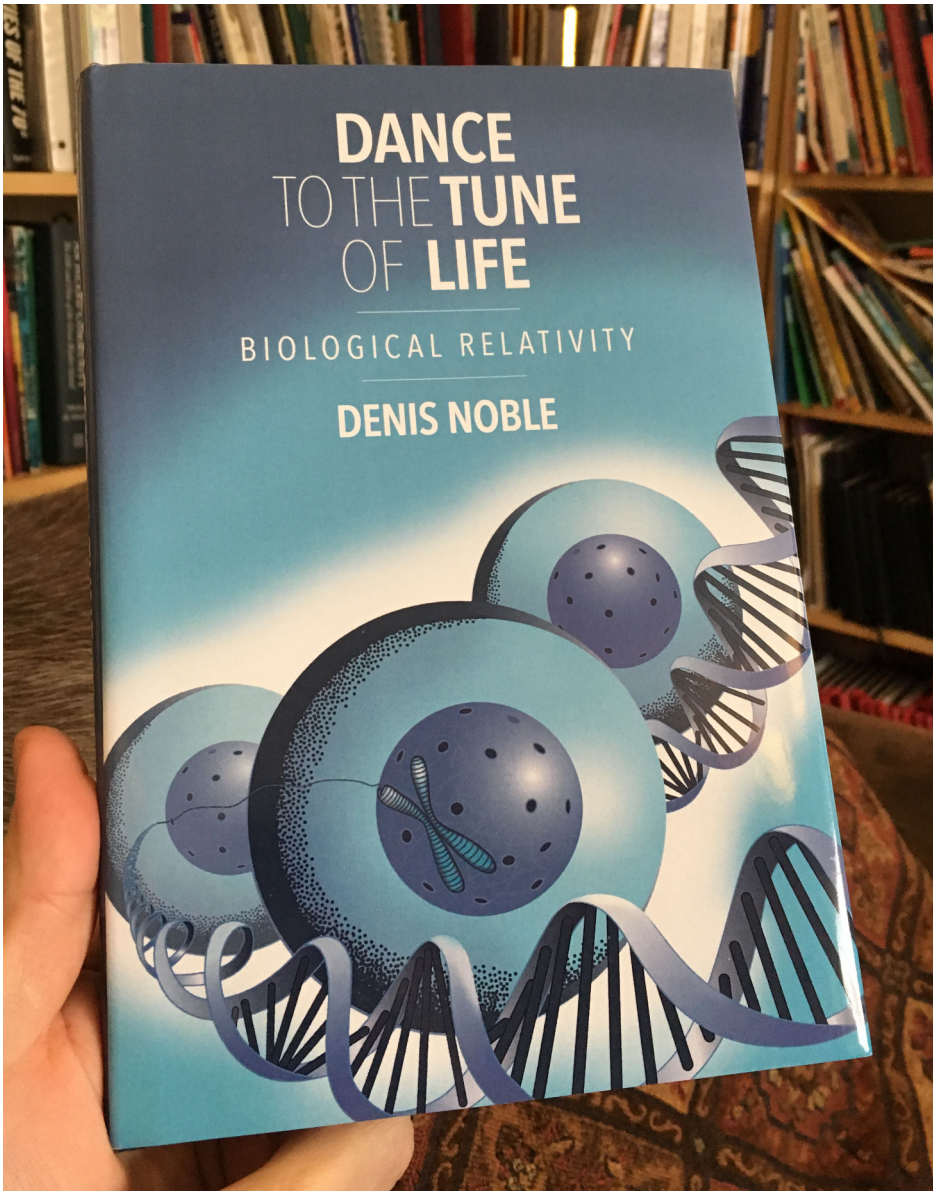
The Dance Sourcebook

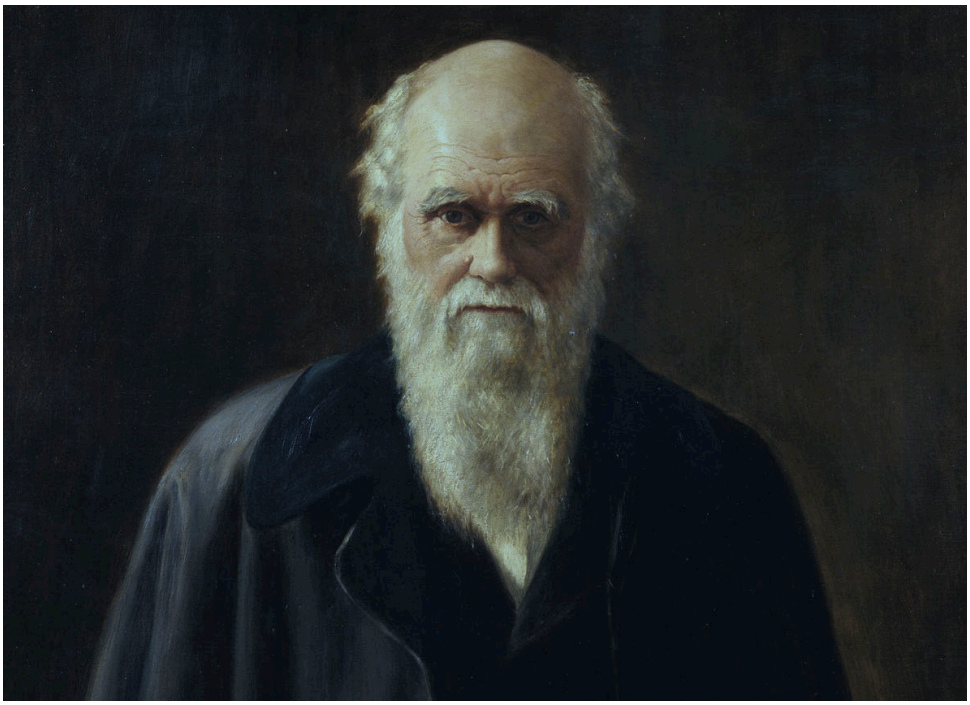


This sourcebook is modelled on the *Music of Life Sourcebook* in collating articles that respond to the wish from readers to have more detail on the sources of the ideas and observations that form the basis of the book. First, though, what is the argument about? Why replace Neo-Darwinism?

Why replace NEO-Darwinism?
The debate in a nutshell

The books on the musicoflife.website, *The Music of Life* (2006) and *Dance to the Tune of Life* (2016), deconstruct standard evolutionary biology, which is usually referred to as Neo-Darwinism or the Modern Synthesis. The books propose an integrated synthesis instead.

Why is that necessary? Isn't Neo-Darwinism simply Darwin's *Origin of Species* updated by 20th and 21st century discoveries in genetics? Isn't it obvious that his central idea of Natural Selection must be correct: successful organisms transmit their characteristics to the next generation? In fact it is almost a tautology. What succeeds must succeed.



From portrait of Charles Darwin in The Royal Society.

Well, yes. But Darwin was not a Neo-Darwinist. In fact he got two other contrary ideas largely correct:

- He acknowledged that Natural Selection was not the only process involved. He realised that organisms actively select other organisms, as for example in sexual selection, and in doing so must partially direct their evolution.

- Second, he agreed with Lamarck on the inheritance of acquired characteristics and even proposed a theory of Pangenesis on how it could work. His idea is almost identical with modern experimental findings on exosomes and transmission of RNAs and DNA to the germline from the soma.

Neo-Darwinism opposes or downplays these processes. In many evolutionary biology textbooks you will not even find them referred to. Moreover, Lamarck is usually presented in very derogatory language. By contrast, Darwin praised Lamarck as a "justly celebrated naturalist …. who upholds the doctrine that all species, including man, are descended from other species." (Preface to fourth edition of *The Origin of Species*). Neo-Darwinism was originally formulated to expunge these Darwinian ideas from evolutionary biology.

The central problems are

- Neo-Darwinist ideas favour gene-centric views of biology, which gets causation in biology the wrong way round. DNA is a completely passive molecule until it is activated by the organism to enable RNAs to be produced that in turn form templates for the production of proteins. DNA cannot even be transmitted faithfully until massively corrected by the organism. It is not therefore the 'immortal replicator'. See articles in this sourcebook: **Evolution viewed from Physics, Physiology and Medicine** and **Central Dogma or Central Debate?**

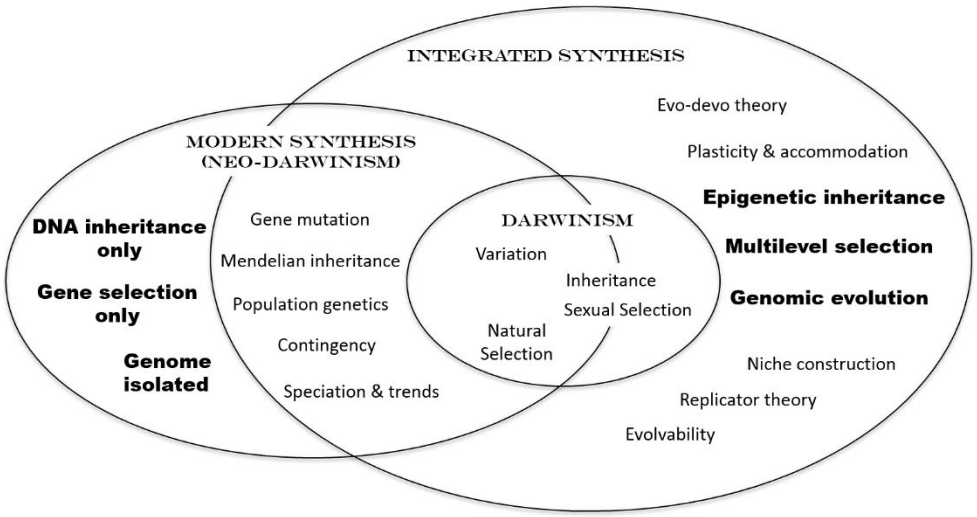
- Neo-Darwinists have problems with the idea that organisms are agents with purposive behaviour. Yet, agency is central to understanding life. It has to be central to the process of evolution. See article in this sourcebook: **Was the Watchmaker Blind?**

- Neo-Darwinism misunderstands the role of biological stochasticity, which is given primacy in the origin of variation. In fact, stochasticity is actively harnessed by organisms to create novelty. See articles in this sourcebook: **Evolution viewed from Physics, Physiology and Medicine, Was the Watchmaker Blind?** and **Harnessing stochasticity: How do organisms make choices?**

Some evolutionary biologists redefine Neo-Darwinism to avoid these problems, but if a theory is redefined to mean the precise opposite of what it was intended to achieve, it is quite simply no longer different from the Darwinism it opposed.

SUMMARY DIAGRAM

How do Darwinism, the Modern Synthesis and the Integrated Synthesis relate to each other?



The diagram represents the fact that Darwin's view of inheritance included the inheritance of acquired characteristics, which was excluded by neo-Darwinism. Darwin's concept of inheritance is therefore shown as being partly outside the neo-Darwinist modern synthesis. So also are his ideas on sexual selection. The diagram also represents the features that lie outside the range of neo-Darwinism as defined by Weismann and Wallace. The features of that theory that were excluded are shown as corresponding bold-face items. The highlighted items on the far left correspond with the highlighted items at the far right (from the article in this sourcebook: **Exosomes, Gemmules, Pangenesis and Darwin**).

As of July 2019, the sourcebook contains 10 articles:

Noble, D. (2017) Evolution viewed from physics, physiology and medicine. *Interface Focus*, **7,** 20160159.

> Introduces the fact that organisms harness stochasticity to generate functionality. Random variations have more significance than they are given in neo-Darwinism.

Noble, R. & Noble, D. (2017) Was the watchmaker blind? Or was she one-eyed? *Biology*, **6**, 47.

> Organisms and their interacting populations have evolved mechanisms by which they can harness blind stochasticity and so generate rapid functional responses to environmental challenges. They can achieve this by re-organising their genomes and/or their regulatory networks. Evolution does therefore have partial direction. The direction is by organisms themselves.

Noble, D. (2018) Central Dogma or Central Debate? *Physiology*, **33**, 246-249.

> The Central Dogma of molecular biology has been widely misinterpreted to be a modern version of the Weismann Barrier. This confuses cellular-level inheritance with DNA inheritance and is therefore incorrect.

Noble, D. (2018) Lost in Translation – a second letter from Lamarck. *Physiology News*, **111**, 6-7.

> Lamarck is represented in standard evolutionary biology textbooks as favouring a ladder of life, as contrasted with Darwin's tree of life. In fact, Lamarck retracted his ladder of life idea and formulated a very clear tree of life well before Darwin.

Noble, R. & Noble, D. (2018) Harnessing stochasticity: How do organisms make choices? *Chaos*, **28**, 106309.

> Harnessing stochasticity is the way in which organisms become free agents. A free choice is both unpredictable in prospect and rational in retrospect.

Neuman, Y, Noble, D. & Cohen, Y. 2018. Is the whole different from the sum of its parts? A proposed procedure for measuring divergence from additivity. *International Journal of General Systems*. **7**, 665-678.

> That the whole is greater than its parts is a central assumption of integrative multi-level interpretations of biology. Demonstrating this mathematically is a big challenge. This article shows how category theory can be used to develop an approach to the problem.

Jablonka, E. & Noble, D. (2019) Systemic integration of inheritance systems. *Current Opinion in Systems Biology*, **13**, 52-58.

> An inheritable trait can be described as an attractor in a developmental landscape constructed by networks of inputs at underlying and overlying levels of organization. This approach could enable the development of a systemic, dynamic and predictive model of inheritance

Noble, D. (2019) Exosomes, Gemmules, Pangenesis and Darwin. In *Exosomes in Health and Disease*, Elsevier

> Lamarck and Darwin both produced theories to explain how acquired characteristics could be inherited. They realized that something would need to transmit information from the soma to the germ cells. Their theories were very similar, i.e. that minute particles or fluids could be transported by body fluids. Lamarck called them 'suble fluids'; Darwin called them 'gemmules'. Today they have been discovered and we call them exosomes. Exosomes and body fluids can transmit both RNA and DNA to the germline.

Noble, R. & Noble, D. (2019) A-mergence of biological systems. In *Handbook of the Philosophy of Emergence* Routledge, 387-399.

> There is therefore no privileged direction of emergence, the upper levels constrain the events at the lower levels just as much as the lower levels are necessary for those upper level constraints to exist. To emphasise this point, we introduce the concept of a-mergence, which expresses the lack of causal directionality. We illustrate these points with a major test case: Schrödinger's distinction between physics and biology in which he proposed that physics is the generation of order from molecular disorder, while biology is the generation of order from molecular order. This characterization of biology is physically impossible. Modern biology has confirmed both that this is impossible and that, on the contrary, organisms harness stochasticity at low levels to generate their functionality.

Noble, R, Tasaki, K, Noble, PJ, & Noble, D (2019) Biological Relativity Requires Circular Causality but Not Symmetry of Causation: So, Where, What and When Are the Boundaries? *Frontiers in Physiology*, 10, 827.

