals (HA), Parts of Animals (PA), Generation of Animals (GA), Movement of Animals, Progression of Animals and On the Soul (OS). On the other hand, careful analysis carried out by some classical scholars in the past century, indicated that Aristotle's biological treatises correspond to his mature philosophical perspective that illuminates his logic, physics and metaphysics (Grene, 1963, During, 1966). However, although others scholars have challenged this conclusion (Graham, 1986) it is hard to refute that Aristotle's biology is a foundation stone for his philosophy (Thomson, 1913; 1940).

3. The concept of nature in Aristotle

For Aristotle the most important concept in relation to nature is that of end or purpose (*telos*). Aristotle applies this concept to both animate and inanimate objects as he assumes that any material body has a specific nature or essence (*physis/ousia*) that rules its behavior and so it drives the material body to find a proper place or to achieve a particular condition. Therefore, terrestrial objects move towards the center of the Earth while fire rises up and away from the center. For Aristotle change/movement is the process by which a given nature (essence) may achieve its inherent purpose. When such a purpose is not yet realized, it is regarded as being potentially (*dynamis/dunamis*) but when that purpose is achieved then it becomes actualized. Thus, Aristotle defines change/movement as the actualization of a potentiality, and such actuality (*entelechy*) of a former potential is an end or completion of something (During, 1990, p. 952-956). This view is quite in contrast with that of contemporary physics in which the laws of movement are the same for any sort of matter. Indeed, modern physics considers matter as constituted by finite kinds of elementary particles and that the whole phenomenology of the universe is the consequence of the positions and movements of such elementary particles. However, Aristotle was skeptic that the mere spatial movement of particles could explain all types of change, for him the qualitative differences among substances were real and

so qualitative changes cannot be reduced to variations in the position or movement of elementary particles. That is why he rejected the atomic theory of Democritus. Thus, Aristotle postulates that there is a prime matter (*proto-hyle*) that is a general, indefinite substrate with potential to be transformed into a specific substance when it is endowed with a specific form (*eidos/morphe*/ soul). Such a form is the essential property of a given substance that becomes what it should be. Therefore, all material bodies are conceptually conformed by matter and form (*hylomorphism*) that are in the same relationship as potency and act: prime, non-differentiated, matter has the potential to become something when it receives a given form (it becomes informed matter able to be perceived by the intellect). Thus. Aristotelian matter is quite different from the matter of contemporary scientific materialism. For example, in Aristotelian terms the head of a sculpture has the shape but no the form of a real head, since it cannot perform the functions and purposes of a real human head. Moreover, for Aristotle a human corpse has lost its soul which is the form of the living human and as such the corpse has lost its human essence, because what a thing is, is always determined by its function: a thing really is itself when it can perform its function; an eye, for instance, when it can see (Meteorology, IV. 12, 390a, 10). It must be stressed that for Aristotle the soul (*psyche*) is not a supernatural entity but it is the 'first actuality' of a natural body that has life potentially (OS, II. 1, 412a, 27). Thus, the soul of a living thing is the capacity to engage in processes or activities that are characteristic of the natural kind to which such a living thing belongs. Soul is the first actuality that drives animal development, that consists in a serial passage from potentiality to actuality, so that each actualization results in a further potentiality until the developing system achieves its end (*telos*): a fully completed and functional embodied form which is the culmination of such a development. Therefore, soul is the form of a living thing understood not as its figure or shape but as its actuality: that in virtue of which it is the kind of living thing that it actually is.

4. Causality in Aristotelian science

For Aristotle in order to proceed to explain something, we must first consider the following questions: what is the thing to be explained? And why there is such a thing? Both questions cannot be answered without the use of reason and so the explanation is equal to a reason for something to be, for something to occur (a *logos*). The *logos* establishes a relationship between the notion to be defined with another, more fundamental and defining notion, or a relationship between a given statement and another, foundational, demonstrative or proving statement. Our rational beliefs are organized upon such explanations.

From the Aristotelian perspective, any theory aiming at explaining the facts in the universe depends on the principle of causality. Therefore, we must ask what is a cause? Perhaps this word of the common language had its origin in the common human experience that through our deliberate actions we can produce changes in the real world. Such changes are the effects and the actions that produce them are the causes. Bertrand Russell in a famous and very critical paper on the classical notion of cause (Russell, 1912) noticed that the concept of cause is somehow linked to the notion of will (volition). Science, for Aristotle, is the knowledge based on causes, and the notion of cause is derived from the notion of principle. Thus every cause is a principle but not every principle is a cause. Therefore, a given cause is a principle for some things. In Aristotelian science a principle is everything from which something begins and a cause is everything from which something starts, either as movement or being. At the ontological level the causes are the principles of being but at the logical level the causes are the principles of knowledge. The demonstrative principles that rule the process of science result from abstractions based on causes, thus science is causal knowledge. The cause gives reason to the effect, phenomenon or thing and as such is both a principle of universality (conceptualization) and a principle of argumentation (demonstration). Therefore, given that cause is a foundation of reason, it becomes the core of any explanation.

5. The four Aristotelian causes

In classical times the notion of cause was loosely defined until Aristotle undertook its analysis. The Greek term *aitia* used by Aristotle refers to everything that contributes to an effect and he suggested that for constituting a new object we should consider four aspects: first we must consider the stuff necessary to make the object, this is the material cause. Then we must consider that which provides its specific nature to the object, this is the formal cause. Next we need to consider that which introduces the formal cause into the material cause, this is the efficient cause. Finally, we need to consider the reason or purpose by which the efficient cause acts on

the formal and material causes, this is the final cause. For example, when producing a statue depicting blind justice, the material cause is the block of marble, the formal cause is the figure to be sculpted upon the marble, the efficient cause is the craftsman with his tools sculpting the marble and the final cause is the concept or idea (blind justice) represented by the statue. Thus the Aristotelian doctrine of causality consists in determining the relationship among the four types of cause and somehow reinforces the link between the idea of cause and the notion of will since for example, in the Aristotelian cosmos the efficient cause of the celestial movements is an intelligence that operates in a fashion analogous to human will. Nevertheless, we must be careful to point out that in the Aristotelian worldview the final cause does not implies the notion of an intentional agent operating for achieving the constitution of a given object. For Aristotle the notion of final cause is close to that of function or purpose, so that the final cause for the eye is vision without implying a conscious designer shaping the eye for the purpose of vision.

The current and common confusion of the final cause with a conscious agent results from a theological interpretation of Aristotelian causality that equals divinity with the final cause. However, since Galileo the trend in science is to consider only material and efficient causes when explaining the natural phenomena, and the ignorance of final causes is perhaps the consequence of avoiding, at any rate, a hint of religious outlook that may interfere with a neutral, objective description of nature. Nevertheless, for Aristotle the final cause is the main cause, because it causes the causality of the other three causes as they align towards an end. However, when considering the explanation of something Aristotle assigns a chief role to the formal cause because the final cause is extrinsic while the formal cause is intrinsic to the process or phenomenon to be explained. Thus the formal cause is the one that unifies all the other causes. The causal perspective on knowledge and explanation in Aristotle aims at achieving the intelligibility of the formal cause that is: of form. Aristotelian science proceeds by means of classification and argumentation, and in these cognitive endeavor the fundamental element is form. Thus in material bodies the part that bears intelligibility is form while the one that poses a limit to cognition is matter. From the epistemological perspective what is truly universal is that which is conceptualized be means of abstraction and this corresponds to form. Therefore, what we may truly know about matter is always through form and in relation with form. Hence, formal cause or form as such is both the principle of intelligibility and of universality.

Aristotle also suggested that causes might be grouped in pairs (GA, I. 1, 715a, 5-10). Sometimes the final and the formal cause can be considered as a single or the same cause, while the material and efficient causes may also be considered as very close entities. In PA book I, Aristotle describes his general method for the study of biological phenomena and yet there is no mention in it of the notion of natural law. In Aristotle's epoch the notion of law was only applied to political or moral issues. However, the Aristotelian way for describing natural phenomena strongly suggests the notion that there are regular, constant relationships between phenomena. Aristotle enunciates rules and principles akin to the contemporary notion of natural law but with a fundamental difference: he never suggests that such principles universally apply in a ruthless fashion, instead he suggests that they correspond to that which more often occurs, to that which generally happens. Moreover, the most fundamental principle that sustains Aristotelian biology it is never explicitly stated, although it is implicitly present in all the biological treatises after HA: all vital phenomena depend on natural causes, since Aristotle never considers non-natural or supernatural causes for explaining biological phenomena. Thus, when Aristotle describes some monstrosity or biological anomaly, he never recurs to the action of displeased or malignant deities but explains these phenomena as the result of the interplay between natural causes. However, like Plato, Aristotle doubts that natural phenomena may occur only for mechanical reasons. Indeed, in the first chapter of GA, Aristotle affirms that the production of natural phenomena requires the four types of cause. However, for Aristotle it is the final cause the one with the largest capacity for explaining biological phenomena, even though he acknowledges that the material cause is important for explaining the accidental differences among members of a species, such as color of the eyes, of the skin, the pitch of voice or even monstrosities, given that such differences have no particular purpose.

6. Aristotle on necessity

Necessity has its origin in matter but Aristotle distinguishes two types of necessity: a simple one that only applies to things that are forever, things the causes of which cannot be other than they are, and another one that operates in the living world; the hypothetical necessity (Physics, II. 9, 200a, 13) that depends on an end

beyond itself, since the nature (form) of a living thing is the internal source of change within itself, the organizing principle that directs its development towards its particular end. If nature as form is prior to nature as matter, nature as that-toward-which, nature as end, is the biological manifestation of nature as form. Therefore, that what shall be, the culmination of development, controls necessity. Such is a necessity that flows backwards from the achieved *telos* to the process that leads to such an end or towards the structure of the parts that contribute to such an end. For example, in contemporary terms, the several global or local organizers described in varied embryonic developmental processes, such as the Spemann organizer in amphibians, the Hensen node in the chick, and the equivalent node region in the mouse, might be the embodied manifestations of the hypothetical necessity that establishes a set of 'attractors' along the developmental pathway that allow us to rationalize in a retrospective fashion the process of ontogeny, in the same way that a satellite view of an earthly landscape allows us to understand and then to predict the course taken by water flowing upon such a landscape in its relentless voyage towards the ocean. Thus, necessity subordinated to end is what according to Aristotle the true naturalist/biologist is seeking to understand. Moreover, for Aristotle the function of each part, of each organ can only be fully understood by relation to the whole:

> For no bone in the body exists as a separate thing in itself, but each is either a portion of what may be considered a continuous whole, or at any rate is linked with the rest by contact and by attachments... And similarly no blood vessel has in itself a separate individuality; but they all form parts of one whole (PA, II. 9, 654a, 34-37; 654b 1-3).

7. Hypothetical necessity and contemporary attractors

For any dynamical system the phase space is the abstract space in which all possible states of the system are represented, with each possible state corresponding to a unique point in the phase space. That part of the phase space corresponding to the typical behavior of the dynamical system is known as the attracting set or attractor. More formally, for a dynamical system an attractor is a closed subset Γ from the system's phase space so that, despite starting from multiple possible initial conditions, the system evolves towards that set. There is a debate on the origin of the concept of attractor, since attractors consisting of more than one point seem to have been first considered by Auslander, Bathia and Seibert, in a mathematical paper from 1964. However, also there is evidence that this neologism was already used in 1966 by the Fields' medal mathematician René Thom to whom Stephen Smale, another Fields medal winner, attributes the neologism, (Thom, 2016). In any case, the concept of attractor reintroduces the final cause in the discourse of contemporary science. Attractors may be classified as steady-state, periodic or chaotic, but in essence any attractor corresponds to a steady-state akin to a state of minimum free-energy at the bottom of a "well of potential" that corresponds to a basin of stability, the basin where the attractor exerts its "strongest attraction", thus precluding the system from leaving it too easily or not at all.

Early in the twentieth century Hans Driesch experimentally demonstrated the teleological behavior of embryonic developing systems, by showing that a living embryo self-regulates to form a whole organism despite the removal of a significant part of its constituting material (in this case, one whole cell or blastomere from an early two-cell stage embryo). Thus, at a difference of a purely mechanical device, the embryo remains a whole after the removal of some of its parts. Driesch fully assumed the epistemological consequences of such finding when suggesting that a guiding *entelechy* explains the wholeness and teleological behavior of embryonic developing systems (Driesch, 1908). This position is quite different to materialistic reductionism in which a living process is just a particular case of material processes in general.

The concept of potentiality generally refers to any "possibility" that a thing can be said to have. Nevertheless, Aristotle did not consider all possibilities the same, and emphasized the importance of those that become real of their own accord when conditions are right and nothing stops them (Sachs, 2015). On the other hand, actuality is the motion, change or activity that represents the exercise or fulfillment of a possibility, when a possibility becomes real in the fullest sense (Durrant, 1993). Entelechy is an ancient Greek neologism (*entelécheia*) coined by Aristotle, that very often has been translated as 'actuality' (anything which is currently happening) but more recent translations suggest "being-at-work-staying- the-same" or "being-at-an-end" (Sachs, 2005). Entelechy is then a kind of completeness, a continuous being-at-work, a specific way of being in motion. All things that actually exist are beings-at-work, and all of them have a tendency towards being-at-work in a par-

ticular way that should be according to their proper and "complete" nature. Thus Driesch suggested that living things develop by entelechy, a purposive and organizing field that he conceived as "mind-like", that is: non-spatial, intensive, and qualitative rather than spatial, extensive, and quantitative (Driesch, 1908). Indeed, Driesch approach for explaining organic development was rooted in vitalism, understood as the notion that the processes of life are not explicable by the laws of physics and chemistry alone and so, that life is somehow self-determining.

The rise of molecular genetics in the second half of the twentieth century leads to a shift in the kind of experiments used in experimental embryology so that now most experiments on this topic are designed for putting into evidence the role of genes and their products as determinants of embryonic development. Obviously, such experimental designs are not the right framework for studying things like entelechy. Indeed, experiments are on the one hand narrow windows and, on the other, contrived schemes for observing or asking questions to natural systems. Any experimental set up depends on implicit and explicit theoretical assumptions and that includes preconceptions or prejudices about the workings of nature. Therefore, experiments can only produce a limited set of answers that may be biased by the theoretical background. In other words, depending on the experimental system used, we may only see what it is already expected to be seen. On that account, the presence or activity of entelechy cannot be documented through the looking glass of the current experimental approach in reductionist biology, that discards formal and final causes from the causal analysis by concentrating only in the material and efficient causes. This is exemplified by the following mock experiment, suggested by René Thom: a fast car coming from an avenue crosses a bridge upon a river and gets into a further road where it hits and kills a passing pedestrian. The authorities want to determine what caused the death of the pedestrian. Thus, they fit a dummy in the original position of the killed pedestrian and then run a fast car starting from the original avenue but then blow up the bridge and so the car fells into the river unable to hit the dummy. From this experiment they conclude that the standing bridge was the cause of the pedestrian's death. As pointed out by Thom, a lot of current experimental biology is carried out according to this weird experimental logic (Thom, 1990a).

Attractors imply the actualization of a potential, hence when the system is at or "within" the attractor it may be said that it is being-at-work-staying-the-same or being-at-an-end. Moreover, since the attractor regulates the behavior of the parts or elements of the system (agents), this is a case of top-down or downward causation (from the complex or global to the simple or partial), completely different from the bottom-up causation that tries to explain the behavior of a complex system as the additive result of the properties of its elementary constituents. In principle, when a dynamical system is not yet in the attractor such an attractor lies in the future of the system. Thus, by definition attractors are non-spatial entities, at least not in Euclidean space. Even more, an attractor corresponds to a form of behavior or activity for the system and as such it is a qualitative entity besides being intensive, as it determines the behavior of the system once "within" the attractor. Therefore, all the properties attributed by Driesch to entelechy can be also predicated about attractors. For many dynamical systems there is more than one attractor, and the development or evolution of very complex dynamical systems (such as living systems) implies visiting several attractors in time until reaching one among those included in the set with foremost stability.

Purely physical self-organizing systems such as the Belousov-Zhabotinsky reaction, currents in electrical circuits or the atmospheric winds, have their specific attractors (e.g., the BZ, van der Pol and Lorenz attractors) for which there are defined mathematical descriptions. However, things like cellular phenotypes or the behavior of living flocks correspond to higher-order attractors for which no thorough mathematical description exists for the time being. We may conceive further higher-order attractors that correspond to the typical morphologies of whole living systems. If such is the case, then evolution of life on earth would not be just a chancy, historical and arbitrary process (as claimed by neo-Darwinism) but an exploration of life's phase space in which there is a collection of attractors that correspond to possible stable typologies that define an Aristotelian *scala naturae* or great chain of being (Bynum, 1975). Therefore, although there is a common basic mathematical definition that may be applied to any attractor, there are different categories of attractors (in the same fashion that Driesch suggested the existence of different sorts of entelechies) which cannot be reduced to a single common mathematical description, and so higher-order attractors cannot be reduced to lower level attractors nor systems bound by nature to lower level attractors can truly interact with higher level attractors.