> The principle of Biological Relativity is that there is no privileged level of causation in organisms. There must therefore be boundaries between the levels of organization across which the levels influence each other. This is the first article to systematically answer the question how the forms of causation between the levels differ. It also reviews the evidence for downward causation in a variety of physiological examples.

INTERFACE FOCUS

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Review

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One contribution of 20 to a theme issue 'New trends in evolutionary biology: biological, philosophical and social science perspectives'.

Subject Areas:
biophysics, biocomplexity, systems biology

Keywords:
evolution and physiology, Schrödinger's error, biological relativity, stochasticity, neo-Darwinism, modern synthesis

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Evolution viewed from physics, physiology and medicine

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Stochasticity is harnessed by organisms to generate functionality. Randomness does not, therefore, necessarily imply lack of function or 'blind chance' at higher levels. In this respect, biology must resemble physics in generating order from disorder. This fact is contrary to Schrödinger's idea of biology generating phenotypic order from *molecular*-level order, which inspired the central dogma of molecular biology. The order originates at higher levels, which constrain the components at lower levels. We now know that this includes the genome, which is controlled by patterns of transcription factors and various epigenetic and reorganization mechanisms. These processes can occur in response to environmental stress, so that the genome becomes 'a highly sensitive organ of the cell' (McClintock). Organisms have evolved to be able to cope with many variations at the molecular level. Organisms also make use of physical processes in evolution and development when it is possible to arrive at functional development without the necessity to store all information in DNA sequences. This view of development and evolution differs radically from that of neo-Darwinism with its emphasis on blind chance as the origin of variation. Blind chance is necessary, but the origin of functional variation is not at the molecular level. These observations derive from and reinforce the principle of biological relativity, which holds that there is no privileged level of causation. They also have important implications for medical science.

1. Introduction: the original formulation of the neo-Darwinist modern synthesis

The theory of evolution by natural selection was formulated by Charles Darwin and Alfred Russel Wallace who presented their ideas to the Linnean Society of London in 1858, followed by Darwin's book *On the Origin of Species* in 1859. Darwin was cautious in the presentation of his ideas. He wrote 'Natural Selection has been the main, but not the exclusive means of modification'. He was concerned that he did not know the origin of variation and he acknowledged the existence of other mechanisms, including the inheritance of acquired characteristics. Ernst Mayr wrote in 1962: 'Curiously few evolutionists have noted that, in addition to natural selection, Darwin admits use and disuse as an important evolutionary mechanism. In this he is perfectly clear' [1]. Although Darwin disagreed with Lamarck on whether evolution had a direction (what Lamarck called *le pouvoir de la vie* [2,3]), he nevertheless acknowledged 'this justly celebrated naturalist . . . who upholds the doctrine that all species, including man, are descended from other species' [4]. However, Darwin's multi-mechanism approach to evolution became significantly narrowed with the rise of neo-Darwinism.

Weismann's formulation of neo-Darwinism involved three major assumptions. First, that all genetic variation is random. Second, that the germline is isolated from variations in the soma. This is the Weismann barrier. Third, together with these two assumptions, that natural selection is then all-sufficient (*allmacht*) to explain evolution [5]. The subsequent integration of Mendelian genetics into this scheme led to the formulation of the modern synthesis [6].

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Several important consequences followed. First, genetic variation is not itself viewed as functional. It becomes so only through the operation of natural selection to weed out harmful variations and promote helpful ones. The origin of variation is therefore completely blind. If this view is correct, we should not explain genetic variation in terms of existing or anticipated functionality. As physiology is the study of functional processes in organisms, physiology is thereby excluded from any direct role in the source of variation. Second, the inheritance of acquired characteristics, often called Lamarckism, cannot occur because it would require either that the germ line is not isolated from influences of somatic variations and/or that some forms of functional genetic reorganization can be triggered as a response to environmental stress. In an 1896 publication [7], Weismann added his theory of germinal selection, involving competition and selection among the hereditary units *within* the germplasm but, as Charlotte Weissman shows, this change in Weismann's view did not make any real concessions to the Lamarckians [8].

The neo-Darwinist modern synthesis was therefore both an extension and a simplification of Darwin's ideas. It was an extension through the incorporation of Mendelian genetics, about which Darwin unfortunately knew nothing. It was a simplification because it excluded the inheritance of acquired characteristics, whereas Darwin not only included this form of inheritance, he even proposed a theory for how it could happen, his pangenesis theory of gemmules [9], which resembles some forms of such inheritance discovered recently (see §6).

2. Purpose of this article

A central thesis of this paper is that blind stochasticity is a misconceived idea as it has been used in evolutionary biology. Stochasticity is used by organisms to generate new functional responses to environmental challenges. Far from proving that evolution is necessarily blind, randomness is the clay from which higher level order can be crafted. But it necessarily works the other way too: higher levels then organize the molecular level through many forms of constraint. The reason we do not necessarily see that organization from the molecular level is that the difference of scale is vast. If we focus on particular molecular events, such as gene mutations at particular loci, they will still appear stochastic. Blind chance can then seem to be the sole determinant of variation even when, in fact, the variation is directed in response to environmental challenges.

I will present the case for the following theses, which run counter to neo-Darwinism and the modern synthesis. With respect to neo-Darwinism, the view in this paper is a replacement more than an extension.

1. Randomness (stochasticity) is what one should generally expect at the molecular level even if determinate functionality rules at higher (cellular, tissue, organ, systems, organisms, sociological) levels. Randomness and functionality necessarily coexist at different levels.
2. Organisms can and do harness stochasticity in generating function.
3. Functional genome reorganization can occur in response to environmental stress.
4. Non-DNA information can be transmitted across generations.
5. By using diverse higher level processes, organisms can resist potentially harmful effects of many random genetic variations, at lower levels of function.
6. Physical constraints can and must influence both development and evolution.
7. The gene-centric view has so far been very disappointing from the viewpoint of medicine.

3. Stochasticity and order coexist at different levels

Physics teaches us that at a molecular level, there must be stochasticity. At any temperature above a value near absolute zero, below which a Bose–Einstein condensate becomes possible [10], molecules have kinetic energy which generates random movement. But physics also teaches us that, once there is a constraint at a higher level, e.g. a gas in a container, thermodynamics can describe determinate behaviour arising from the averaged behaviour within the constraint. This is the reason why Schrödinger argued correctly in *What is life?* that physics generates order from disorder [11].

Yet he contrasted this with biology, which he described as generating order at a high level from *order* at a molecular level, i.e. that the functional order at a high level actually results directly from order at the molecular level. But this is highly problematic from a physical viewpoint. Why then did he propose a theory that even he initially characterized as ridiculous? The reason is that following Delbrück [12], he predicted that the genetic material would be found to be an aperiodic crystal, which is a good description of DNA sequences if one thinks of a polymer as a kind of crystal. Crystal structure can be investigated accurately using diffraction. I believe he saw the 'read-out' of genetic sequences as determinate in the same kind of way. In this respect, he anticipated the formulation of Crick's central dogma of molecular biology [13]. Francis Crick and James Watson both acknowledged Schrödinger's influence in their thinking about the central dogma.

There are two fatal problems with this approach, as noted by Kupiec [14,15]. The first is that, as is clear from Crick's original statement, the central dogma refers only to the fact that *sequence* information passes one way, from DNA to proteins:

> The central dogma of molecular biology deals with the detailed residue-by-residue transfer of sequential information. It states that *such information* cannot be transferred back *from protein* to either protein or nucleic acid. [16, p. 561]

I have italicized '*such information*' and '*from protein*' because it is evident that the statement does not say that *no* information can pass from the *organism* to the genome. In fact, it is obvious that it must do so to produce many different patterns of gene expression, which enable many different phenotypes (e.g. many different cell types in the same body) to be generated from the same genome. In addition to controlling relative expression levels, the organism also makes use of protein-mediated protein processing to add yet another layer of control following transcription.

This information from organisms is conveyed to their genomes by patterns of transcription factors, genome marking, histone marking and many RNAs, which in turn control the patterns of gene expression. These controls are exerted through preferential targeted binding to the

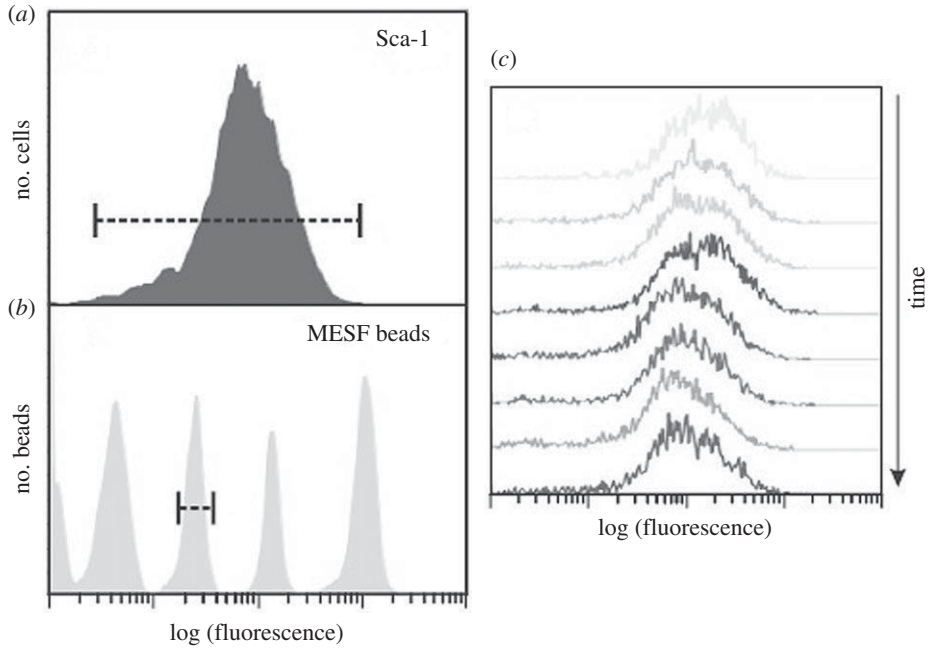

Figure 1. The robustness of heterogeneity of expression of Sca-1 protein expression in a cloned cell population. Heterogeneity detected by immunofluorescence flow cytometry (*a*) was significantly larger than the resolution limit of the method (*b*). (*c*) The stability of the clonal heterogeneity over a period of three weeks. Note that the spread of gene expression levels is three orders of magnitude [21].

genome or histone proteins. For example, methylation of cytosines preferentially occurs at CpG sites. Binding to histones preferentially occurs at the histone tails. Even though these are the targeted molecular mechanisms by which the functional control is exerted, there is no guarantee that the functionality will be evident at the molecular level. It would require many correlations between the *patterns* of binding and the functional processes at a higher level to identify the functionality involved. Without that correlation, the binding patterns will appear random. There are simply far too many sites. There are millions of CpG sites in the whole genome and tens of thousands of CpG clusters, which significantly are located near gene regulatory sites [17].

The second problem is that, as Schrödinger must have understood as a physicist, there is no way in which the molecules in an organism can avoid stochasticity. He wrote:

> We seem to arrive at the ridiculous conclusion that the clue to understanding of life is that it is based on a pure mechanism, a 'clock-work' in the sense of Planck's paper. [18, p. 101]

But he then confuses the logic by continuing: 'The conclusion is not ridiculous and is, in my opinion, not entirely wrong, but it has to be taken "with a very big grain of salt"'. He then explains the 'big grain of salt' by showing that even clock work is, 'after all statistical' (p.103). This seriously compromises the logic because the stochasticity in clockwork has to be negligible. We now know that the stochasticity in biology is far from negligible.

Schrödinger realizes that something is far from right but is struggling to identify what it might be. We would now say that the molecules involved (DNA) *are* subject to frequent statistical variations (copying errors, chemical and radiation damage, etc.), which are then corrected by the cell's protein and lipid machinery that enables DNA to become a highly reproducible molecule [19]. This is a three-stage process that reduces the copy error rate from 1 in 10^4 to around 1 in 10^{10}, which is an astonishing degree of accuracy. In a genome of 3 billion bp, this works out as less than 1 error in copying a complete genome, compared to millions of errors without error correction. The order at the molecular scale is therefore actually created by the system as a whole, including lipid components that are not encoded by DNA sequences [20]. This requires energy, of course, which Schrödinger called negative entropy. Perhaps therefore this is what Schrödinger was struggling towards, but we can only see this clearly in retrospect. He could not have known how much the genetic molecular material experiences stochasticity and is constrained to be highly reproducible *by the organism itself*. The order at the molecular (DNA) level is actually imposed by higher level constraints.

4. Organisms can and do harness stochasticity in generating function

4.1. Stochasticity is a population-level attractor

Experiments on the stochasticity of gene expression in cell populations show that, at least in some cases, it is the population as a whole that controls the stochasticity. Figure 1 is taken from Chang *et al.* [21].

The results show that in this case, the range of gene expression is 1000-fold and it follows a simple bell-shaped curve. The range is a population-level attractor, which is stable over long periods of time. That the population controls the heterogeneity is shown by experiments of the kind illustrated in figure 2. In a cell population showing a bimodal distribution, new populations of cells were cloned from one of the peaks (left), while in a monomodal distribution, cells were cloned from outliers. In both cases, after a few days, the original heterogeneity became re-established.

Cell populations can therefore control stochasticity.

4.2. Cells can harness stochasticity to generate function

That cells can also harness stochasticity to generate specific function is known from experiments on the cells of the immune system that show the phenomenon of somatic

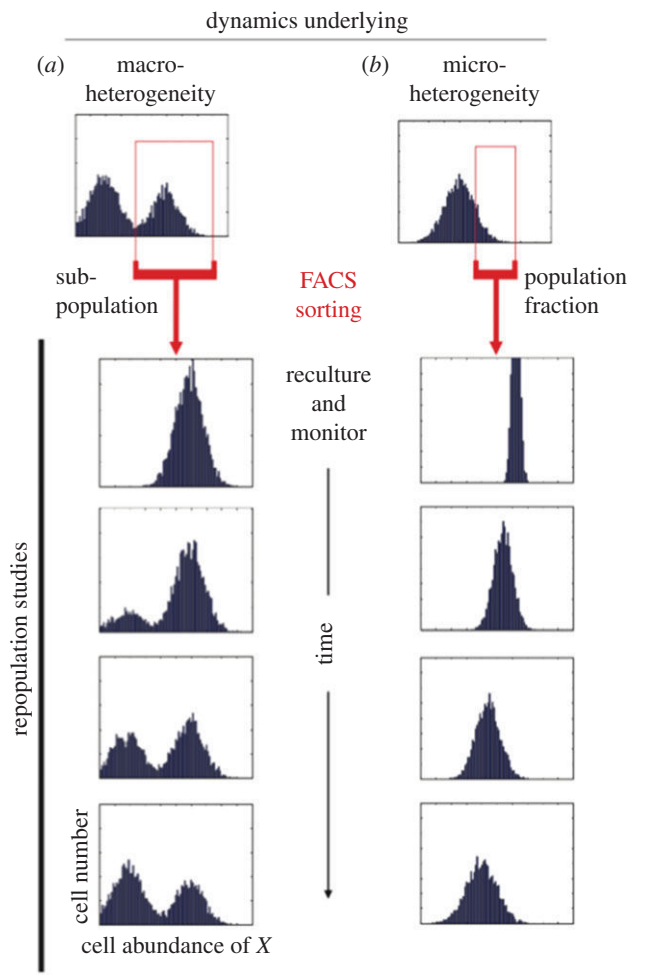

Figure 2. Two examples illustrating experiments in which populations were produced by cloning either from one of the peaks in a bimodal distribution (*a*) or from outliers in a monomodal distribution (*b*). In both cases, the new population initially exhibits the range of expression of the parent subpopulation. Over time (several days), however, the heterogeneity reverts to the original distribution [22]. (Online version in colour.)

hypermutation. Figure 3 summarizes what we know. Faced with a new antigen challenge, the mutation rate in the variable part of the genome can be accelerated by as much as 1 million times. So far as we know, the mutations occur randomly. But the location in the genome is certainly not random. The functionality in this case lies precisely in the targeting of the relevant part of the genome. The mechanism is directed, because the binding of the antigen to the antibody itself activates the proliferation process.