Do attractors exist or are they mere intellectual constructions? And if such is the case, how it is possible for an abstract entity to influence a process with a material substrate? This sort of vexed question is characteristic of current biological science that is trapped within the mindset of naïve positivism and its fear of metaphysical entities. However, Thom suggested that science recurs to the theoretical perspective for reducing the arbitrariness of phenomenological descriptions engaged by proximate causes (Thom, 1980; Thom, 1990b) and for him any theory implies the existence of imaginary entities that are postulated to exist and correspond to the vectors of causality linking cause and effect (Thom, 1990a). Thus, in cosmology and physics one may speak of "superstrings", "time-warps", "gluons" or "charmed quarks" without worrying about the fact that such entities are not endowed with rock-hard materiality. The explanatory and predictive success of deep physical theories is based on introducing many levels of abstraction, from objects to microscopic entities to particles to force fields to probability distribution functions, and the like. All these theoretical entities are based on metaphysical requirements that are applied de facto by scientists when working with such theories (Margenau, 1977). On the contrary, in experimental biology there is fear, for example, of exploring a morphogenetic field that cannot be weighed, measured with a ruler or observed under the microscope. This limitation of current biology for assuming virtual or theoretical entities makes it walk in circles and thus hinders its possibilities for reaching deeper understanding.

8. The limited role of chance in Aristotelian biology

Chance is excluded from Aristotelian causality since for Aristotle fantasy and disorder cannot be causal factors in nature. Indeed, Aristotle considers that the same causes generally produce the same effects as he acknowledges a regular behavior in nature from which some general rules may be inferred. For example, he suggests that animals endowed with a large number of teeth usually live longer than those with a reduced number. Also, he suggests that animals that produce less yellow bile live longer than those that produce more of it. To the contemporary mind such statements may look useless or naïve but nevertheless they reflect the will to find general principles that correlate with specific biological phenomena. Thus Aristotle proposes that the character and sensitivity of an animal depends on the quality of its blood so that an animal with blood of a lesser density is more intelligent and vivacious, while animals devoid of red blood are generally fearful (PA, II. 4, 650b, 20-35). Animals with a large heart are generally shy while those with a relatively compact heart are assertive (PA, III. 4, 667a, 15).

Aristotle also derives some principles from his studies on comparative anatomy. For example, he proposes that only viviparous animals with lungs have epiglottis. Also he proposes that red-blooded animals always move using at most four points of mechanical support. Therefore, those animals that use more than four points of support are unlikely to be red-blooded. Thus, starting from the previous principle Aristotle explains why birds while red-blooded are biped, as they have two wings and so if they were endowed with four legs they would have more than four points of support, something that is impossible for red-blooded animals. Another Aristotelian rule of animal movement is that all animals with legs have them in pairs. In the case of quadrupeds Aristotle notes that such animals always move by a diagonal movement of their legs: the movement of the right anterior leg is followed by that of the left posterior leg, that one of the left anterior leg is always followed by that of the right posterior leg.

Some Aristotelian principles apply to the whole of the animal kingdom: all animals are made from the same natural substances or elements: earth, wind, fire and water, and all animals inhabit in one of these elements or in a milieu dominated by one of them. For example, fish in water and birds on air. Another general principle is that all animals must feed themselves in order to grow and develop; no animal escapes this rule no matter how ephemeral it might be. Indeed, in modern physiology survives the Aristotelian rule that when in an animal a small change in a first principle (such as gender/sex) undergoes a sudden change, then a number of details that depend on such a principle are also modified (GA, I. 2, 716b, 2-10). Aristotle offers the example of castrated animals, in which the elimination of small distinctive organs (testicles) leads to a transformation in body appearance, physiology and behavior of the animal. Thus:

> small changes are the causes of great ones, not per se but when it happens that a principle changes with them. For the principles, though small in size, are great in potency (GA, V. 7, 788a, 11-13).

Moreover, differences between the major animal families are also explained on the basis of the previous rule, for example:

> And so by the occurrence of modification in minute organs it comes to pass that one animal is terrestrial and the other aquatic, in both senses of these terms (HA, VIII. 2, 590a, 4-6).

Aristotle establishes a correlation between the celestial bodies, not generated and imperishable, and living beings, subjected to generation and corruption, for then affirming that both kinds of beings are worth of study and admiration since there is beauty in every work of nature. In animals, beauty is rooted in the subordination of the parts to become a whole so as to achieve an end or purpose, while in the case of celestial bodies the regularity of their movements are a manifestation of order in nature. Thus for Aristotle the vital functions are the subject of wonder in the same fashion as the regular movements in the heavens, as they bear witness to the existence of purpose in nature (PA, I. 5). Moreover, according to Aristotle the observation of the universe leads to the conclusion that nature makes nothing in vain and such a principle is also manifested in the properties of animals (OS, III. 12, 434a, 30-32). In both Aristotelian cosmology and biology nature always knows what it wants and where it goes, never acting lightly or capriciously. For example, the fact that fish do not have eyelids is not by chance but the consequence that such structures made for protecting the eyes from dust and air impurities are completely useless in water that poses a hindrance to sharp vision but where, according to Aristotle, there are less objects that may knock against the eyes and so, instead of providing eyelids to fish, nature has given them eyes of fluid consistency so a to counterbalance the opacity of water (PA, II. 13, 685a, 7-10).

9. The equilibrium and the economy principles of Aristotelian biology

The conformation of animals is for Aristotle the source of important considerations. He notices that a large number of animals present a bilateral symmetry and so they have right and left halves, therefore most organs are distributed in pairs. Such a symmetry is a manifestation of equilibrium and beauty. Thus the principle of equilibrium is fundamental for explaining the forms of animals:

> all influences require to be counterbalanced, so that they may be reduced to moderation and brought to the mean (for in the mean, and not in either extreme, lies their substance and account (PA, II. 7, 652b, 16-18).

Therefore, nature always knows how to compensate the excess of something by the juxtaposition of its contrary. The equilibrium principle is also the basis for the Aristotelian way of explaining the place occupied by certain organs and for justifying their role in the physiology of the corresponding animal. For example, given that Aristotle had no real clue about the role of the brain, but starting from the principle of equilibrium coupled to the notion that nature makes nothing in vain, he suggests that the brain is a counterpoise to the heart as container of vital heat, because the body needs a structure for attenuating the heat emanating form the heart, and such is the brain (PA, II. 7, 652b, 20-26). Moreover, the fact that in humans the tip of the heart is displaced towards the left side is not by chance, but for compensating the heat loss from the left half of the body which, according to Aristotle and for reasons related to the actual distribution of tissues, it cools down in man quicker than in other animals (PA, III. 4, 666b, 8-11). On the other hand, the spleen has its place in the left upper abdominal quadrant so as to be the counterpoise of the liver located in the right upper abdominal quadrant.

In Aristotelian biology the exceptional development of a function or organ always occurs at the expense of another function or organ. This is a most fundamental rule. Therefore, no animal possesses both tusks and horn, nor yet do either of these exist in any animal endowed with saw-teeth (HA, II. 1, 501a, 18-19) accordingly then:

> it would appear consistent with reason that the single horn should go with the solid rather than with the cloven hoof. For hoof, whether solid or cloven, is of the same nature as horn; so that the two naturally undergo division simultaneously and in the same animals. Again, since the division of the cloven hoof depends on deficiency of material, it is but rationally consistent, that nature, when she gave an animal an excess of material for the hoofs, which thus became solid, should have taken away something from the upper parts and so made the animal to have but one horn (PA, III. 2, 663a, 28-35).

In the case of birds, the development of legs can only occur at the expense of the development of wings for flying. Thus wading birds have solid legs but fragile wings and they have reduced the size of their caudal feathers because, according to Aristotle, the matter necessary for increasing the size of the legs it is obtained at the expense of the stuff necessary for making feathers. That is why wadding birds when flying use their legs as rudders, as they lack the large caudal feathers that other birds use for the same purpose. In case of crustaceans, the lack of claws in shrimp is explained on the fact that

they possess a larger number of legs than their lobster relatives.

The equilibrium principle also allows Aristotle to explain how the organism works. Thus for Aristotle it is not by chance that during pregnancy and lactation the menstrual cycle is suspended since the stuff for nourishing the embryo is equivalent to milk and similar to that shed with menstruation and so:

"*if the secretion is diverted in the one direction it must needs cease in the other, unless some violence is done contrary to the general rule. But this is as much as to say that it is contrary to nature, for in all cases where it is not impossible for things to be otherwise than they generally are but whether they may so happen, still what is the general rule is what is according to nature*" (GA, IV. 8, 777a, 16-21).

Aristotle also establishes a correlation between the typical size of the animals of a given species and their progeny, so that large bodied animals have less progeny than small bodied animals, and even in the vegetal world the smaller plants produce a larger number of seeds than the larger ones. Indeed, the following principle: "*every organism constitutes an ensemble, a unique and closed system in which all parts are mutually interlocked and concur towards the same action by means of reciprocal reaction*", known as the principle of organic and functional correlation, enunciated in the XIX century by Georges Cuvier, was based on the equilibrium principle of Aristotelian biology.

The principle of economy is another fundamental principle of Aristotelian biology. Such principle establishes that for obtaining a specific end or result nature always uses the least quantity of matter enough for achieving such a purpose. Thus the bones of vertebrates are not completely solid but more like thick but hollow tubes. The great length of the intestines is justified because it allows for a slower but more complete assimilation of food so as not to waste too much of it. In sharks the location of the mouth is justified so that they cannot swallow too much food in a single bite. Moreover, Aristotle notices that often nature use the very same organ for different functions. Thus the mouth has as primary function to be the gate for food ingestion but it also functions for the emission of voice and even as a defense or weapon (PA, III. 1, 662a, 20-24). However, this is not a universal rule as shown by the separation of the proboscis and the sting in bees, while in dipterans both parts and functions are integrated in a single organ. Therefore, nature does not apply the economy principle at any rate, instead the economy of organs and functions it is always for the sake of obtaining the best result for each particular species. For Aristotle the principle of economy is also manifested in the fact that nature provides specific organs only to such animals able to use them. Thus nature always provides the organ compatible with the function:

> For it is better plan to take a person who is already a flute-player and give him a flute, than to take one who possesses a flute and teach him the art of flute-playing. For nature adds that which is less to that which is greater and more important, and not that which is more valuable and greater to that which is less (PA, IV. 10, 687a, 13-17).

Aristotle differs from Anaxagoras who suggested that man was the most intelligent animal because it is endowed with hands. Instead, Aristotle suggests that man has hands because it is the most intelligent animal and as such man is able to use properly a large number of tools. Therefore, given that man is able to acquire and practice diverse techniques, nature has provided man with the most useful tool of all: the hand. The Sophists philosophers liked to suggest that man was an inadequate, badly constituted being since it comes about naked and barefooted but Aristotle challenges this view:

> For other animals have each but one mode of defense, and this they can never change, so that they must perform all the offices of life and even so to speak, sleep with sandals on, never laying aside whatever serves as a protection to their bodies, nor changing such single weapon as they may chance to possess. But to man numerous modes of defense are open, and these, moreover, he may change at will; as also he may adopt such weapon as he pleases, and at such places as suit him. For the hand is talon, hoof, and horn, at will. So too it is spear, and sword, and whatever other weapon or instrument you please; for all these can it be from its power of grasping and holding them all (PA, IV. 10, 687a, 25-30; 687b, 1-5).

10. Conclusion

Undoubtedly, for the contemporary mind many rules of Aristotelian biology are plainly mistaken or supported by erroneous observations and premature generalizations. However, Aristotle was the first thinker suggesting the need of finding general rules or principles derived from observations and not from *a priori* philosophical considerations, since as shown by Aristotelian scholars: searching the works of Aristotle for scientific demonstrations based on *a priori* first principles is rather fruitless. Indeed, most of the arguments from

most of the treatises do not look like assertions of defining phrases followed by deductions from these. Most of them appear not demonstrative, but inductive, dialectical, or aporetic. They move from common experience or common opinions, weighing the views of others or analyzing difficulties, in hope of arriving at (but not starting from) an insight into some specific nature (Grene, 1972). Yet, Aristotelian scientific explanation aims at establishing the reasons for phenomena to occur. Thus Aristotle outlook differs from that of contemporary science more interested in how phenomena occur so as to achieve predictive power upon them, instead of searching for a deep understanding of occurring phenomena.

A basic Aristotelian principle that still permeates contemporary science states that the same causes must produce the same effects. This statement acknowledges the regularity of nature and so the possibility of achieving stable, communicable knowledge about nature. Inspired by this principle Aristotle tried to find rational explanations for biological phenomena.

However, an essential function attributed to causality is the possibility of inferring the future from the past and any system in which such inference is possible it is considered a "deterministic" system in which an event or sets of events are the determinants, that is the factors determining the system. Russell, as previously mentioned, was very critical of the old notion of cause and so he considered that that the statement "the same causes produce the same effects" was an unduly simplified, given that when the whole context of a phenomenon is considered then it looks very unlikely that the same cause produces the same effect as a matter of ruthless repetition (For example, while striking a dry match usually leads to ignition, striking a wet match not necessarily leads to its ignition). Instead, he suggested that the assumed sameness of causes and effects actually rests on the sameness of relations among factors involved in determining a phenomenon, as this is implied in the assumed constancy of natural laws (Russell, 1912). Thus, this sameness of relations is an empirical generalization from a number of natural laws which are themselves empirical generalizations (something completely in agreement with the Aristotelian outlook that derived its rules and principles from empirical observations). Therefore, instead of the old formulation that the same causes produce the same effects, what it is really assumed by modern science is the uniformity of nature that implies the permanence of natural laws, as precondition for the possibility of scientific knowledge.

Nevertheless, despite assuming the regularity of nature the Aristotelian outlook is not really concerned with the study of causes as a mean for achieving control upon nature (by being able to predict the future behavior of the natural system studied). Indeed, Aristotelian explanation is deeply associated with the need for making sense of natural phenomena, of finding meaning in them. This corresponds to understanding the form (or *logos*) of the phenomenon as well as its end or purpose (*telos*). This in contrast with current biology that explains all vital phenomena as events derived from chance and necessity within a universe lacking any sense or meaning (Monod, 1970; Dawkins, 1986). And yet, modern biology, that stretches from molecular biology to ecology, regularly uses teleological explanations (e.g., the shape of the beak in Darwin's finches is the right one for chipping the sort of seed that constitutes the meal proper to each kind of finch) which are usually understood as a way of talking, imposed on us by the limitations of human language. Thus, it is quite a paradox that contemporary biologists continuously recur to meaning despite their sustained effort for avoiding it.

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Original Articles

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Mathematical Modelling and Simulation of EMT/MET Biological Transitions

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Abstract

The capability of cells to alter their phenotype in response to signals is crucial to the understanding of different morphogenetic pathways. We focus presently on the case of Epithelial-to-Mesenchynaml Transition (EMT) and its reverse Mesenchymal-to-Epithelial Transition (MET), which are considered as a plausible mechanism at the base of tumours onset and spread. We propose a simplified mathematical model, consisting of two coupled differential equations, aiming to describe the minimal dynamics of Epithelial and Mesenchymal cells. Differently from many previous models arising in various contexts, the basic assumption is the presence of a cooperative-like structure between the two families determined by the presence of a source term (possibly nonlinear) involving cells of the opposite compartment, in addition to an inherent apoptosis term. Finally, being the Mesenchymal phenotype characterised by high-level motility, the presence of motion is included into its dynamics by means of a diffusive-like term. In case the source term is truly nonlinear and, as a consequence, multiple equilibria may coexist, propagating fronts connecting such different states can be numerically observed. For different values of the parameters, specifically the relaxation times σ and τ, the measure of invasiveness λ and μ, together with functions f and g, the model is capable to describe various directions of propagation, also suggesting a possible simple mechanism responsible for tumour reversion.

Keywords: phase transitions, reaction-diffusion systems, propagating fronts, finite difference schemes, wave speed approximation

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Introduction

In order to pursue investigation concerning cancer occurrence and development, mathematical modelling turns out to be a powerful tool of analysis experimental studies might rely on. Nowadays, indeed, trials in cancer research are one of the most challenging and interdisciplinary contexts, so that the possibility of improving strategies for approaching the subject to deliver better and faster results is imperative. A lot of effort is particularly made with the aim of developing suitable models that could account for the processes leading to tumour cells production and spreading. Although such techniques are typically bounded by limitations mathematical abstraction inevitably brings with it, the recognition of their relevance is increasingly perceptible inside scientific groups: the contribution in terms of predicting cells evolution, and potentially forecasting treatments, is remarkable, thus constituting an effective research path worth being deeply investigated.