This example from the immune system shows that functionally significant targeted hypermutation can occur in the lifetime of an individual organism. There is no reason why this kind of mechanism should not be used in evolutionary change, as shown in the next example.

A well-known functionally driven form of genome change is the response to starvation in bacteria. Starvation can increase the targeted reorganizations of the genome by five orders of magnitude, i.e. by a factor of over 100 000 [24,25]. This is one of the mechanisms by which bacteria can evolve very rapidly and in a functional way in response to environmental stress.

A similar targeting of location where genomic change can occur has been found in experiments on genetically modified fruit flies. One of the common ways in which genetic modification is achieved is to use a particular kind of mobile genetic element that can move around the genome using a cut-and-paste mechanism that does not require an RNA intermediate. Most often, the insertions occur in a random way. But when DNA sequences from certain regulatory regions are used, they get inserted preferentially near the gene from which the sequence was derived [26]. This process targets the changes in a way that is clearly not random with respect to possible function.

5. Functional genome reorganization can occur in response to stress

5.1. Barbara McClintock and the genome as an organ of the cell

Barbara McClintock first observed that whole domains of genetic material move around the genome, even from one chromosome to another. She was working on Indian corn in the 1930s and 1940s, but it was much later, in 1983, that she was recognized with the award of a Nobel Prize. In her Prize lecture, she was very clear about the functional significance of her discovery. She described the genome 'as a highly sensitive organ of the cell, monitoring genomic activities and correcting common errors, sensing the unusual and unexpected events, and responding to them, often by restructuring the genome' [27].

She could not have anticipated the extent to which her idea would be confirmed by the sequencing of whole genomes. From the 2001 *Nature* paper on the first draft sequence of the human genome, we have comparisons between sequences in completely different species of eukaryotes for two classes of proteins, transcription factor proteins and chromatin binding proteins [28]. These show that the evolution of these proteins must have involved the movement of whole functional domains. This is far from the idea of slow progressive accumulation of point mutations. And it has much greater evolutionary significance because the rearrangement of whole domains including the functionality of those domains in response to stress could have been the origin of creativity in the evolutionary process. It is obvious that combining two or more domains each of which already has functionality is much more likely to produce a viable solution to a problem than waiting for random sorting of point mutations. This is why McClintock characterized the genome as a *highly sensitive organ of the cell*.

5.2. Can we observe genome reorganization happening in evolutionary experiments?

We can now observe organisms making use of this ability to reorganize their genomes. Bos *et al.* have observed the emergence of antibiotic resistance from multi-nucleated bacterial filaments. They write:

> the strategy of generating multiple mutant chromosomes within a single cell may represent a widespread and conserved mechanism for the rapid evolution of genome change in response to unfavorable environments (i.e. chemo-therapy drugs and antibiotics). [29, p. 182]

Jack *et al.* [30] have shown that

> signaling pathways that sense environmental nutrients control genome change at the ribosomal DNA. This demonstrates that not all genome changes occur at random and that *cells possess*

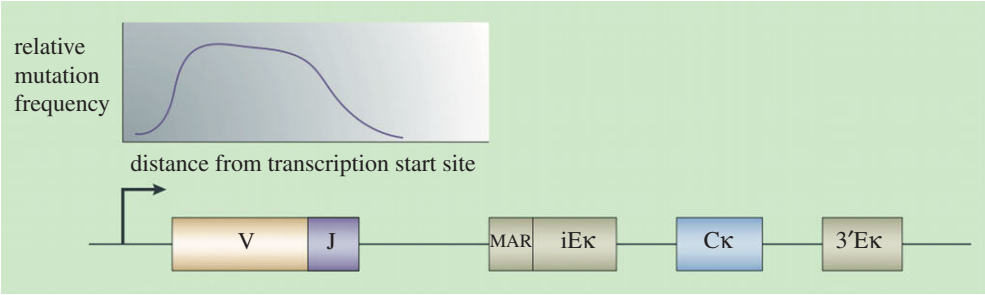

Figure 3. Schematic diagram of gene-specific targeted hypermutation in immunoglobulin gene loci. The mutation rate is greatly increased only in the variable part of the genome, which is an approximately 1.5 kb region in each of the three immunoglobulin loci. In this figure, the graph above the rearranged variable (V) and joining (J) gene segments that form the variable region of Igκ depicts the mutation domain in the κ-light chain (Igκ) locus. 3′Eκ, Igκ 3′ enhancer; Cκ, Igκ constant; iEκ, Igκ intronic enhancer; MAR, matrix attachment region [23]. (Online version in colour.)

> *specific mechanisms to optimize their genome in response to the environment*. (my italics) [30, p. 9674]

How can genomes know about what is happening at the cell surface? The physiological mechanisms by which events in tiny micro-domains near the cell surface signal to the nucleus to control specific gene expression levels have now been studied in fine detail [31,32]. There is no longer any mystery in understanding the highly specific transmission of information to the nucleus that can control gene expression. There is no reason why genomes should not use similar communication pathways in response to stress signals received by cells and organisms.

6. Non-DNA information can be transmitted across generations

Recent experiments have demonstrated that non-DNA information can be transmitted between generations [33], and this rapidly growing field has been reviewed in an important paper in *Science* [34]. Two quotations from that review are relevant:

> Many phenomena and mechanisms of nongenetic and/or non–DNA sequence–based inheritance have been described in a range of model organisms, challenging our perception of the well-established relationship between transmitted genotype and phenotype. [34, p. 59]

They conclude

> The idea of certain sequences that might be refractory to germline epigenetic reprogramming provides a compelling mechanism for the inheritance of modulated epigenetic states. [34, p. 63]

To illustrate the range of processes that can be involved, I will briefly describe three examples.

Rechavi *et al.* [35] investigated the inheritance of resistance to viral infection in the nematode worm, *Caenorhabditis elegans*. The resistance is acquired when infected worms have the DNA required to make a viral-silencing RNAi, which is triggered by viral replication. They cross-bred these worms with a wild-type population, including worms that do not have the required DNA. Some of the later generations have the required DNA, others do not. Yet subsequent generations inherited the acquired silencing response irrespective of whether they had the required DNA. The RNAi is inherited through the germline, and is then amplified by RNA polymerase in each generation. This non-DNA inheritance was followed successfully for 100 generations. It resembles Darwin's gemmule theory (see Introduction).

Nelson *et al.* [36] found robust inheritance of epigenetic marking in mice with Apobec1 deficiency. They found that 'these [epigenetic] effects persist for many generations and are as strong as conventional genetic inheritance'. The journal, *PNAS*, published a commentary article in the same issue, which concludes: 'the belief that the soma and germline do not communicate is patently incorrect' [37].

The question whether epigenetic transmission of acquired characteristics could have been responsible for the evolution of separate species has been answered by Skinner *et al.* [38] who investigated the DNA mutations and non-DNA epigenetic changes in one of the icons of Darwinian speciation, the Galapagos finches. Five species were studied with different phylogenetic distances between them. Figure 4 shows the results. Both DNA mutations and epigenetic variations increase with the phylogenetic distance, with the epigenetic changes correlating better with distance. The authors conclude that both changes were involved in speciation and that they must have interacted.

7. Organisms can resist the harmful effects of many molecular-level variations

One of my own fields of research is cardiac rhythm and arrhythmias. The main pacemaker in the heart, the sinus node, is an example of a robust functional process. Several different ionic transporter circuits are involved, any one of which could generate rhythm. The evolutionary advantage of this situation is obvious: if one mechanism fails, another can take over the function. In 1992, we investigated this robustness by reverse engineering an experimentally based computer model. We found that removing a transporter that could carry as much as 80% of the ionic current necessary for generating the rhythm would change the overall frequency by only around 10–15% [39]. Reverse engineering studies using a physiological model reveals the mechanism of the substitution. The small voltage changes that occur when one component is knocked out are sufficient to activate the substituting mechanism. This discovery formed the basis of the development of a safe heart slowing medication, ivabradine [40].

This kind of 'back up' of important physiological functions is ubiquitous. A systematic study of gene knockouts in yeast showed that 80% of knockouts have little or no effect on physiological functions under normal physiological conditions [41]. Metabolic stress was needed to reveal the functional roles of most of the genes involved.

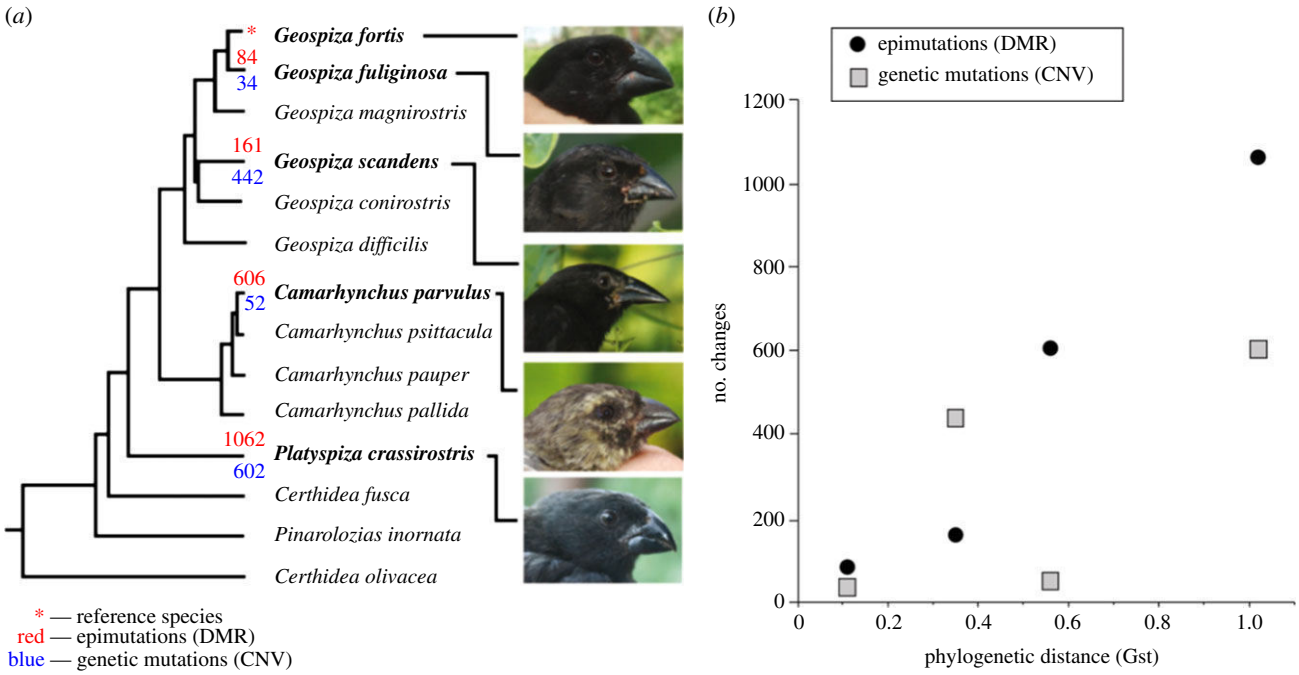

Figure 4. (*a*) Five of the Galapagos finch species were studied, the reference species *Geospiza fortis* and four others. The graph in (*b*) shows the number of genetic and epigenetic changes plotted as a function of phylogenetic distance. The epigenetic changes correlate well with phylogenetic distance, the genetic mutations do not correlate as strongly [38]. (Online version in colour.)

These studies pose a serious problem for bottom-up gene-centric theories of biology. The functionality will simply not be seen at that level or may be far from quantitatively accurate. Organisms seem to be very resourceful when challenged with knockouts, blockers or absence of nutrients. If we look for that ingenuity at the molecular level, we may not find it.

Again, we can ask the question whether such processes can be demonstrated in actual evolutionary time. This was done recently by Taylor *et al.* [42] who have shown that bacteria that have lost their flagella through deletion of the relevant DNA sequence can evolve the regulatory networks required to restore flagella and so restore motility in response to a stressful environment within just 4 days. Specifically, Taylor *et al.* show that deletion of FleQ (Flagellar transcriptional regulator) in *Pseudomonas fluorescens*, and starvation of the bacteria, produces mutations that enable the regulatory role to be taken over by a different pathway, normally involved in nitrogen uptake and assimilation. The genes required to produce flagellae are then reactivated by the new regulatory pathway. The authors interpret their work as showing how selection can rapidly produce this kind of substitution to restore activation of flagella genes. But, equally clearly, the mutations are targeted in a remarkably precise way. They are not randomly occurring anywhere in the genome. This example is therefore somewhat comparable to the cardiac pacemaker example I discuss earlier in this section, in that one network takes over the lost function when another network is no longer functional. That ability is a property of the bacterium regulatory networks and of the ability of the organism to signal the environment pressure to the genome to activate mutation.

It is important to note that such examples, and the earlier ones I quoted above in §5, involve what, so far as we know, are random mutations. At each location on the DNA sequence level, this will therefore appear as 'blind' variation. At that level, there will also be a form of Darwinian selection operating [14]. But the targeting of particular locations, which is what enables the response to the environmental challenge to be effective, is not blind. Nor does targeting necessarily require differential mutation rates in the genome. Buffering of non-functional genome changes by regulatory networks can also ensure the preservation of existing functionality, just as the regulatory networks involved in cardiac rhythm can ensure insensitivity to molecular-level changes, as I described at the beginning of this section.

Differential mutation rates have been extensively investigated by Moxon *et al.* [43] who use the term 'contingency locus' to characterize the targeted loci of hypermutable DNA. In bacteria, these loci are simple sequence repeats in which the repeating unit is one to several nucleotides. In eukaryotes, these loci are called microsatellites and often consist of hundreds of repeats. As 'mutation rates vary significantly at different locations within the genome', they propose that 'it is precisely in the details of these differences and how they are distributed that major contributions to fitness are determined'. In an earlier article, Moxon & Thaler [44] write 'This phenotypic variation, which is stochastic with respect to the timing of switching but has a programmed genomic location, allows a large repertoire of phenotypic solutions to be explored, while minimizing deleterious effects on fitness'.