Presently, we focus on the application to Epithelial-to-Mesenchymal Transition (EMT) and its reversal mechanism Mesenchymal-to-Epithelial Transition (MET). These are comprised among the experimental hallmar-

ks of cancer, as responsible also for the activation of invasion and metastasis (Hanahan and Weinberg, 2020, 2011; Magi et al., 2017; Thiery, 2006; Thiery and Sleeman, 2006; Thompson and Newgreen, 2005). Altogether, EMT and MET display dynamical behaviours which resemble those observed in physical systems during abrupt macroscopic changes between qualitatively separated stable states, also known as *phase transitions* (Davies et al., 2011). Also, such transitions are active in other important morphogenetic processes, such as early embryogenesis, tissue generation and wound healing (Kalluri and Weinberg, 2009).

More precisely, EMT is the process which allows a polarised epithelial cell, interacting with the basement membrane via its basal surface, to experience a phenotypical switch that permits it to acquire a mesenchymal phenotype. Such new epiphany is characterised by the loss of connectivity (usually as a consequence of down regulation of internal E-cadherin), improved migratory skills, enhanced invasiveness and elevated resistance to apoptosis. From a biomedical point of view, cells phenotypic differentiation turns out to be a crucial step for determining cancer onset and evolution. A characteristic element to be taken into consideration is that even gradual variation in a few control parameters and/or unknown densities can switch cells into distinct and specific phenotypes. The possibility of inducing MET, namely the reverse process of EMT, by means of some external stimuli, is giving rise to perform promising studies at the base of which lies the ultimate ambition to revert an apparently already sealed fate for cells having acquired malignant features.

Apparently, after some previous pioneering work, the first experimental studies showing the presence of a phenotypic transition have been published during the '80s (Greenburg and Hay, 1982). Nevertheless, the attention to this phenomenon has been limited until 2000, when the number of publications on these topics has drastically increased (Nieto, 2011). Since then, the exploration of the subject is considered as an emerging research front.

The most traditional approach is based on a bottom-up procedure, supposed to be predominant for describing how global structures are the result of underlying microscopic counterparts. Indeed, a large part of the literature is devoted to a detailed description of activators and inhibitors of the transitions as determined at molecular level, sometimes comparing an appropriately proposed mathematical model with experimental results. As an example, it is a well-known fact that transforming growth factor β (TGF-β) plays a pivotal role in EMT (Xu et al., 2009) and, thus, finding which elements could enhance its presence has been an issue in the last years (Snail, ZEB, Slug, Twist, ...). Correspondingly, up regulation of E-cadherin promotes the epithelial phenotype; thus, a big effort has been devoted to understand which are the specific ingredients (at the molecular scale) making its level growing (e.g. myo-inositol). In addition, the eventual appearance of an intermediate cells phenotype between the epithelial and mesenchymal ones has also been considered (Jolly et al., 2014).

However, more recently, such point of view has been widely disputed, leaving the space to different approaches based on special types of modelling programmes (Bertolaso, 2016). Actually, it has been proposed that critical events are the result of emerging properties at a scale that is larger than the microscopic ones, according to the influence of external constraints. Therefore, a novel strategy should be applied, grounded on what it is nowadays a well-established discipline, the so-called *Systems Biology approach* (Bizzarri et al., 2008; Bizzarri et al., 2013; Hornberg et al., 2006). More specifically, instead of focusing on the role of individual genes, proteins or other local pathways in biological phenomena, a pertinent alternative is to characterise the ways molecular parts adopt for interacting with each other to determine the collective dynamics of the system as a whole.

Therefore, following the same philosophy, we concentrate on the mechanisms emerging from a cumulative account of the different elements contributing to EMT and MET. Indeed, the main idea underlying the Systems Biology approach is to replace the reductionist paradigm by describing biological systems as a whole, through an holistic view by virtue of which the biomedical processes cannot be exhausted considering the system as the mere sum of its components; that is why, instead of focusing on the role of individual agents, the purpose is to define how essential components interact to characterise the collective dynamics.

In this direction, we propose a simplified mathematical model, which does not pretend to provide any quantitative description of the phenomenon under scrutiny, but only to attempt a qualitative analysis. Such a model considers a very limited number of unknowns —one for the epithelial and one for the mesenchymal

phenotype— and also a minimal set of parameters, then concentrating on the presence of *propagating fronts* which typically correspond to the invasion of one state into the other. We stress that the inclusion of a nonlinear term is crucial for the dynamics, since it guarantees the existence of fronts through the emergence of several discrete stationary states (we shall introduce the definition of these mathematical objects later on).

The majority of mathematical models relevant to the description of EMT/MET is based on *ordinary differential equations*, i.e. systems where the unknowns are considered as functions solely of the time variable. The resulting models are usually rather entangled, often consisting of very large systems, with each unknown variable possessing a stringent biological meaning (Bocci et al., 2018; Gasior et al., 2017; Guerra et al., 2018; Laise et al., 2012; MacLean et al., 2014; Turner and Kohandel, 2010).

Regrettably, the absence of physical space dependency limits dramatically their range of application, together with the capability of catching the correct and complete biological behaviour. As a matter of fact, cells migration requires some (spatial) destination. Thus, more recently, attention has been paid to a different type of modelling based on *partial differential equations* (Hellmann et al., 2016; Sfakianakis et al., 2018). In such an extended framework, as a consequence of the presence of a single (and simple) term accounting for the motility of cells with mesenchymal phenotype, propagation fronts take place and such appearance participate to the investigation in a crucial way.

In this article, EMT/MET analysis is carried out by exploiting a simplified one-dimensional hyperbolic-parabolic partial differential system. Specifically, the densities of epithelial and mesenchymal phenotypes are the unknown variables, and the main purpose consists in establishing the existence of traveling waves by means of numerical simulations. A reliable approximation of the propagating fronts speed is provided by the so-called *LeVeque-Yee formula* (LeVeque and Yee, 1990). One of the most interesting results arising from the model currently under investigation lies in the possibility of reproducing the property of being/not being invasive, according to the dependence on a reduced number of control parameters. Numerical simulations are based on *finite difference schemes* and carried out by employing an Implicit-Explicit strategy (Quarteroni, 2017).

1. A simple mathematical model

Following Simeoni et al., 2018, we propose a prototypical model which consists of two coupled differential equations aiming to reproduce the EMT/MET dynamics. The first is an ordinary differential equation for the nonnegative density $u = u(t,x)$ and it illustrates the time variation $\frac{\partial u}{\partial t}$ of the amount of cells displaying the epithelial phenotype. The second is a partial differential equation of reaction-diffusion type for the time variation $\frac{\partial v}{\partial t}$ of mesenchymal cells, with density denoted by $v = v(t,x)$. After rescaling the variables, the system reads as

$$(1)\quad \begin{cases} \sigma \dfrac{\partial u}{\partial t} = -u + \lambda f(v) \\ \tau \dfrac{\partial v}{\partial t} = -v + \mu g(u) + \dfrac{\partial^2 v}{\partial x^2} \end{cases}$$

for some given positive parameters σ, τ, λ, μ and functions f and g.

The two equations of system (1) have a strong similarity in their structure, guaranteeing a sort of symmetry of the underlying physical mechanisms. However, there are two crucial differences. Firstly, the presence of the parameters σ and τ is needed to incorporate different time-scales in the phenomena, often required when dealing with non-equilibrium thermodynamics. We focus mostly on the choice of values $\sigma = \tau = 1$, corresponding to the case of same time-scale for both the unknowns, although individual choices could be more appropriate, depending on the context. Secondly, the last term in the equation for v, which represents a motility given by the second order space derivative $\frac{\partial^2 v}{\partial x^2}$ of the unknown, is expressed as a diffusive term, which, on its turn, is the macroscopic appearance of an underlying *brownian random walk* (Taylor, 1920). Such term is mandatory, since one hallmark of the mesenchymal phenotype is its high degree of motility. Possible alternatives of modelling could also be considered, essentially corresponding to different types of random walk, such as the *correlated random walk* (Zauderer, 1983). Let us stress that modelling motility terms is one of the major issues in order to obtain a reliable model, but we made the choice of the above form to keep the presentation as simple as possible.

The unknowns u and v are interpreted as the amount of cells having epithelial and mesenchymal phenotype, respectively, inside some tissue under observation. The choice of the functions f and g is also crucial, and it con-

stitutes one of the points where the interaction between applied mathematicians and theoretical biologists is pivotal, as mentioned in the introduction. We assume that both functions f and g are nonnegative and monotone increasing, according to the modelling assumption that the system (1) is cooperative, i.e. a higher presence of epithelial phenotype determines a higher production of mesenchymal phenotype and viceversa.

Disregarding the coupling term f, the density u of the epithelial phenotype is destined, asymptotically in time, to the extinction (due to the apoptosis-like term $-u$ in the first equation), that suggests a stabilisation towards the equilibrium point with an exponential decay rate (as characteristic for solutions to linear equations). A similar fate is expected for the other unknown v when neglecting the coupling term g, thus providing convergence —again, with an exponential decay rate— to its asymptotic equilibrium.

Examples for f and g, being considered in the forthcoming discussion, are

$$(2)\quad f(v) = v \quad \text{and} \quad g(u) = \frac{u^p}{1+u^p},$$

for some p > 1. The former is a standard linear function, while the latter is an S-shaped function, as for the classical saturating Hill form (Gesztelyi et al., 2012; Weiss, 1997). Such choice is motivated by the assumption that the default state of mesenchymal cells is prone to become motile without any limitation. On the contrary, epithelial cells have an inherent tendency to generate mesenchymal cells with an asymptotic bounded range of availability. Different choices can be easily implemented without specific and/or additional difficulty.

The presence of the coupling terms f and g determines the possible existence of a second stable equilibrium point with larger coordinates (u,v). The parameters λ and μ are interpreted, respectively, as a factor enhancing cell-cell adhesion (hence, structural stability typical of epithelial phenotype) and an inflammatory factor, inducing the transition towards a motile mesenchymal phenotype.

A reaction-diffusion system similar to (1) has already been discussed in Capasso and Maddalena, 1981. In that (epidemiological) context, the meaning of the variables u and v is different: the unknown u represents the average concentration of bacteria and v the infective human population inside an urban community; moreover, attention is drawn to convergence towards constant equilibrium states. Presently, we concentrate on the existence of propagating fronts, which are interpreted as epiphanies of EMT and/or MET, depending on the sign of the propagation speed.

In Section 1.1, we preliminarily consider basic properties of the ordinary differential equations obtained by disregarding the second order spatial derivatives. Then, we move to the case where space dependency is taken into account, hence leading, in particular, to the emergence of propagating fronts for describing invasion of one-state into another. Depending on the values λ and μ, invasion can be modulated and also reverted, thus corresponding to a possible tumour reversion scenario. Of course, the present model is too simple to be capable of providing quantitative predictions of such phenomenon, but is regarded as an attempt at a qualitative description of the basic elements at the core of tumour reversion.

1.1 Space independent solutions

Neglecting the space dependency, system (1) reduces to standard ordinary differential equations, usually describing the dynamics of a *well-stirred mixture*, that is

$$(3)\quad \begin{cases} \sigma \dfrac{du}{dt} = -u + \lambda f(v) \\ \tau \dfrac{dv}{dt} = -v + \mu g(u) \end{cases}$$

Analogous models are already present in the literature since decades. Among others, we quote Green and Sleeman, 1974 and its descendants, where the FitzHugh-Nagumo system is proposed in the context of axon signalling, with variables u and v denoting approximately the potential of nerve axons and a (qualitative) feature of the ionic channels opening/closure mechanism, respectively. The effect of the variable u inside the equation for v is completely different with respect to the model (3) presently considered: indeed, we attempt at simulating a particular type of cellular mechanism, distinguished by a cooperative-type coupling, for which each variable positively contributes to the increase of the other. Finally, in Jones et al., 2004, a system with analogous cooperative structure —arising in the context of wound healing experiments (Barriere et al., 2015)— is proposed, but with a mixed product $u \cdot v$ as consequence of the mass action law assumption, with the variables u and v describing the area of dead tissue and the

spatially-evolving section of the wound, respectively.

A standard procedure for analysing differential equations consists in evaluating constant steady states, i.e. special constant solutions which are preserved by the dynamics. For system (3), these are given by

$$\sigma \frac{du}{dt} = 0 \quad \text{and} \quad \tau \frac{dv}{dt} = 0$$

which correspond to the request that

(4) $u = \lambda f(v)$ and $v = \mu g(u)$.

As an example, we consider the functions in (2) with $p = 2$, for some parameters $\lambda, \mu > 0$. In such a case, the modelling function g is said to have a Holling type III response form (Holling, 1959). Substituting into (4), we deduce the polynomial equation

$$(1 - \lambda\mu \cdot u + u^2)u = 0,$$

which admits one, two or three solutions depending on the value of the product $\lambda\mu$. Indeed, for $0 < \lambda\mu < 2$ we compute a single (physically meaningful) solution u_0, for $\lambda\mu = 2$ two solutions $u_- = u_+$ and, finally, for $\lambda\mu > 2$ three solutions always with coordinates

$$u = u_0 = 0,\ u = u_-,\ u = u_+,$$

where

$$u_- := \frac{1}{2}\left(\lambda\mu - \sqrt{(\lambda\mu)^2 - 4}\right)$$

and

$$u_+ := \frac{1}{2}\left(\lambda\mu + \sqrt{(\lambda\mu)^2 - 4}\right).$$

In the latter case, the constant solutions $u_0 = 0$ and u_+ are shown to be asymptotically stable equilibria of the system (3), while the intermediate state u_- is unstable. In the present context, *stability* is referred to the behaviour of small perturbations to the corresponding state: stability being a (local) synonym of attractive, and instability of repulsive dynamics, respectively.

The limiting regime of system (3) as $\sigma \to 0^+$ is said to be a singular dynamics, since the first equation reduces to the algebraic identity $u + \lambda f(v) = 0$,which turns out to be a constraint for the overall dynamics. Correspondingly, there is no need of specifying an initial condition for the unknown u, thus being determined by the relation itself. In such regime, system (3) reduces to the first order differential equation

$$(5)\quad \tau \frac{dv}{dt} = -\frac{\partial H}{\partial v}(v;\lambda,\mu)$$

where

$$(6)\quad \frac{\partial H}{\partial v}(v;\lambda,\mu) = v - \mu g\big(\lambda f(v)\big).$$

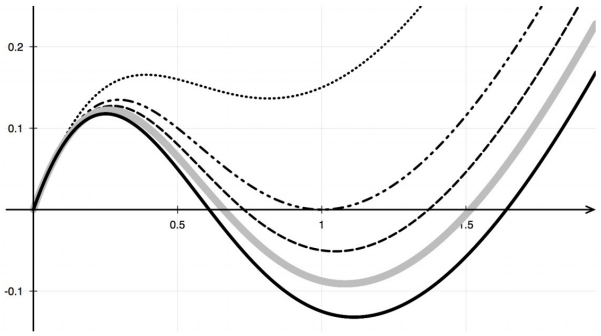

Figure 1. Graphs of the potential function H given in (7) for different values of the parameters.

Therefore, in the singular limit $\sigma \to 0^+$, the equation for v has a special form, which is usually called a *gradient-like structure*. Indeed, after multiplying both sides of (5) by $\frac{\partial v}{\partial t}$, we deduce the identity

$$\frac{d}{dt}H(v;\lambda,\mu) + \tau\left(\frac{dv}{dt}\right)^2 = 0,$$

which shows that the function H is dissipated (it has nonpositive variation) along trajectories of the variable v and thus, in principle, the solutions converge to its minima. In the specific case (2) with $p = 2$ and λ, μ appropriately chosen, the potential H has two distinct minima. Hence, H actually acts as a switch separating solutions which asymptotically converge to one of the two achievable phenotypes. Entering into details, for $f(v) = v$ and $g(u) = \frac{u^2}{1+u^2}$, from (6) there holds

$$(7)\quad \begin{aligned} H(v;\lambda,\mu) &= \frac{1}{2}v^2 - \frac{\mu}{\lambda}\int_0^{v/\lambda} g(\sigma)\,d\sigma \\ &= \frac{1}{2}v^2 + \frac{\mu}{\lambda}\Big(\arctan(\lambda v) - \lambda v\Big) \end{aligned},$$

by using the explicit form of the functions given in (2) with $p = 2$. For $\lambda\mu > 2$, the function H has the typical shape of a double-well potential with wells located at $u_0 = 0$ and u_+ defined above (see Figure 1). In particular, for a specific choice of the product $\lambda\mu$, the two wells have the same depth, with significant consequences in the space-dependent case of system (1), as we shall discuss in the following section.