8. Physical constraints can and must influence both development and evolution

Natarajan *et al.* [45], in a paper significantly entitled 'Predictable convergence in hemoglobin function has unpredictable molecular underpinnings', have examined the molecular basis of convergence in haemoglobin function involving 56 avian taxa that have contrasting altitudinal range limits. They found that 'Convergent increases in hemoglobin–oxygen affinity were pervasive among high-altitude taxa,

but few such changes were attributable to parallel amino acid substitutions at key residues. Thus, predictable changes in biochemical phenotype do not have a predictable molecular basis'. This article beautifully illustrates the main point I am making in this paper, which is that unpredictability at the molecular level, which would lead one to think the changes are random, can be perfectly compatible with predictability and functionality at a higher level. This is biology's equivalent of the physical principle that determinate thermodynamics can coexist with unpredictable stochastic behaviour at a molecular level. The difference is that, in biological systems, through the process of evolution, the higher level becomes functional. That is the level at which the functionality can be seen. It is then the level from which the lower level stochasticity can be understood, including the functional constraints.

If physics can be so important by using stochasticity in convergent evolution, can it also be important in a similar way in constraining development? It is tempting to think so because early embryonic development is similar in all multicellular eukaryotes, despite many differences in genome sequences. Edelman *et al.* [46] have explored this question by showing graphically how some simple physical constraints might be sufficient to explain certain aspects of embryonic development without having to assume that there must always be a specific DNA basis for all such processes. Their images are speculative and would require computational modelling to develop and test the ideas. Stuart Newman, Santiago Schnell and Philip Maini have led the way on this approach [47,48]. There must be interaction between overall physical constraints and molecular-level specifications. Ehrlich *et al.* [49] show how modelling such physical constraints can account for the evolution of shell form in ammonites.

These examples illustrate a general point. Nature does not need to write to the 'hard disc' of the organism, its DNA, when it can get functions automatically from physical 'free rides', i.e. by letting physics do what it will do naturally. There is no need for DNA to be involved, for example, in ensuring that lipid membranes naturally fuse and form vesicles and many of the other properties of thin oily bilayers. And, of course, there is no DNA forming templates for the wide variety of lipids in organisms.

9. The gene-centric view has so far been very disappointing from the viewpoint of medicine

There is another field of science where focusing on the molecular level has blinded us to functional processes at higher levels. That is the field of medicine. But before I explain why that is the case, I want to make it quite clear that I fully recognize the great scientific value of genome sequencing.

Sequencing whole genomes has been of immense value in evolutionary biological studies. The benefits for phylogeny and in discovering new parts of the 'trees' or 'networks' of life are obvious. It was sequencing that enabled Carl Woese to make his fundamental discovery of the archaea and how they differ from bacteria and eukaryotes [50]. Sequencing also enabled us to identify the extent to which mobile genetic elements must have been involved in the evolution of many proteins. In this sense, describing the genome as the 'book of life' has been a useful metaphor. But, as a metaphor used to publicize the health benefits that would accrue from genome sequencing it has been misinterpreted. The promise was that by a decade or so following sequencing of the human genome, the 'book of life' would reveal how to treat cancer, heart disease, nervous diseases, diabetes and many others through the discovery of many new pharmaceutical targets. This did not happen. An editorial in *Nature* in 2010 spelt this out:

> But for all the intellectual ferment of the past decade, has human health truly benefited from the sequencing of the human genome? A startlingly honest response can be found on pages 674 and 676, where the leaders of the public and private efforts, Francis Collins and Craig Venter, both say 'not much'. [51]

The targets were identified all right. At least 200 new possible pharmaceutical targets are now known and there may be more to come, but we simply do not understand how to use them. The problem does not therefore lie in the absence of knowledge about the sequences. The problem is that we neglected to do the relevant physiology at the higher levels. A valuable critique of genotype–phenotype relations as a basis for the common disease–common variant hypothesis has been published by Joyner & Prendergast [52].

Before the shift towards genomic approaches to pharmacology, we did in fact have reasonably adequate methods for developing new drugs against specific diseases. The method was to work initially at a phenotype level to identify possible active compounds, and then to drill down towards individual protein or other molecular targets. This was the approach used so successfully by Sir James Black, the Nobel laureate discoverer of β-blockers and H2 receptor blockers [53]. It is the method by which the work of collaborators in my laboratory eventually led to the successful heart drug, ivabradine, to which I have already referred.

But the consequence of diverting large-scale funding towards the search for new drugs via genomics has been that the Black approach is now much less common and that the pharmaceutical industry is producing fewer new medications at vastly greater cost. Of course, the Black approach could and should be complemented by genomics, and there are successful cases where protein targets found by classical methods were later also identified as coding templates formed by particular genes. A good example is Duchenne muscular dystrophy, where the gene for the protein utrophin that can substitute, in mice at least, to cure the disease was discovered before the DNA sequence was identified [54].

10. Conclusion

There has been much debate about whether the neo-Darwinist modern synthesis needs extending or replacing. Both views are correct. It depends on the context in which they are assessed. Theories in biology, as in any branch of science, can be judged by several criteria.

10.1. Falsifiability

The original neo-Darwinist assumptions of the modern synthesis have been clearly falsified. I will consider the three basic assumptions outlined in the Introduction.

10.2. The Weismann barrier

The Weismann barrier should be seen as a relative not an absolute barrier. Strict isolation of the genome was required

in order to exclude the inheritance of acquired characteristics. As we now know that acquired characteristics can be inherited, I believe it is more honest to admit that this reason for departing from Darwinism is no longer valid. In any case, the barrier could only apply in those organisms that have a separate germ line. For the great majority of the duration of life on the Earth, there was no separate germ line. And plants can reproduce separately from their germ line. Quite simply, then, two of the original basic assumptions, isolation of the germ line and the impossibility of inheritance of acquired characteristics, can be seen to be incorrect.

Some criticisms of this conclusion refer to the rarity of experiments showing intergenerational transmission of epigenetic mutations. Originally, this was based on the idea that the genome was always wiped clean of epigenetic marking, so that it was thought that the idea was misconceived and impossible. As I have shown, this is simply not correct.

Another criticism was that it would not be robust. It has been demonstrated to persist for as many as 100 generations, and that it can, in some cases, be as robust as DNA transmission. Moreover, it does not need to be robust in all cases. As the review by Burggren [55] shows, the softness and therefore reversibility of epigenetic inheritance is one of its evolutionary virtues. Sultan and co-workers [56] have also identified the factors that may determine the transience or persistence of epigenetic variation.

The third criticism is that it is observed in only rare cases. My reply is that so is speciation. Speciation is such a rare event that in thousands of years of selective breeding of cats, dogs, fish, etc., we have not succeeded in producing new species, as defined by reproductive isolation.

Note also that these criticisms obviously do not apply to functionally significant reorganization or hypermutation of genomes.

10.3. Blind stochasticity

The other basic assumption is blind stochasticity, meaning that what are seen as random genetic variations are not functionally directed. The concept of randomness is a major topic of research in philosophy, mathematics and physics. One way to by pass these highly technical issues is to ask the question 'random with respect to what'? The key in relation to evolutionary biology is whether variations are random with respect to function and whether they can be seen to be so. Even if the molecular-level variations do in fact represent functional order at a higher level, we will almost certainly require insight from the functional level to appreciate the functional nature of the molecular variations. The randomness I am referring to is therefore epistemological: without knowing the constraints by higher levels, the variations will *appear* to be random and unpredictable. Once we know those constraints the possibility of prediction at the molecular level begins to exist. Whether it is computable is a very different question. Given the huge differences of scale, e.g. between molecular and cellular, it is implausible to expect molecular-level computation alone to reveal the functionality.

Even before we consider whether a theory based on blind stochasticity has been falsified, we have to examine its conceptual status. A very basic lesson from physics is that stochasticity at lower, such as molecular, levels is not only inevitable as a consequence of molecular kinetic energy, it is also perfectly compatible with regular law-like behaviour at higher levels, a fact that was appreciated long ago by one of the founders of population genetics, Fisher [57]. Even if behaviour at a high level is directed, stochasticity is what we can expect at lower levels. The example in this paper concerning the evolution in different species of haemoglobins at high altitude illustrates that point perfectly. As the authors of that paper say 'predictable changes in biochemical phenotype do not have a predictable molecular basis' [45]. It is the physics of oxygen transport in organisms living at low partial pressures of oxygen that dictates the changes that occur to adapt to such environments, not specific changes in the genome.

From a gene-centric viewpoint, it could be objected that the genome changes are nevertheless those that enable the beneficial changes in oxygen transport to happen. That is certainly true. But it is precisely the higher level perspective that enables us to show that fact. What we can see here is that a conceptual issue, which is the question of the level at which functionality occurs, interacts with an empirical issue, which is whether the changes at the molecular level are predictable, *from that level alone*. Another way to put the conceptual issue is to say that, in any information transmission system, whether languages or genomes, sequences by themselves do not have meaning. They acquire meaning through their context, which can only be understood at a much higher level. As a linguistic example, the three letter alphabetic sequence 'but' has two totally different meanings and pronunciations in English and French. Similarly, genome sequences acquire meaning in their context. Sequences enabling arms, legs and eyes derive from organisms that had none of these.

10.4. Unravelling the problem

My paper unravels this problem by showing where some aspects of biological thought went wrong in the twentieth century. Schrödinger's book, *What is life?*, was a landmark in predicting correctly that the genetic material would be found to be an aperiodic crystal. But it contained the seeds of a major misunderstanding, leading Schrödinger, and then Crick and Watson, to maintain that, like a crystal, the genetic material could be read in a determinate way. That could be true only if the 'crystal', that is the linear polymer DNA, could be read and copied faithfully, with few or no copy errors. As we can now see, that is not an inherent property of DNA alone. On the contrary, it is a property of the complex system by which the copy error rate can be reduced from an unacceptable frequency of millions per genome to less than 1. That is a higher level systems property of cells, including an army of proteins and lipids, not of DNA alone. In life as we know it on the Earth, this process occurs only in the context of living cells.

A possible objection to this conclusion is that all proteins have DNA templates that determine their amino acid sequences. That includes the proteins that contribute to the error-correcting systems for DNA. That is true, but it is usually taken a step further to mean that therefore the genome determines everything. That is not true. The error-correcting systems operate within cellular structures that contain molecular elements, such as lipid membranes, that do not require DNA templates in order to exist. Elsewhere, I have shown that the structural information in cells can be represented as comparable to that in the genome [58].

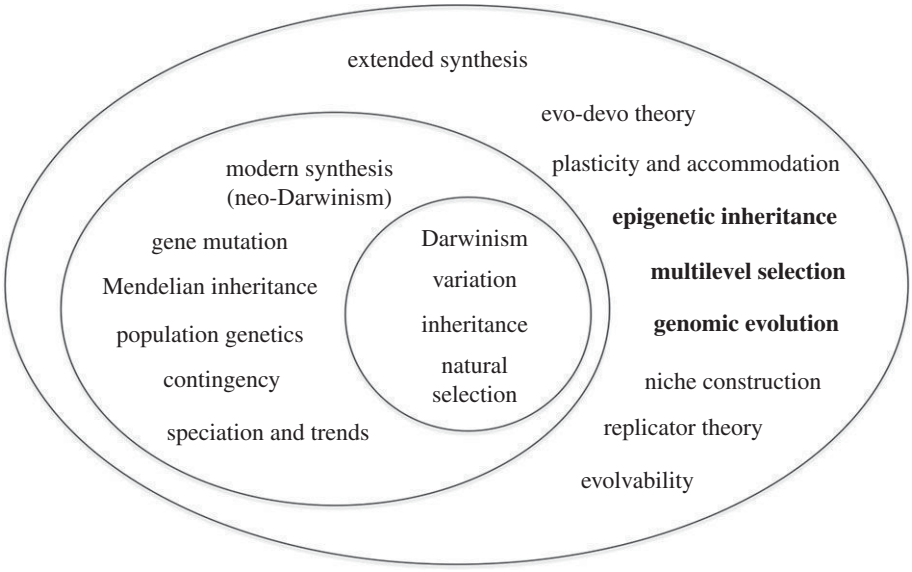

Figure 5. The extended evolutionary synthesis representing the extension as extensions of Darwinism and then of the neo-Darwinist modern synthesis (from [62]).

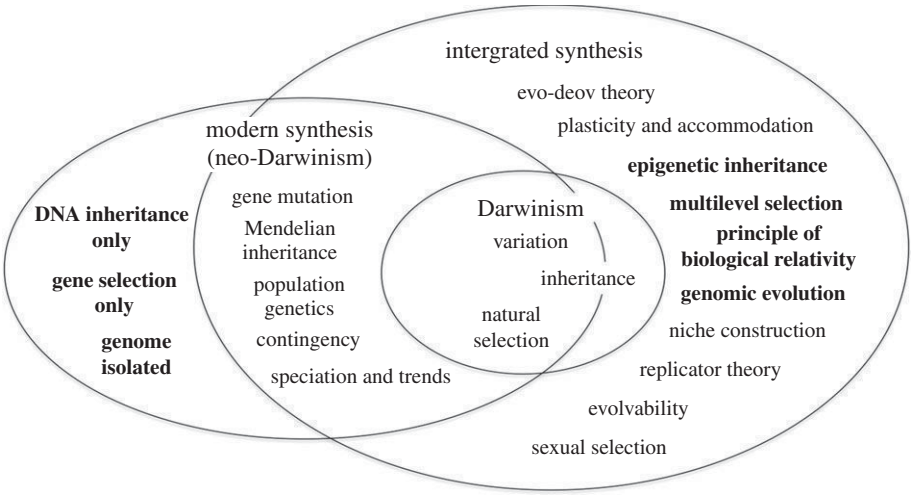

Figure 6. The integrated synthesis representing the extensions as extensions of Darwinism but only partially from neo-Darwinism. Darwin's view of inheritance is also represented as extending outside the boundary of neo-Darwinism/(developed for this article from [61], based on [62]).

Organisms always inherit both. In one of the rare examples of a successful clone from the nucleus of one species inserted into the enucleated but fertilized egg cell from another species, both the cell and the nucleus contribute to the final structure of the adult. Reproductive hybridization between species has also been shown to produce intermediate forms which can generate speciation [59].

Experimentally, we need to re-examine the way in which functional change in organisms can harness stochasticity at lower levels to create new functionality. Huang and his co-workers have shown the way forward here by demonstrating that stochasticity in gene expression is an attractor produced by a cell population. The many studies of targeted hypermutation, e.g. by Moxon's group, also show the way forward. Organisms in their evolution had to harness stochasticity because at a low enough level, this is an inevitable property of the physics of molecular-level systems that have kinetic energy.

We can now return to the question whether the assumption of blind stochasticity has been falsified. If the case presented in this paper is correct, then one answer would be that it is very difficult for it to be falsified because stochasticity necessarily reigns at a low enough level, even if functionality reigns at higher levels. The constraints may have too subtle an effect at the molecular level. The falsifiability then depends on a prior conceptual question, which is whether one accepts multi-level functionality. A purely gene-centric theory does not accept multi-level functionality and can therefore maintain its view of everything being 'blind chance followed by natural selection'.

To a physiologist or a medical scientist, this is not a useful viewpoint. Functionality arises in organisms at many different levels. This is one of the bases of my formulation of the principle of biological relativity, first proposed in a previous article in this journal, and developed more completely in a book, *Dance to the tune of life. Biological relativity* [60].

10.5. Utility

These points naturally lead to the other main criterion for judging a theory, which is its utility. Theories can be useful, even if they are false. Indeed, on a Popperian view of the logic of science, that must always be true. We can only ever falsify theories about the natural world, never conclusively

prove them. I want therefore to acknowledge the fact that the neo-Darwinist modern dynthesis was very useful. Whole fields of mathematical biology, such as population genetics, would not have flourished in the twentieth century without the modern synthesis as a framework.

But, I also think that we have reached a watershed in relation to the issue of the utility of the neo-Darwinist modern synthesis. As I have argued in detail elsewhere, there are too many experimental breaks with the original theory as formulated by Weismann & Wallace [61]. Moreover the metaphorical language of neo-Darwinism is a problem. The metaphors used strongly reinforce a simplistic gene-centric view. The time has come to see that evolutionary biology would progress faster if we used a different framework to develop a more inclusive theory, as illustrated in figures 5 and 6.

Figure 5 shows the extended evolutionary synthesis, which is represented as a development from the neo-Darwinist modern synthesis, in turn developed from Darwinism.

Figure 6 shows the version of this diagram that better represents the conclusions of this paper. There are several important differences. First, it represents the fact that Darwin's view of inheritance included the inheritance of acquired characteristics, which was excluded by neo-Darwinism. Darwin's concept of inheritance is therefore shown as being partly outside the neo-Darwinist modern synthesis. Second, it represents the features of the extended synthesis (highlighted in bold in both figures 5 and 6) that lie outside the range of neo-Darwinism as defined by Weismann and Wallace. The features of that theory that were excluded are shown as corresponding bold-face items. The highlighted items on the far left correspond with the highlighted items at the far right. Also included as a bold-face item is the principle of biological relativity. Although beyond the scope of this paper, I have included sexual selection.

In spirit, this approach inherits an important part of Darwin's more nuanced philosophical approach. I emphasize *philosophical* here because it is obvious that we have moved way beyond what Darwin knew experimentally, as figures 5 and 6 also show. But we can learn from his approach. Darwin was cautious in acknowledging the limits of what he knew. He was even unsure whether he had discovered the title of his book, because he did not know what produced variations in organisms, and he did not exclude the inheritance of acquired characteristics. Unjustified certainty is not the best way forward in scientific research. It remains open to further experimentation to clarify the extent of the many mechanisms now known to be available to nature, and to determine how she used them, alone or more probably in various combinations, to evolve life as we now know it.

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Review

Was the Watchmaker Blind? Or Was She One-Eyed?

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Abstract: The question whether evolution is blind is usually presented as a choice between no goals at all ('the blind watchmaker') and long-term goals which would be external to the organism, for example in the form of special creation or intelligent design. The arguments either way do not address the question whether there are short-term goals within rather than external to organisms. Organisms and their interacting populations have evolved mechanisms by which they can harness blind stochasticity and so generate rapid functional responses to environmental challenges. They can achieve this by re-organising their genomes and/or their regulatory networks. Epigenetic as well as DNA changes are involved. Evolution may have no foresight, but it is at least partially directed by organisms themselves and by the populations of which they form part. Similar arguments support partial direction in the evolution of behavior.

Keywords: blind chance; harnessing stochasticity; hypermutation; evolutionary hold mechanisms; adaptability driver; internal and external goals

1. Introduction

We again use our computer monkey, but with a crucial difference in its program. It again begins by choosing a random sequence of 28 letters, just as before ... it duplicates it repeatedly, but with a certain chance of random error—'mutation'—in the copying. The computer examines the mutant nonsense phrases, the 'progeny' of the original phrase, and chooses the one which, *however slightly*, most resembles the target phrase, METHINKS IT IS LIKE A WEASEL. (Richard Dawkins, *The Blind Watchmaker*).

1.1. Background

In chapter 3 of his book, *The Blind Watchmaker* [1], Richard Dawkins produces his famous Weasel program. He shows that a monkey writing out 28 characters randomly on a typewriter would require much more than the whole lifetime of the universe to arrive by pure chance at a correct 28 letter sequence to match Shakespeare's text [2]. But if each correct character were to be held constant between successive generations of random typing, it would require only a modest number (43) of iterations to achieve a correct result. The program resembles the operation of an old-fashioned three-wheel fruit (slot) machine. If the target for a reward is, say, three lemons and a spin of the wheels produces two, the best strategy might be to hold the wheels with the two lemons and spin the remaining wheel until that also shows a lemon. The number of 'wheels' (28 in the Weasel example) doesn't change the principle of this mechanism.

The example shows that, however unlikely a pattern might be, it might evolve in a reasonable amount of time by using such an incremental strategy.

Dawkins acknowledges that the original program does not truly represent the process of blind variation followed by natural selection assumed in neo-Darwinist evolutionary models since it uses a long-term goal set by the computer program writer. The program only 'knows' when to hold a character constant between generations by comparing it with the long-term goal. As Dawkins writes:

> "Life isn't like that. Evolution has no long-term goal. There is no long-distance target, no final perfection to serve as a criterion for selection, although human vanity cherishes the absurd notion that our species is the final goal of evolution. In real life, the criterion for selection is always short-term, either simple survival or, more generally, reproductive success."

It is also important to acknowledge that more complex versions of the Weasel program have been produced that do not require that a correct character should be completely fixed. In those cases back-mutation is also possible. But all these programs still require various kinds of comparison, in the selection process, with the long-term goal in order to succeed. When we refer to the 'hold' metaphor in this article it is important to note that this does not completely exclude mutations. It refers to the ability to preserve existing functionality sufficiently well for subsequent generations to inherit.

A further important deficiency in the Weasel program as originally formulated is that it assumes that the goal would be correctly represented by a particular genome sequence. From the viewpoint of organisms and their functionality, that is not correct. Genomes and phenotypes are far from being equivalent. The mismatch works both ways. The same genome can be used to generate many different phenotypes and the same phenotype can evolve through many different genome variations, to such an extent that the sequence variations may even be unpredictable [3]. It is of course the functional phenotype that is 'seen' by natural selection. DNA sequences are not directly available for selection other than through their functional consequences in the production of RNAs and proteins, and even most of those variations are effectively buffered by the regulatory networks and so may also be invisible to natural selection. For example, 80% of DNA knockouts in yeast are 'silent' in controlled experimental conditions [4]. We will return to this important point later (see Section 4.2) since it is the fundamental reason why gene-centric views of evolution are incorrect. Evolution is a high-level *forming* process, not simply a matter of genome informatics.

1.2. Purpose and Organization of This Article

In this article we will agree with Dawkins that (a) completely stochastic processes with no 'hold' or similar 'guiding' mechanism would require impossibly long periods of time for successful evolution to occur, and (b) there is no need to assume that evolution has a long-term goal. This is where both he and we part company with Intelligent Design (ID) and creationist theories.

But we will nevertheless show that organisms and populations of organisms do have identifiable and empirically testable goals, and that variations on the theme of the Weasel program found experimentally in nature, show this to be true. The key to understanding why we differ from neo-Darwinists on this matter lies in multi-level feedback processes that have been shown to exist which enable organisms and populations to direct their evolution in response to stimuli from the environment and so achieve the inheritance of acquired characteristics. These feedback processes require analysis of function at a high (systems) level, e.g., networks, cells, tissues, organs and the organism as a whole in interaction with the environment, including other organisms. Multi-level feedback is a requirement of goal-directed behaviour. A purely gene-centric view will not necessarily 'see' such feedback. Empirical tests used routinely in physiology and engineering do so readily.

Our article is not intended to be a systematic review. It is rather the development of a conceptual interpretation of the process of evolution that differs from neo-Darwinism in implementing the principle that there is no privileged level of causation [5,6]. We believe this is a novel conceptual advance. It differs radically from views of evolution that privilege the role of DNA sequences in the intergenerational transmission of inheritance [7–9], and is therefore more sympathetic to

views of evolution that emphasise the active role of functional regulatory networks and behavior in organisms [6,10]. These are the processes that endow organisms with what we will call natural purposiveness. DNA alone cannot do that. Outside the regulatory network environment of the complete cell it is inactive.

We develop our case in stages: first, to show how multicellular organisms use targeted evolution of their cells to respond to environmental challenge; second, to show how populations of microorganisms achieve similar targeted responses; third, to show how epigenetic inheritance occurs in multicellular organisms with separate germ-lines; fourth; to show how the evolution of behavior can use similar processes that have developed agency in their evolution. In all these cases, variation is not random with respect to genome location and/or organism functionality. The targeting of variation and the preservation of existing functionality ensure that evolution is not entirely blind. Where relevant we reference alternative viewpoints, including standard neo-Darwinist interpretations. But this article does not analyse where we believe those viewpoints are deficient. That was the purpose of a recent related article [11].

2. Definitions

Agency: an agent acts, it does not just react in the way, for example, in which a billiard ball is caused by another ball to move. Organisms are agents to the extent that they can interact socially with other organisms to choose particular forms of behavior in response to environmental challenges. This definition of agency can therefore apply to microorganisms, such as bacterial films and eukaryotic slime moulds, that form interacting communities [12,13] as well as to multicellular organisms. In principle, it can also apply to the subcellular networks responsible for buffering organisms against many forms of DNA variation.

Goals: Goals can be ascribed to agents since choice of action involves directionality in their actions. A goal in this sense is the situation towards which the agent's action leads. Goals arise naturally from within the agent's cognitive behavior, albeit in interaction with other agents. This kind of behavior can be called natural purposiveness. Goals can therefore be ascribed empirically on the basis of observation of the behavior of organisms.

Teleology: The possession of goals is what defines teleology. Some biologists prefer the word teleonomy [14] to emphasise the view that goals in organisms (sometimes with the qualification 'other than humans') are only apparent. Since our use of the word 'goal' enables empirical physiological tests for the presence of the required natural purposiveness we see no need to avoid the word teleology.

Natural purposiveness: Natural purposiveness is an emergent property of multi-level evolved systems. It is easier to understand and appreciate its significance within the principle of biological relativity, i.e., no privileged level of causation [6].

Neo-Darwinism: Classical neo-Darwinism was formulated by August Weismann [15,16] and others in the late nineteenth century to expunge the inheritance of acquired characteristics from Darwin's theory. Blind variation followed by natural selection was claimed to be *entirely sufficient* (Weismann's *allmacht*). This is clear from his extensive argument with Herbert Spencer [17–19]. Many biologists today redefine neo-Darwinism in various ways (see e.g., the on-line dialogue between one of us and David Sloan Wilson (https://thebestschools.org/dialogues/evolution-denis-noble-david-sloan-wilson/) and the relevant entry in the Encyclopedia of Evolution [20]). Redefining a term does not however change the fact that the original theory using that term is no longer the complete story. Our position can therefore, to some degree, be seen to return to Darwin's multi-mechanism viewpoint, though with vastly extended empirical evidence (see Figure 6 in reference [11]).

Gene-centrism: We will refer to gene-centric views of evolution several times in this article. There are two senses in which we view neo-Darwinist theories as gene-centric. The first is the view that the genome is "the Book of Life" [21], i.e., that the development of an organism is essentially a read out of the DNA sequences, in interaction with the environment. The hidden assumption here is that inheritance depends on DNA alone. Sometimes this is spelt out, as in the distinction between the 'replicator' (DNA) and the 'vehicle' (the rest of the 'disposable' organism). The second sense is that, even though it is the phenotype that is selected in evolution, only those aspects of the phenotype that are represented in DNA are inherited.

These definitions are conceptual, as are all definitions, but they endow the theory we develop here with empirically testable predictions.

3. Goals within Organisms

3.1. Regulated (Directed) Hypermutation Processes

The Weasel program example shows that the monkey at the keyboard needs some kind of guidance to have any chance at all of reaching the goal. In the evolutionary process the 'monkey at the keyboard' is blind chance mutations, the process assumed in neo-Darwinism to be the only process producing genetic variation. The assumption that all mutations are produced by blind chance is central to the theory. This is the assumption that appears to exclude goal-directed behavior [6].

Yet, as we will show in this paper, organisms have demonstrably evolved *guided* random mutation mechanisms that can respond rapidly and correctly to environmental challenges. These mechanisms allow organisms and populations to harness stochasticity to evolve a solution to such challenges at high speed compared to what could be achieved by blind chance alone. It is the harnessing of stochasticity in guided response to environmental challenges that achieves what blind chance alone could not possibly do [11].

One way in which the guidance can occur is through the process of natural selection. Progressively, through the generations, selection acts as a filter. Neo-Darwinism assumes that this is the only guide. We disagree with that view because it is demonstrably insufficient: nature also uses other faster guidance processes.

How can that be achieved? The answer is already implicit in our fruit machine analogy. The quickest way to achieve the fruit machine target is to hold correct wheels while spinning the others to let chance find the target. By analogy, this is precisely what the immune system does within our bodies.

Figure 1 summarizes how this is achieved. Faced with a new antigen challenge, the mutation rate in the variable part of the genome can be accelerated by as much as 1 million times. So far as is known, those mutations all occur stochastically. But the location in the genome is certainly not a matter of chance. The functionality in this case lies precisely in the specific targeting at the relevant part of the genome. The mechanism is directed, because the arrival of the antigen itself activates the hypermutation process, and its binding to a successful antibody triggers proliferation of those cells that make it. What this mechanism achieves is that all the other 'wheels' in the DNA sequence forming a template for the immunoglobulin protein are held sufficiently constant for functionality to be retained. Even more remarkably, all the functionality in the rest of the genome is also maintained. Considering the huge size of the complete genome, this is pin-point targeting requiring highly specific feedback processes to be successful.

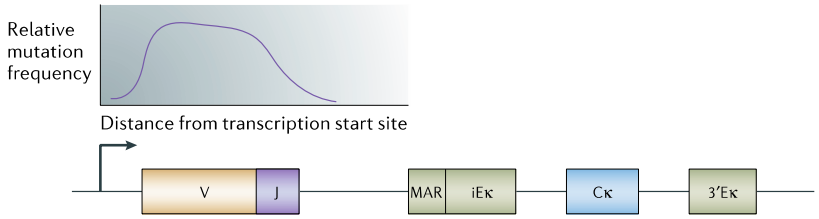

Figure 1. Schematic diagram of gene-specific targeted hyper-mutation in immunoglobulin gene loci. The mutation rate is greatly increased only in the variable part of the genome, which is a ~1.5 kilobase region in each of the three immunoglobulin loci. In this figure, the rectangular elements (V, J, MAR, iEκ, Cκ, 3′Eκ) represent different functional parts of the DNA sequence for the immunoglobulin protein. V is the variable part, subject to hypermutation, while the other parts are fixed. For further details on the functions of the parts see Odegard and Schatz [22]. Those details are not important for the purposes of this article.