1.2 Accounting for space dependency

The original model (1) is a simple instance within a wider class, usually referred to as *reaction-diffusion systems*, which very often support special solutions exhibiting a wave-like structure. Roughly speaking, the

interest in such mathematical objects is that they are designed to reproduce invasive patterns, which are a key-feature of many biological applications, properly starting from cancer modelling. A complete review on this issue and its ubiquity in biological modelling can be found in Volpert and Petrovskii, 2009.

As already mentioned in Section 1.1, the limit dynamics of system (1) as $\sigma \to 0^+$ is said to be singular since the mathematical object obtained by (formally) setting $\sigma = 0$ is not a differential equation, but rather an algebraic relation. This fact has a number of significant consequences, relatively to the number of initial/boundary conditions that can be imposed. In such regime, the system reduces to the identity $u = \lambda f(v)$ together with the scalar reaction-diffusion equation

$$(8)\quad \tau \frac{\partial v}{\partial t} = F(v) + \frac{\partial^2 v}{\partial x^2}$$

where $F(v) = -v + \mu g(\lambda f(v))$.Depending on the specific form of function F, the equation (8) may support special solutions of a traveling wave type, namely given by $v(x, t) = V(x - ct)$ for some profile V and *propagation speed* c (Volpert and Petrovskii, 2009). In addition, if the profile function V is such that there exists finite limits at $-\infty$ and $+\infty$, i.e.

$$\lim_{x\to-\infty} V(x) = v_- \quad \text{and} \quad \lim_{x\to+\infty} V(x) = v_+,$$

with $F(v_-) = F(v_+) = 0$, then the solution is said to be a *propagating front* with speed of propagation c. Let us stress that both the profile function V and the speed parameter c are unknown, and have to be determined by imposing that they satisfy the scalar reaction-diffusion equation (8) with boundary data v_- and v_+.

Inserting the above *ansatz* into equation (8) gives an ordinary differential equation for the profile V, parametrised by the speed value c, that is

$$(9)\quad \frac{d^2V}{dx^2} + \tau c \frac{dV}{dx} + F(v) = 0$$

which satisfies the boundary conditions $V(-\infty) = v_-$ and $V(+\infty) = v_+$. In mathematical terms, if $v_- \neq v_+$, we are looking for a so-called heteroclinic orbit (we note that, since the equation (9) is autonomous, the solutions are translationally invariant).

In the prototypical case (2) with $p = 2$, the reaction function becomes $F(v) = -v + \mu \dfrac{\lambda^2 v^2}{1 + \lambda^2 v^2}$,which has a bistable shape if $\lambda\mu > 2$, and then equation (8) supports propagating fronts. In general, the speed c in equation (9) does not have an explicit formula, nevertheless it can be approximated numerically, usually furnishing a value which depends on the parameter τ and the form of the function F.

When the parameter σ is non zero, similar properties hold for the complete system (1). More precisely, a traveling wave solution is a special solution of the form

$$(10)\quad \begin{cases} u(x,t) = U(x - ct) \\ v(x,t) = V(x - ct) \end{cases}$$

And we notice that, by definition, both components are assumed to travel with the same propagation speed. The system of ordinary differential equations for U and V is obtained by substituting (10) into (1), so that

$$(11)\quad \begin{cases} \sigma c \dfrac{dU}{dx} + U - \lambda f(V) = 0 \\ \dfrac{d^2V}{dx^2} + \tau c \dfrac{dV}{dx} + V - \mu g(U) = 0 \end{cases}$$

As before, the solution is said to be a *propagating (or invasion) front* if it defines a heteroclinic orbit of the dynamical system (11) with constant (and different) boundary values

$$(12)\quad \lim_{x\to\pm\infty} U(x) = u_\pm \quad \text{and} \quad \lim_{x\to\pm\infty} V(x) = v_\pm,$$

where the asymptotic states $(u_\pm, v_\pm)$ are forced to be equilibria of system (1) as given by equations (4).

Incidentally, let us observe that being system (11) autonomous, the profile functions U and V, whenever they exist, are determined up to a translation of the independent variable, as for the scalar case (9).

The parameter c has to be appropriately tuned in order for the boundary conditions (12) to be satisfied. Determining the exact —or, at least, an approximate— value of the propagation speed c is crucial for the understanding of the EMT/MET phenomenon under investigation, since it provides the velocity of invasion of phenotype fronts. Actually, computing an exact solution for c is, in general, not possible; thus, it is crucial to develop a suitable algorithm producing a reliable numerical estimate of the speed (refer to Section 2.2).

1.3 A short overview of rigorous results

For the sake of simplicity in the presentation, we focus on the case of modelling functions (2) with $p = 2$.

In the singular limit $\sigma = 0$, the model system (1) reduces to the scalar reaction-diffusion equation

$$\frac{\partial v}{\partial t} = -\frac{\partial H}{\partial v}(v;\lambda,\mu) + \frac{\partial^2 v}{\partial x^2},$$

where the potential H is given in (7). Whenever the function H has two wells, there exists a propagating front connecting the two minima of this potential, with a unique propagation speed c, whose value is linked to the difference of depth of the potential wells. Moreover, its stability is rigorously established as in Fife and McLeod, 1977. An identity for the propagation speed is indeed available and it shows that, in the particular case of two wells of equal depth, the traveling wave is, in fact, a steady state ($c = 0$).

Similarly, for $\sigma > 0$,when H has the same properties as mentioned above, there exist positive values λ_0 and μ_0 such that system (1) possesses a standing wave with profiles $(U, V) = (U(x), V(x))$ corresponding to the speed $c = 0$. Such value separates positive and negative speeds of propagation, and it is determined by the requirements

$$H(v_0;\lambda_0,\mu_0) = \frac{1}{2}v_0^2 + \frac{\mu_0}{\lambda_0}\left(\arctan\left(\lambda_0 v_0\right) - \lambda_0 v_0\right) = 0$$

and

$$\frac{\partial H}{\partial v}(v_0;\lambda_0,\mu_0) = v_0 - \mu_0\frac{\lambda_0^2 v_0^2}{1+\lambda_0^2 v_0^2} = 0\,.$$

The first condition corresponds to the requisite that the two wells of H have same depth; the second one translates the fact that v_0 is a zero of the variation $\partial H/\partial v$ — hence a singular point of the potential $H(\cdot;\lambda,\mu)$— and consequently a candidate for the asymptotic state v_+. Together, they imply that the speed is zero and the wave is stationary.

Finally, in that framework, one can compute the stationary traveling fronts U and V by using the standard construction of a steady heteroclinic orbit for the double-well potential with wells of equal depth (Mascia et al., 2019). Incidentally, we recall that a rigorous proof of the existence of propagating fronts for the system (1) is an open problem in full generality.

2. Numerical simulations

In the mathematical literature, there is a number of numerical schemes of different types for approximating partial differential equations. The choice depends mainly on the dynamical features of the numerical solution one is interested in and, even within the same framework, various algorithms could be implemented (Quarteroni, 2017). As regards the numerical strategy to be applied to the model system (1), we have chosen to employ an implicit-explicit finite difference algorithm. Such a choice allows adopting larger time steps compared to fully explicit schemes, which are instead heavily conditioned by the restrictions that stability requires, thus leading to less computationally expensive simulations. As a matter of fact, our numerical algorithm discretises implicitly all the linear terms, whilst the nonlinear reaction functions f and g are treated explicitly.