3.2. Is the System Purposive?

Holding correct parts of the immunoglobulin sequence constant is the way rapid mutation can then be restricted to only very small *and relevant* parts of the whole genome. Hyper-mutation of all the immunoglobulin sequence, and even more so everywhere in the genome, would not work. As Odegard and Schatz say:

> "Somatic hypermutation (SHM) introduces mutations in the variable region of immunoglobulin genes at a rate of ~10^{-3} mutations per base pair per cell division, which is 10^6-fold higher than the spontaneous mutation rate in somatic cells. To ensure genomic integrity, SHM needs to be targeted specifically to immunoglobulin genes."

What this example shows is that the basic idea in Dawkins' Weasel program is actually broadly correct. Imagine that the monkey already has XYZHINKS IT IS LIKE A WEASEL. Then the best strategy is to treat only the XYZ sequence with stochastic mutation until MET turns up. Within the Weasel program analogy, it would be essential to hold the sequence HINKS IT IS LIKE A WEASEL constant.

At this point it is important to recall what we emphasized in the INTRODUCTION: Evolution is a high-level forming process, not simply a matter of genome informatics. The more correct way to look at the process therefore is that it is the high-level functionality that corresponds to HINKS IT IS LIKE A WEASEL and to any equivalently functional sequence that needs to be maintained. Any low-level sequence changes that are neutral with respect to phenotype functionality would not matter. The targeting may therefore be attributable to higher-level buffering by regulatory networks in addition to differential genome mutation rates. This point is important since not all the examples we discuss later in this article necessarily involve differential rates of mutation.

This is also why it is misleading to talk of the 'language of the genes' [23] or the 'book of life' [21]. In a language, the sequence *is* the written language's 'phenotype'. That is even more obvious in languages employing idiograms. By contrast, the genome is a template resource used by the organism, and is far from identical with or simply translatable into the phenotype.

The targeted mechanism in the immune system has been known and intensely studied for many years [24]. So, how did many people not realise that it is a physiologically guided process? The answer is that the guidance does not lie at the genome level. At the genome level the process appears blind. It depends on stochastic mutation. The functionality enabling the process to be described as guided lies in the system as a whole.

The system includes: (a) sensing the environmental challenge, i.e., the antigen invasion, (b) transmitting this signal to the nuclei of immune system cells to trigger hyper-mutation in just a tiny fraction of the genome. (c) Then sensing of the correctness or otherwise of the outcome, followed by the "reproduce or die" signal: cells that do not produce an antibody that fits the antigen do not survive. At this stage, natural selection occurs amongst the population of immune system cells [25]. This is a complete finely-tuned physiological feedback system that rapidly generates an acquired characteristic in response to an environmental challenge, which is then inherited in the surviving population of cells. This is what is *meant* by a goal-oriented system. By all the usual criteria this is a teleological, i.e., goal-directed, process (see Section 2).

It may not be perfect; it doesn't have to be. Not all keys have to be perfect to open a lock. The system feels its way forward, harnessing stochasticity to create novelty while using targeted preservation of what already works. The targeted preservation is what gives the system its purpose: to maintain its own integrity. It uses stochasticity to change what it must change, precisely because that is the part that doesn't work.

It is important moreover to see that the goal, the directionality, exists *within* the organisms and their populations. The goals of organisms have developed during the evolutionary process. Our position does not therefore require the ideas of Intelligent Design. In agreement with this aspect of Dawkins' position, we do not have to assume there is a long-term goal.

At this stage it is also important to clarify that we partly agree with alternative (such as neo-Darwinian) views of hypermutation mechanisms, to the extent of saying that such differential mutation rates must have evolved, and that the neo-Darwinian mechanism of stochastic variation combined with natural selection has operated [26–31]. The point to understand is that, once hypermutation has evolved and is linked to environmental feedback that endows the organism with natural purposiveness, subsequent evolution is not purely neo-Darwinian. Natural purposiveness evolves and then changes the nature of subsequent evolution. There is a transition, one of many transitions in evolution [32], the most spectacular of which has been the transition to enable cultural evolution leading to the development of humans, to which we will return in Section 6.

3.3. Natural Genetic Engineering

Such physiologically functional feedback leading to genomic change in response to an environmental challenge is not restricted to the immune system. In fact, responsiveness of the genome generally to environmental stress was discovered by the Nobel laureate, Barbara McClintock, more than 70 years ago. Working on Indian corn, she showed that in response to stress genetic material can move around even between different chromosomes [33]. She was therefore the discoverer of what are now called mobile genetic elements, known more colloquially as 'jumping genes'. In her 1983 Nobel Prize lecture she wrote:

> "In the future attention undoubtedly will be centered on the genome, and with greater appreciation of its significance as a *highly sensitive organ of the cell*, monitoring genomic activities and correcting common errors, sensing the unusual and unexpected events, and *responding to them, often by restructuring the genome*. We know about the components of genomes that could be made available for such restructuring. We know nothing, however, about how the cell senses danger and instigates responses to it that often are truly remarkable" (our italics). [34]

This was highly perceptive since it was written before whole genome sequencing. By 2001 with the publication of the first complete draft of the human genome, it became possible to compare genome sequences in different organisms. The results show that movements of whole domains of sequences corresponding to functional domains of transcription factor proteins and chromatin proteins must have occurred as evolution diverged to produce organisms as different as worms, yeast, flies, mouse and human [6,24,35].

Movement and rearrangement of functional domains of proteins can also function as a mechanism for speeding up evolutionary change. Like targeted hypermutation it also avoids having to wait for very slow accumulation of small (point) mutations. To appreciate this in less technical language, imagine two children given Lego bricks to construct a model bridge. To the first child we give a pile of the original small Lego bricks which have to be laboriously pieced together to form an architectural feature like an arch. To the second child we give some preformed Lego structures. It is obvious that the second child will construct a realistic bridge much faster than the first.

Moving complete functional domains around the genome is therefore a bit like the mirror image of hypermutation since it recombines already functional parts of proteins. In terms of the Weasel program, imagine already having METHINKS IT IS and LIKE A WEASEL. Joining them up is worth trying. Of course, not all joined up sequences will produce new functionality. What the mechanism gives is a much improved chance of obtaining new functionality. There is a bias in the process, which is precisely the extent to which it is not blind. It plays with existing functionality. As we will show later in this paper, behavioural evolution can use comparable mechanisms in which existing functionality is preserved and rearranged.

4. Goals within Populations

The cells of the immune system can evolve extremely rapidly to achieve the goal of the system, where the goal is the protection of the organism, and the system is the organism itself. But each organism does not transmit all of this information to its progeny. In this section we will look at ways in which populations of organisms can use stochastic mutation to evolve inheritable functional responses to environmental challenges very rapidly.

4.1. Contingency Loci in Bacteria

A comparable mechanism to that employed by the immune system has been extensively investigated by Richard Moxon and his colleagues who use the term 'contingency locus' to characterise the targeted loci of hypermutable DNA [36]. In bacteria, these loci are simple sequence repeats in which the repeating unit is one to several nucleotides in length. In eukaryotes these loci are called microsatellites and often consist of hundreds of repeats. In both kinds of organism these microsatellites are prone to high rates of mutation through slippage during strand repairing, leading to either increases or decreases in the number of repeat units. When these mutations occur within functional gene sequences, they can therefore produce high frequency reversible switching of genotype. Since "mutation rates vary significantly at different locations within the genome" they propose that "it is precisely in the details of these differences and how they are distributed that major contributions to fitness are determined." In an earlier article, Moxon and Thaler write

> "This phenotypic variation, which is stochastic with respect to the timing of switching but has a programmed genomic location, allows a large repertoire of phenotypic solutions to be explored, while minimizing deleterious effects on fitness." [37].

Moxon and Thaler's conclusion is correct. If the hyper-mutation were not restricted to a small subset of the genes, the results would certainly be deleterious, just as non-targeted hypermutation of immunoglobulin genes would rapidly destroy the functional proteins of the immune system.

The phenotype effects can also be combinatorial. The example given by Moxon et al. [36] is that switching in just seven independent loci to produce two genotypes in each case could generate up to 128 phenotypes. Many of these phenotype changes occur significantly in bacterial cell surface structure, which is the structure through which organisms detect changes in the environment and foreign invaders. They can also generate switching between metabolic and regulatory cell networks: Ritz et al. discovered a triplet repeat enabling *E. coli* to switch between adaptation to reducing and oxidizing environments [38].

Can such processes be demonstrated in actual evolutionary time? An example of organisms making use of this ability to reorganise their genomes is the study of Bos et al., who have observed the emergence of antibiotic resistance from multinucleated bacterial filaments. They write:

> "The strategy of generating multiple mutant chromosomes within a single cell may represent a widespread and conserved mechanism for the rapid evolution of genome change in response to unfavorable environments (i.e., chemo-therapy drugs and antibiotics)" [39].

Similarly, Jack et al. (2015) have shown that

> "Signaling pathways that sense environmental nutrients control genome change at the ribosomal DNA. This demonstrates that not all genome changes occur at random and that *cells possess specific mechanisms to optimize their genome in response to the environment*." (our italics) [40].

It is important at this stage in the argument to note that, in addition to the functional feedback, an essential property in purposive genome adaptation in response to environmental stress is the 'hold'

mechanism, by which existing functionality is preserved. This mechanism can operate whether or not hypermutation comparable to that in the immune system and many bacteria occurs. Hypermutation is simply an extreme example of the non-random location of mutations. Any differential mutation rate in genomes *might* be exploited by organisms and so improve their chances of generating new functionality, though equally clearly differential mutation rates alone do not *necessarily* indicate functionality. The relevant feedback loops with environmental interaction must also exist. That is an essential part of how a goal-oriented system is defined (see Section 2). The evolution of such links is a major transition.

4.2. Genetic Buffering by Regulatory Networks

Buffering of genome variation by regulatory networks may also be involved. This is a further important part of our argument so we have represented it in a development of Waddington's famous landscape diagram shown in Figure 2. As in the original diagram, genes (as DNA sequences) are represented by the pegs at the bottom. The regulatory networks are represented as lying between the genes and the phenotype. Genes can only influence the phenotype through the networks; they do not do so directly. They themselves do not exhibit agency in the sense in which we define it. This is one of the reasons why we believe the 'selfish gene' metaphor is misleading. Moreover, from a physiological perspective, selfish gene theory is not testable [41].

We have added two new features to the diagram. The first is the inclusion of environmental interactions above the phenotype landscape. The second is a cloud which we have placed to represent buffering of genomic change by the networks. Inspired by Hillenmeyer's work on yeast [4], we have represented the cloud as covering as much as 80% of the genome, which is the proportion of silent knockouts in Hillenmeyer's experiments, to which we have already referred in the INTRODUCTION. Similar robustness has been found in the networks involved in the natural pacemaker of the heart, where multiple mechanisms exist that can maintain rhythm if one is disabled either by genetic change or by pharmacological blockers [42].

Of course, that percentage will vary with different species, cell types and many other parameters. The buffering cloud may cover different proportions of genomic change under different environmental and experimental conditions. Thus, Hillenmeyer et al.'s experiments to which we referred earlier [4] also showed 97% of genes to be functional by varying metabolic conditions. The controlled experimental conditions in which 80% were silent are unlikely to match actual wild population conditions. The cloud is therefore dynamically variable, as its name suggests. Note also that the cloud is not a separate structure from the networks. It represents the filtering (buffering) action of the networks: a process of the networks, not an object in its own right.

The idea of the cloud mechanism was already implicit in Waddington's early work on what he called epigenetics [43] and has been confirmed by many physiological and developmental studies since then on the robustness of organisms in accommodating genomic variations, the most recent studies being the comparative failure of genome-wide association studies to reveal very much about the genetic origins of health and disease [44,45]. This is one of the most important empirical findings arising from genome sequencing. But its implications for evolutionary biology have not yet been sufficiently well appreciated.

The main implication that is relevant to this article is that the hold mechanism need not require targeted differential mutation rates. In the current stage of our knowledge we do not believe it is possible to estimate how often evolutionary change might depend on targeted differential mutation compared to the operation of regulatory networks protecting themselves via 'cloud' mechanisms. Those mechanisms are of course a further aspect of the robustness of regulatory networks in the face of genetic changes. Nor are the mechanisms only epigenetic. The phenomenon of epistasis by which different genes can influence each other's effects can play a similar role, particularly when the interactions, mediated through the networks, are to cancel each other's effects [46].

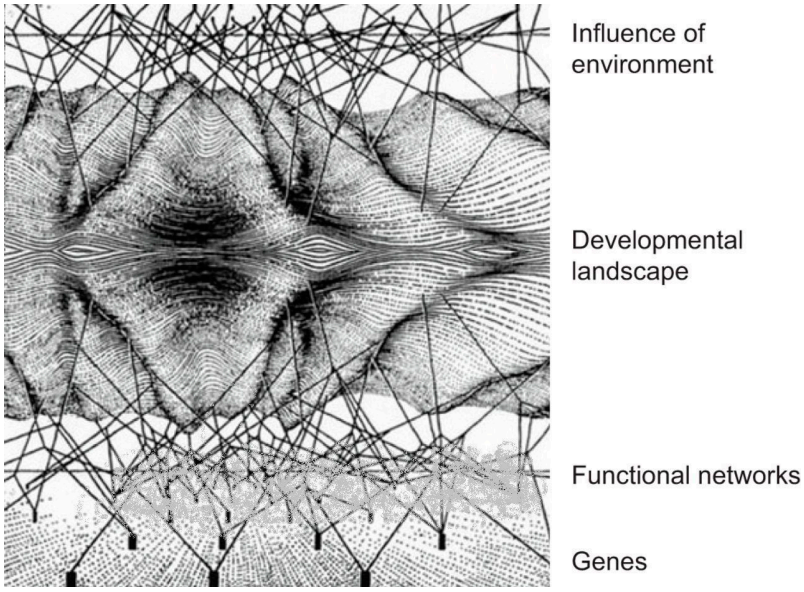

Figure 2. Development of Waddington's (1957) landscape diagram [43]. The original diagram was simply the lower half of this diagram, which Waddington used to indicate that the developmental phenotype (the landscape) is not directly dependent on the genes (the pegs at the bottom) but also depends on the regulatory networks represented as lying in between genes and the phenotype. Our version of the diagram incorporates two new features. First, organisms are open systems sensitive to the environment. This is represented by the top half of the diagram. Second, the regulatory networks in the lower half (the original half) of the diagram act to buffer genetic variation. This is represented by a 'cloud' covering a large fraction of the genome, corresponding to the fact that many mutations at the genome level are silent functionally. The regulatory networks can buffer many variations at the genome level. The filtering action of the 'cloud' performs a function similar to that of the 'hold' mechanism in this article. Differential mutation rates are not therefore essential to enable organisms to guide their own evolution.

4.3. Switch of Function in Regulatory Networks

Networks can not only act as buffers, they can also switch function. Our next example is from the work of Taylor et al. [47] who have shown that bacteria that have lost their flagella through deletion of the relevant DNA sequence can evolve the regulatory networks required to restore flagella and so restore motility in response to a stressful environment within just four days.

That ability is a property of the bacterium regulatory networks and of the ability of the organism to signal the environment pressure to those physiological networks to enable them to adapt. It is that feedback that makes such a rapid and clearly functional response possible. Two mutations were involved:

> "Step one mutations increase intracellular levels of phosphorylated NtrC, a distant homolog of FleQ, which begins to commandeer control of the FleQ regulon at the cost of disrupting nitrogen uptake and assimilation. Step two is a switch-of-function mutation that redirects NtrC away from nitrogen uptake and toward its novel function as a flagellar regulator. Our results demonstrate that natural selection can rapidly rewire regulatory networks in very few, repeatable mutational steps".

Viewed from the level of the genome site(s) where the mutations are occurring, there is a process of Darwinian selection amongst the results of stochastic mutation, as the authors themselves say. But the response clearly exploits the physiological regulatory properties of *existing* cell regulatory networks, resulting in a switch of function at the regulatory network level.

4.4. Roles of Stochasticity and Natural Selection

This is also a suitable point at which to emphasise that we are not denying the essential contribution of a neo-Darwinian process, i.e., mutation followed by natural selection. Natural selection necessarily operates within the context of these evolved functional characteristics. The key to our argument lies in the way in which organisms maintain and develop existing functionality *either* through exploiting differential mutation rates *or* through buffering the effects of mutations in many parts of the genome, *or* combinations of the two processes. It requires a multi-level systems-level approach to see that the neo-Darwinist process is harnessed, so that it is not sufficient in itself to explain the functionality of what is happening.

This point reflects once again our insistence that evolution is a high-level forming process. It may help to clarify that there are two senses in which this point has force. The first is conceptual. Even when a process is entirely neo-Darwinian when viewed from the genome sequence level, that viewpoint is not necessarily the most productive way to view it. Much more than the genome is inherited. The roles of regulatory networks, which are relatively well buffered against sequence changes, and the physiological feedback processes that ensure that the process leads to the inheritance of a characteristic in response to an environmental change, are better characterized from a physiological systems perspective. They also function within a cellular structural environment involving lipids and other components not coded for by the genome.

The second sense is empirical. It may, as a matter of fact, be the case that at the molecular level targeted differential mutation rates form an essential part of the process that explains the speed with which the adaptation occurs. In this article we have given examples of both of these senses.

4.5. Communication to the Genome

How can genomes know about what is happening at the cell surface? The physiological mechanisms by which events in tiny micro-domains near the cell surface signal to the nucleus, and so control specific gene expression levels, have now been studied in fine detail [48,49]. There is no longer any mystery in understanding the highly specific transmission of information to the nucleus that can control gene expression. There is no reason why genomes should not use similar communication pathways in response to stress signals received by cells and organisms.

5. Speculation 1: Goals Achieved through Epigenetic Inheritance

5.1. Different Forms of Epigenetics

Epigenetics was originally introduced and defined by Conrad Waddington [43] to refer to the role of networks in organisms in interpreting and controlling their genetic inheritance. Waddington was a developmental biologist and he correctly identified the general mechanisms by which development, in interaction with the environment and genes, could canalize both development itself, and subsequently also inheritance, towards specific phenotypes. His experiments on fruit flies showed that selection for environmentally-induced variants could become assimilated into the genome within a relatively small number of generations, as few as around 14 or 15. In so doing, he produced one of the first examples of the inheritance of acquired characteristics based on rigorous multigenerational experiments.

In addition to Waddington's mechanism of genetic assimilation of acquired characteristics, there was the discovery of transcription factors, i.e., proteins that convey signals to the genome from higher level networks to control levels of gene expression. This mechanism is what makes it possible for cells as different as bone cells and heart cells to be developed using the same genome. In vertebrates, around 200 clearly distinct cell types are produced in this way during development and in the maintenance of tissue types in the adult.

Recently, the mechanisms of epigenetic control of the genome have been greatly extended, through the discoveries of DNA marking, histone marking, and many processes by which the germline can be

either side-stepped [50] or itself marked, or modified by the transmission in the germline of functional RNAs [51,52].

5.2. Experimental Examples

These developments are transforming the study of genetics in evolution. As just one well-documented example, Michael Skinner and his colleagues have experimented on one of the icons of Darwinian evolution, the Galapagos finches, to find that there are as many epigenetic as genetic variations and that the number of epigenetic variations between the species correlate rather better with phylogenetic distance between them than do the genetic variations [53]. But it will be almost impossible to determine which came first in the evolution of the different species, since epigenetic and genetic changes necessarily interact. Moreover, some authors have highlighted the important role that 'soft' (epigenetic) inheritance can play in evolutionary change [54,55].

Given that organisms are active agents and can choose their behavior in response to the environment (including other organisms), they must be able to mark their genomes with variations as a consequence of their behavior. This is the mechanism described by Michael Meaney and his colleagues in showing how stroking behavior in rodents can mark the genome in the hippocampus to predispose the young to adopt the same affective behavior as adults [56].

Similarly, many life-time choices can now be shown to act epigenetically to influence health and disease in subsequent generations. Hanson and Skinner have written a valuable review of these effects [57]. They include all the evidence for environmentally-induced inheritable epigenetic impacts shown in Table 1, taken from their review.

Table 1. Environmental epigenetic impacts on biology and disease.

• Worldwide differences in regional disease frequencies
• Low frequency of genetic component of disease as determined with genome wide association studies (GWAS)
• Dramatic increases in disease frequencies over past decades
• Identical twins with variable and discordant disease frequency
• Environmental exposures associated with disease
• Regional differences and rapid induction events in evolution

Regional differences and the dramatic increases in disease frequencies are hard to explain without recourse to epigenetic mechanisms, just as identical twin studies show that genome differences alone are insufficient.

All of the epigenetic effects referred to in this section are well-documented experimentally (see Menger [58] and Skoblov et al. [59] for further examples). We come now to our first main speculation. If organisms have agency and, within obvious limits, can choose their lifestyles, and if these lifestyles result in inheritable epigenetic changes, then it follows that organisms can at least partially make choices that can have long-term evolutionary impact.

5.3. Role in Speciation

We refer to this as a speculation because, as Skinner says, it may be very difficult to disentangle epigenetic and genetic changes using estimations based on the differences between living species. Ideally, we would need to document both genetic and epigenetic changes as a function of time during the developments that led to the speciation. Given the long periods of time over which speciation occurs, it is difficult to see how such experiments could ever be performed. Like astronomers, we usually have to infer the past from what we observe now. Moreover, as Waddington showed, epigenetic changes can become assimilated into the genome: what begins as 'soft-wired' may become 'hard-wired'. One of the mechanisms by which such assimilation may occur is analysed in Noble, reference [6] (pp. 216–219) which is also the mechanism Waddington himself proposed. More generally

epigenetic changes produce changes in function and behaviour that lead to organisms choosing different niches. Genetic change can then follow. That process itself would usually 'hide' evidence of what initiated the change.

Exceptionally, the speciation process can be sufficiently rapid to be observed during very few generations. This is so for the remarkable case of an immigrant finch to one of the islands in the Galápagos archipelago which initiated a new genetic lineage by breeding with a resident finch. Genome sequencing of the immigrant identified it as a male that belonged to another species more than 100 km from the island. To quote the paper:

> "From the second generation onwards the lineage bred endogamously, and despite intense inbreeding, was ecologically successful and showed transgressive segregation of bill morphology. This example shows that reproductive isolation, which typically develops over hundreds of generations, can be established in only three." [60].

The rapidity with which this reproductively separate line was established is expected since one of the most important triggers for animal and plant speciation is interspecific hybridization. An important feature of hybrid germlines is disruption of normal epigenetic control which translates into increased genome restructuring and mobile element activity. Thus, in addition to forming novel combinations of genome components, hybridization triggers genome innovation by modification of a higher-level control regime, as outlined in more detail in the review by Shapiro [61].

6. Speculation 2: The Evolution of Goal-Directed Behaviour

The effect of behavioural control on evolutionary change could be especially great when the social environment is a major component of the challenges faced by animals. The result would be that individuals would evolve to understand and predict what other members of the social group are about to do [62].

6.1. The Continuity of Animal and Human Evolution

This article is itself part of the proof of what we are leading up to. We and the imagined monkeys at their keyboards in the Weasel example evolved from common hominoid ancestors several million years ago. They in turn evolved from the ancestors of all mammals, in turn from the pre-Cambrian fauna, and so on as we stretch back to the last eukaryotic ancestor, or even the last universal common ancestor, maybe 3 billion years ago. Unless we are to return to a theory of special creation, humans capable of writing this article form a complete evolutionary continuum with the whole of the rest of life on earth.

It is implausible to suppose that goal-directedness and creative purpose suddenly appeared only with the first humans [63]. It is therefore important to identify the roots of such mechanisms that have evolved in other organisms. If goal-directedness is identified as the ability to anticipate what other organisms may do and then act on that ability, then there is no serious difficulty in observing that many organisms in addition to humans can do this [10,62,64–68]. Animals like monkeys, dogs and wolves are sensitive to inequity in the behaviour of others [66,69] and so can favour the formation of cooperative groups. An important criterion is the ability to show unlimited associative learning, which is necessary for such anticipatory and innovative behavior. Bronfman et al. show that this can be observed in animals ranging from vertebrates to arthropods and cephalopods [70].

We contend that neo-Darwinism doesn't have the conceptual tools necessary to even begin to understand the transition to goal-directedness and creative purpose since it assumes a priori that these do not exist, they are only 'apparent'. By insisting on the necessarily exclusive role of blind chance in generating novelty it ignores the fact that such chance has to be, and has been, harnessed by organisms, which are therefore also *active* agents in their own development and evolution [62,71]. As Bateson says so succinctly:

> "the picture of the external hand of natural selection doing all the work is so compelling that it is easy to regard organisms as if they were entirely passive in the evolutionary process." (p. 105).

If we really insist on the passive role of the organism, we will not even recognize the significance of active agency in evolution. Assuming that natural selection is the only directing process is equivalent to denying that organisms have agency, or at least that, if they do, it does not play any role in their evolution. Darwin would not have agreed. He clearly identified the role of sexual selection in evolution [72].

6.2. The Adaptability Driver

Novelty, creativity, can only emerge if stochasticity is harnessed, rather than given free rein. Stochasticity everywhere is destructive, not constructive. But this requires the progressive building-in of mechanisms that include various forms of 'hold' when partial solutions have already been found. This is how organisms come to 'know' [73] what to keep and what to reject. Of course, all of this 'knowledge' is built on the processes of stochasticity and selection. Equally clearly, those processes on their own are insufficient. Furthermore, what becomes built-in includes much more than the genome since it also includes regulatory networks and 3D membranous systems that are also inherited and without which DNA is inactive. This is yet another example of our point that evolution is a high-level forming process, not simply a matter of genome informatics.

Can we therefore generalize the targeted mutation examples to apply not only to physical but also to behavioural evolution and the various forms of active role that organisms may play in their own evolution?

That is surprisingly easy. Targeted mutation allows stochasticity to be harnessed precisely because the organism's use of it is not blind. On the contrary, it is linked to higher-level processes that enable the organism to be an agent (see Section 2). The organism combines mutation with buffering of all that it is important to retain. Change is always combined with preservation. That ability is active in the sense that the knowledge required to identify what to preserve and what to change originates within the organism itself. How it originally evolved to have that knowledge is important, but not immediately relevant to the case being made here. Like the emergence of attractors in physical systems, once they have emerged, the clock can't be turned back.

Very early in the development of Darwin's theory of evolution it was noticed that organisms have the adaptability to choose or even create new niches for themselves and so to partially direct their own evolution. Darwin himself drew attention to the idea as it applied to sexual selection [72], where it is clearly true that choices of mate according to desired characteristics would influence future evolution. Once again, we quote Bateson on this aspect:

> "Charles Darwin (1871) argued that choice of a mate could drive evolution. He called the evolutionary process 'sexual selection'. Alfred Russel Wallace, although the co-author with Darwin of the first clear statement about the role of Natural Selection, did not like the new idea. Indeed, for many years most biologists did not take sexual selection seriously. When I was an undergraduate I was told confidently that, even if it were possible in theory, the process probably played little part in biological evolution. In recent years, however, many experiments have supported Darwin's thinking." [67].

The generalization of the idea of organism choice in evolution was originally attributed to Baldwin and became known as the Baldwin effect [74,75]. Bateson has researched the history and development of the idea [76]. The process was first identified by Douglas Spalding in 1873 [77]. To avoid historical confusion and to let the name of the process be easier to understand it, Bateson chose to call it the *adaptability driver*. We like this nomenclature for two reasons. First, adaptability is the behavioural equivalent of the targeted mutation process. The key lies in the restriction of change only to *parts* of

the behavioural repertoire. Second, the word 'driver' captures the active role of the organism itself. Blind chance is then not the only driver of novelty.

6.3. The Role of Contextual Logic in the Behavior of Organisms

We will conclude this article with a brief sketch of how contextual logic enters into the behaviour of organisms so that they do not react purely passively, but rather become active agents in interaction with their environment. From an evolutionary perspective, organisms have become active agents behaviourally because there are obvious advantages in being able to anticipate the behavior of other organisms. For a predator, anticipating the behavior of prey may be the difference between lunch and no lunch. Conversely, for prey, to be able to anticipate the behaviour of a predator could be the difference between death and survival. Such anticipation assumes rule-guided behavior by organisms for prediction and anticipation to be possible. What results is like a game. Iterative game-playing is clearly not unique to humans.

We may then extend the analogy with the processes of targeted genome mutation and re-organisation outlined earlier in this article. Winning games, including those between predator and prey, depends precisely on a combination of preservation and change. On the one hand, organisms learn the repetitive rules of interaction. The better those rules are known, the more effective will be the organism's anticipation of the behavior of others. On the other hand, innovation requires the ability to break out from the rules, to foil the anticipation of others. We speculate that this is where stochasticity plays a role similar to that of the harnessing of stochasticity in targeted genome variation. Organisms that can combine rule-guided anticipation with occasional innovative behavior will have a selective advantage. Our speculation is that selective harnessing of stochasticity enables innovation, just as it enables targeted genome variation, but the benefits depend also on combining innovation with conservation of routine behavior.

Once organisms have acquired such ability, they become active agents, and all the well-known evolutionary consequences of the 'adaptability driver' then follow.

7. Conclusions

7.1. The Harnessing of Stochasticity

In this article we have outlined mechanisms by which organisms and populations harness stochasticity and so improve their chances of developing functional responses to environmental challenges. Provided that we correctly interpret the targeted nature of genome variation and its necessary correlate, i.e., the preservation of already functional genome sequences and/or the buffering of genome change by the regulatory networks, the principle of Dawkins' Weasel program becomes broadly correct. The 'hold' mechanism corresponds to the spatial targeting of mutation or the buffering of many genetic variations by the active networks: all other sequences and network properties are sufficiently well preserved ('held') to maintain functionality.