We denote by dx and dt the space and time steps, respectively, and by $x_j = j\,\mathrm{dx}$, $j = 1,2,\ldots$, the discretisation points located on a uniform mesh, together with $t^n = n\,\mathrm{dt}$, $n = 1,2,\ldots$, the discrete times. Moreover, the symbols u_j^n and v_j^n indicate numerical approximations of the values $u(x_j, t^n)$ and $v(x_j, t^n)$, respectively. Then, the corresponding numerical scheme reads as

$$\begin{cases} \sigma\dfrac{u_j^{n+1}-u_j^n}{\mathrm{dt}} = -u_j^{n+1} + \lambda v_j^{n+1} \\ \tau\dfrac{v_j^{n+1}-v_j^n}{\mathrm{dt}} = -v_j^{n+1} + \mu g(u_j^n) + \dfrac{v_{j+1}^{n+1} - 2v_j^{n+1} + v_{j-1}^{n+1}}{\mathrm{dx}^2} \end{cases}$$

in the model case (2) with $p = 2$, since the function f is linear. After simple algebraic manipulations, the above algorithm becomes a linear system for the unknown couple (u_j^{n+1}, v_j^{n+1}) to be computed in term of the parameters $\sigma, \tau, \lambda, \mu$ and the (known) couple (u_j^n, v_j^n). By iterations, one ends up with an explicit approximation for the unknown variables at time t^{n+1} (for more details, see Mascia et al., 2019). In the general case of a nonlinear function f, the same implicit-explicit strategy would have suggested the modified scheme

$$\begin{cases} \sigma\dfrac{u_j^{n+1}-u_j^n}{\mathrm{dt}} = -u_j^{n+1} + \lambda f(v_j^n) \\ \tau\dfrac{v_j^{n+1}-v_j^n}{\mathrm{dt}} = -v_j^{n+1} + \mu g(u_j^n) + \dfrac{v_{j+1}^{n+1} - 2v_j^{n+1} + v_{j-1}^{n+1}}{\mathrm{dx}^2} \end{cases}$$

An approximated solution to system (1) is thus the result of time iterations starting from some spatially discretised initial datum which has to be furnished. Presently, we consider initial data of *Riemann type*, i.e. discontinuous profiles consisting of two different constant states at the left and the right of some given point, usually located at $x = 0$, namely

$$(13)\quad u(x,0) = \begin{cases} u_- & \text{for} \quad x < 0 \\ u_+ & \text{for} \quad x > 0 \end{cases}$$

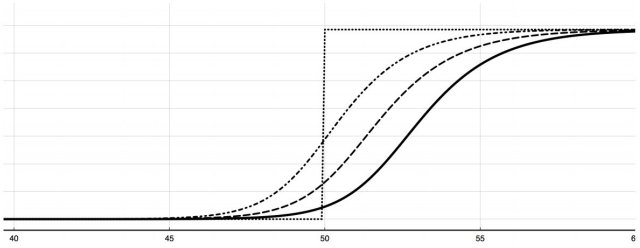

Figure 2. Graphic illustration of case 1 (propagation from right to left)

with the analogous definition for $v(x, 0)$, where $(u_{\pm}, v_{\pm})$ are constant steady states of system (1).

2.1. Computational results for EMT/MET

As mentioned in Section 1.2, in the singular regime $\sigma \to 0^{+}$, the system (1) reduces to a standard parabolic reaction-diffusion equation (8) for the mesenchymal phenotype. The behaviour of such a model is essentially well-known, separating EMT invasion and MET regression regimes by appropriately tuning the model parameters. The general case, for $\sigma > 0$, follows the same qualitative analysis with respect to the model parameters.

In particular, for the reaction functions given in (2) with $p = 2$ and $\mu = 1$, threshold values for the dynamics can be explicitly computed, which are

$0 < \lambda_{*} = 2.0 < \lambda_{0} = 2.175063$.

The analysis is straightforward for $0 < \lambda < \lambda_{*}$, since any positive initial datum generates a solution (u, v) which converges to $(0,0)$ as $t \to +\infty$ with exponential rate. Next, we concentrate on the regime $\lambda > \lambda_{*}$. The numerical results reported below illustrate only the profile for the component u, the profile of the component v being qualitatively very similar. We also limit the presentation to the dynamics exhibited by choosing an initial datum of Riemann type (13).

Case 1: $\lambda = 2.1 > \lambda_{*}$. For this choice of the parameter λ, numerical evidence of the existence of a traveling front is obtained. Moreover, being the stable state u_{+} closer to the critical state corresponding to the threshold value λ_{*}, the solution exhibits a regressive behaviour, namely the front travels towards the right-hand side with positive speed (see Figure 2).

Case 2: $\lambda = \lambda_{0}$. Since the two wells of the potential function H have the same depth for this value of λ, system (1) possesses a stationary solution with the required asymptotic behaviour for $\sigma = 0$. In particular, the dynamics is independent from the relaxation parameters σ and τ, and the existence of a traveling wave in the regime $\sigma > 0$ is a straightforward consequence of the observation that the fronts are actually steady states.

Case 3: $\lambda = 2.25 > \lambda_{0}$. Again, numerical evidence of the existence of propagating fronts emerges as the long-time behaviour of the solution to a Riemann problem (13). The traveling wave has positive speed, so that we are in a situation for which invasion is possible, corresponding to the typical EMT/MET phenomenon (see Figure 3). For more general initial data, competition between different branches of the solution starts playing a crucial role in featuring the large-time behaviour.

As far as λ increases, the numerically computed speed of the propagating fronts increases in absolute value and, thus, invasive EMT/MET regimes are more and more probable.

2.2. Approximation of the speed

Finding a reliable approximation for the velocity of propagating fronts when an explicit formula is not available is crucial for many theoretical reasons. In particular, the speed of propagation c provides an additional parameter which, in principle, could be used to calibrate the model in practical situations. The numerical approximation of the propagation speed relies on the approach originally proposed in LeVeque and Yee, 1990, and successfully applied to systems of reaction-diffusion equations in Lattanzio et al., 2019a, 2019b, Moschetta and Simeoni, 2019.

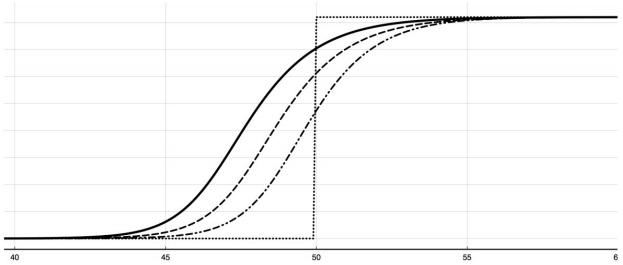

Figure 3. Graphic illustration of case 3 (propagation from left to right)

We provide a brief recasting of the basic idea behind such method: given a smooth function φ with asymptotic states $\varphi_{\pm} = \varphi(\pm\infty)$, there holds

$$\int_{\mathbb{R}} \left[\phi(x+h) - \phi(x)\right] dx = h \int_{\mathbb{R}} \int_{0}^{1} \frac{d\phi}{dx}(x + \theta h)\, d\theta\, dx = h\,[\phi]\,, \tag{14}$$

for any $h \in \mathbb{R}$, where $[\varphi]: = \varphi_+ - \varphi_-$, this formula being obtained by interchanging the order of integration.

Choosing the shift value $h = - cdt$ we infer that

$$c = \frac{1}{[\phi]\,\mathrm{dt}} \int_{\mathbb{R}} \left[\phi(x) - \phi(x - c\,t)\right] d\,x \quad .$$

Denoting by φ_j^n the approximation of $\varphi\,(x_j - ct^n)$, the numerical counterpart of identity (14) is given by

$$(15) \quad c^n := \frac{\mathrm{dx}}{\mathrm{dt}} \sum_j \frac{\phi_j^n - \phi_j^{n+1}}{[\phi]} .$$

The approximation (15) is indeed exact whenever φ_j^n is related to a traveling wave solution with constant speed c and asymptotic states $\varphi_\pm$. In general, the value c^n can be regarded as a space-averaged propagation speed, which is suppose to stabilise towards the correct speed c when φ_j^n converges to the traveling profile.

Because the model system (1) has two dynamical variables u and v, the respective speed values can be computed through the LeVeque-Yee formula (15) by applying it either to u_j^n or to v_j^n. Actually, for large n, the two numerically estimated values appear to be close one to the other, as expected from the theoretical analysis (refer to Section 2.2).

Conclusions

In the recent years, EMT and MET have been considered as an important emerging research subject, constituting a crucial event in cancer onset and spread, also strictly related with invasive features and linked to the consequent formation of metastasis. We have proposed a simplified mathematical model, consisting of a system of coupled partial differential equations for two variables, describing, in principle, two different cells phenotypes, namely epithelial and mesenchymal. The model system is of reaction-diffusion type and often supports the emergence of propagating fronts under minimal assumptions on the physical parameters. Of course, the presence of a space-dependent diffusion term is crucial, together with a little amount of nonlinearity. We also illustrated a numerical algorithm able to furnish reliable approximations for the propagation speeds, which could be useful to calibrate the model with respect to experimental data and realistic situations.

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