Some evolutionary biologists have attributed what we describe as an active 'hold' mechanism to a passive 'ratchet' mechanism [32,78–81]. A process now known as Muller's Ratchet was introduced in 1964 following a series of papers in which he explored the evolutionary significance of sex. He showed mathematically that the damage that would result from accumulation of deleterious mutations can be ameliorated by recombination, either by exchange of DNA between organisms or by recombination through sexual reproduction. Muller's Ratchet idea captures a form of blind directionality (ratchets go forwards not backwards) in evolution but it does not capture the agency of organisms themselves which is implied by the 'hold' metaphor.

Maynard Smith and Szathmary included the ratchet mechanism in their 1995 book on *The Major Transitions in Evolution* [32]. A further advance in this idea was described by Lukeš et al. [79] to demonstrate how a ratchet process which, following Stoltzfus [82] they term Constructive Neutral Evolution can generate cellular complexity. They come close to our idea of the functional significance

of genetic buffering (represented as the cloud in Figure 2) when they write "The interaction (between two biochemical components, A and B), though not under selection, permits (suppresses) mutations in A that would otherwise inactivate it." Our reading of "permits (suppresses)" is precisely the cloud mechanism. While their paper is an undoubted advance on the ratchet idea, it does not include a targeting of the process of mutation, nor does it include feedback from and to the environment.

This is the reason why functionally significant 'hold' mechanisms forming parts of purposive feedback systems should not be interpreted simply as a 'ratchet' mechanism. It is precisely the targeted nature of the complete physiological feedback system *using* the 'hold' mechanism that generates functionality and gives the subsequent evolutionary process a direction, driven by organisms themselves. To repeat what we wrote earlier in Section 3, "this is what is *meant* by a goal-oriented system."

7.2. Organisms as Agents

Yet, our interpretation of the correctness of the Weasel program uses a mechanism that Dawkins did not himself acknowledge. The reason is that neo-Darwinists generally eschew the concepts of goal-directedness or teleology. The idea of active agency in organisms runs counter to neo-Darwinist thought. We suggest that this view is motivated partly by what are perceived as the dangers of misinterpretation of teleological explanations, which are thought to support the ideas of creationism and intelligent design. But attributing agency and directedness to organisms themselves makes no commitment whatsoever to ideas of long-term goals in evolution. We therefore believe that this concern is misplaced. In any case, the scientific investigation of goal-directed behavior should not be restricted by perceived opportunities for misinterpretation.

This also explains why classical neo-Darwinism [15,16], and modern versions of it [1,8] completely excluded the inheritance of acquired characteristics (see Section 2). Such inheritance is sometimes thought to open the door to theories of long-term external directionality in evolution. Confusingly also, this kind of directionality is often associated with Lamarck and his concept of "le pouvoir de la vie" [83]. This phrase was mistakenly interpreted to become identified with 'vital force', which is a serious misreading of Lamarck's writing [84,85]. Lamarck was a thorough-going materialist and was opposed to the vitalists of his time. As the French historian of genetics André Pichot writes:

> 'Lamarck's claim that . . . there is a radical difference between living beings and inanimate objects might lead people to think that he was a vitalist. But he is not. On the contrary, his biology is a mechanistic reply to the physiological vitalism of Bichat, which was then the dominant theory' [86]. (Our translation of Pichot's French).

Lamarck's "pouvoir de la vie" would therefore be better interpreted as an innate tendency in organisms to evolve in a directed way [87]. This is precisely what the mechanisms described in this article achieve. The directionality is innate in organisms and populations as active agents.

We have also shown that some forms, at least, of behavioural evolution can be interpreted within the same scheme of harnessed stochasticity combined with preservation of existing functional forms.

In an important article analyzing many aspects of this issue in the context of a review of Dawkins' *The Extended Phenotype*, Eva Jablonka covers some of the points we make here, including this quotation:

> Being scared of Lamarckism leads to the neglect of the evolutionary effects of evolved systems that allow the inheritance of targeted and acquired variations. When the fact that variation is highly constrained and is shaped (or, rather, drafted) by the rules of the generating system is ignored, evolution cannot be properly understood [88].

To which we can add that this neglect is inhibiting the development of a fully integrated physiological (i.e., functional) interpretation of evolutionary biology [6]. Some of the problems with neo-Darwinist interpretations arise from conceptual limitations, including privileging causation from the molecular genetic level, for which there is no empirical justification [89]. One of the aims of our

article is to encourage empirical investigations of the ideas we put forth. Theories of goal-directedness can be tested, and engineers and physiologists have the tools to do so.

7.3. Organisms and Their Populations Are the One-Eyed Watchmakers

We return now to where this article began: *The Blind Watchmaker* and the monkey at the keyboard. The Watchmaker analogy is a powerful one, both for those who follow Paley's original use of it to argue for creationism or intelligent design, and for Dawkins' use of it to argue for nothing but blind chance. But consider this: the only watchmakers we know are organisms (humans). They evolved from other organisms. The ability to be a watchmaker therefore evolved. There is therefore nothing surprising in the fact that goal-directed agency occurs also in other organisms and is capable of influencing evolution.

Our overall conclusion is that there are several processes by which directed evolutionary change occurs—targeted mutation, gene transposition, epigenetics, cultural change, niche construction and adaptation. Evolution is an ongoing set of iterative interactions between organisms and the environment. Evolution is a continuous organic process. Directionality is introduced by the agency of organisms themselves as the one-eyed watchmakers. Evolution itself also evolves [90].

Acknowledgments: We would like to acknowledge Sir Anthony Kenny's suggestion that led to the title of this article. The kind of direction that organisms and populations exert over their own evolution is best represented as 'feeling a way forward'. The 'sight' involved is always partial. The metaphor 'one-eyed', which Kenny suggested to us, nicely captures the essence of this idea. We are grateful to Sir Patrick Bateson, Eva Jablonka, Perry Marshall, David Miller, Richard Moxon, Anant Parekh, Dan Rubenstein, James Shapiro and Michael Yudkin for kindly criticizing drafts of the paper. We acknowledge in particular the books we have consulted by James Shapiro, Eva Jablonka and Patrick Bateson, as well as the papers of Richard Moxon, all of which are to be found in the reference list. We acknowledge also valuable criticisms and comments from three anonymous journal referees. While this article was in revision we learnt the sad news that Patrick Bateson had passed away. We dedicate this article to a great evolutionary biologist who championed the agency of organisms and from whom we learnt a lot.

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Central Dogma or Central Debate?

Introduction

The Central Dogma of molecular biology has been widely misinterpreted to be a modern version of the Weismann Barrier. This confuses cellular-level inheritance with DNA inheritance and is therefore incorrect. The consequences for biology generally and for physiology in particular are profound. Removing the confusion completely alters our understanding of the relationship between physiology and evolutionary biology. The Weismann Barrier is permeable, and organisms are capable of transmitting non-DNA inheritance.

Lay Summary of Physiological Importance

A central feature of evolutionary biology as it developed during the last century was that acquired characteristics could not be inherited. Physiologists now know that there are many paternal and maternal effects transmitted to subsequent generations. The implications for health are important. The way in which parents live inevitably influences their children even from birth. It is not "all in the genes."

"Weismann's barrier, as now embodied by the Central Dogma of molecular biology (DNA makes RNA and RNA makes protein, and not the reverse) has not yet been falsified. No one has yet shown that protein sequences can be translated into nucleic acid sequences."
Anonymous referee, 2017[1]

What is the Debate About?

About 125 years ago, the hugely influential German biologist August Weismann gave an inaugural lecture, "On Inheritance,"[2] in which he set out his agenda to remove the possibility of the inheritance of acquired characteristics from Darwin's theory of evolution.[3] He wrote:

"In my opinion this [the hereditary substance] can only be the substance of the germ cells; and this substance transfers its hereditary tendencies from generation to generation, at first unchanged, and always uninfluenced in any corresponding manner, by that which happens during the life of the individual which bears it. If these views . . . be correct, all our ideas upon the transformation of species by means of exercise (use and disuse), as proposed by Lamarck, and accepted in some cases by Darwin, entirely collapses."

Thus Weismann knew that Darwin had accepted Lamarck's idea. In fact, that kind of inheritance was widely assumed well before Lamarck, who did not invent the theory that usually carries his name. Darwin did not just passively accept the idea. He also developed a theory on how it could occur. He supposed that tiny particles, which he called gemmules, could pass from the soma to the germline and so modify what was passed on to subsequent generations (5). Physiologists today know that Darwin was right. We characterize this kind of transmission as maternal and paternal effects (8). We have even identified some of Darwin's imagined particles; they are the innumerable RNAs in sperm in the male line (23), and the many cytoplasmic materials in the inherited egg cell in the maternal line, including the eukaryotic cell structure and metabolism (25).

Weismann did not know any of this, of course. But he did have a brilliant simplifying thought. This was that he could explain *all* the examples of the phenomenon given by Darwin in *The Origin of Species* even if one supposed that there were no such particles and/or that there was a completely tight barrier between the soma and the germline. His idea was that random variations in the inherited material would be sufficient, together with Darwin's theory of Natural Selection, to explain evolution entirely. As the evolutionary biologist and historian Ernst Mayr showed very clearly in his magisterial book, *The Growth of Biological Thought* (12), this idea, together with Mendelian genetics, formed the cornerstone of what became called The Modern Synthesis, often also called neo-Darwinism.

The origin of what I refer to here as the "Central Debate" of modern biology therefore came about through a disagreement between Darwin and Weismann. This is the reason why I often say that Darwin was not a neo-Darwinist (Ref. 15, p. 126). But the debate never became a reality between the two men since Darwin passed away in 1882, a year before Weismann gave his 1883 lecture. It is hard though to imagine that Darwin would have agreed with Weismann, since he included not only the inheritance of acquired characteristics but several other processes as also necessary, including sexual selection in which individual organisms give evolution a direction (4). The more general version of this idea, now called the adaptability driver (1), is also central to physiology since it restores functionality and purpose to our discipline (19).

Why is the Debate Important to Physiology?

The reason that the debate is important is that three major errors were made during the process by which the Weismann

[1] The idea that the Weismann Barrier is now "embodied by the central dogma" is widespread. It even appears on the Simple Wikipedia entry on the central dogma: "The dogma is a modern version of the Weismann barrier" (https://simple.wikipedia.org/wiki/Central_dogma_of_molecular_biology). The statement on the main Wikipedia page is more circumspect: "This [the Weismann Barrier] does not predict the central dogma, but does anticipate its gene-centric view of life, albeit in non-molecular terms." The grossly misleading statement on Simple Wikipedia is repeated on a website designed for schoolchildren (https://wiki.kidzsearch.com/wiki/Central_dogma_of_molecular_biology). I want to acknowledge, however, that more careful and scholarly textbooks on evolutionary biology do make the distinction clear.

[2] The lecture entitled *Über die Vererbung* was delivered in 1883 and published in 1889 in English translation by OUP in *Essays upon Heredity* (chapt. 2).

[3] The idea is present in ~12 places in Darwin's *Origin of Species* (1859).

Barrier became considered to be "embodied by the Central Dogma of molecular biology." These errors were in turn responsible for completely and unnecessarily sidelining physiology during the second half of the 20th century.

The first error was to think that the barrier had indeed become so embodied. As Steele writes in Ref. 24:

> *"Indeed the rigid dictum DNA→RNA→Protein is the earlier 1960s rendition which is often mistakenly confused with* Weismann's Doctrine. *It must be made clear that* Weismann's Barrier *enshrines a* cellular theory *of information flow whereas the Central Dogma is a theory of information flow at the* molecular level."

The difference is fundamental. The cell contains much more than its genome. In most of life on earth, it is the complete organism. Moreover, it can be shown that the information content of the rest of the cell matches that of the genome (16). So how did the idea that the barrier could be embodied in the dogma come about?

This development is explained by a shift in the definition of a gene. When Johannsen first introduced the word in 1909, it was defined as an inheritable *phenotype* characteristic (9). This was also essentially Mendel's concept. Their concept of a gene would therefore have included anything that went through the germline *cells*. Johannsen made this clear when he explained that the gene could be anything (*ein etwas*) in the organism that was responsible for inheritance of the characteristic. Had he known of them, RNAs, and epigenetic marks on DNAs and histones, would have been included, as would cellular structures that make the replication and inheritance possible. This is clearly not the modern molecular biological definition of a gene, which is restricted to a DNA sequence forming a template for a protein (10).

Why Does it Matter to Evolutionary Biology?

This major shift in definition was not known at the time the Modern Synthesis was formulated. DNA was not even known to be the genetic material, so it is not surprising that the shift in definition did not matter to those who formulated the Modern Synthesis. The great advances in, for example, the mathematical theories of population genetics (7) worked perfectly well with the gene being defined as a phenotype characteristic. In fact, for most applications of genetics to the social sciences, such as economics and sociology, retaining the phenotype definition is important and even necessary. As with the results of GWAS (genome-wide association studies) generally, the associations at the genome sequence level are remarkably weak and, with the exception of certain rare genetic diseases, may even be meaningless (13, 21). The reason is that if you gather a sufficiently large data set, it is a mathematical necessity that you will find correlations, even if the data set was generated randomly so that the correlations must be spurious. The bigger the data set, the more spurious correlations will be found (3). The current rush to gather sequence data from ever larger cohorts therefore runs the risk that it may simply prove a mathematical necessity rather than finding causal correlations. It cannot be emphasized enough that finding correlations does not prove causality. Investigating causation is the role of physiology.

Nor does finding higher overall correlations by summing correlations with larger numbers of genes showing individually tiny correlations solve the problem, even when the correlations are not spurious, since we have no way to find the drugs that can target so many gene products with the correct profile of action.

But, to return to the definition of a gene, the difference between phenotype and genotype definitions matters enormously to versions of neo-Darwinism, such as selfish gene theory, based on distinguishing the replicator (regarded as DNA) from the vehicle (the phenotype).

There are two fatal difficulties in the *selfish gene* version of neo-Darwinism. The first is that, from a physiological viewpoint, it doesn't lead to a testable prediction. The problem is that the central definition of selfish gene theory is not independent of the only experimental test of the theory, which is whether genes, defined as DNA sequences, are in fact selfish, i.e., whether their frequency in the gene pool increases (18). The second difficulty is that DNA can't be regarded as a replicator separate from the cell (11, 17). The cell, and specifically its living physiological functionality, is what makes DNA be replicated faithfully, as I will explain later.

This difficulty leads to the next error in the development toward the Central Dogma, since the fact that the cell, not its DNA, is the real replicator is fundamental. This is the core issue in the Central Debate. I will now explain how the development toward the Central Dogma got this part of the story wrong.

When the Central Dogma was first formulated, Watson and Crick acknowledged their indebtedness to Schrödinger's famous book *What is Life?* (6, 22). In that book, Schrödinger made two predictions, one of which was spectacularly successful, the other is necessarily incorrect (Ref. 15, p. 176–181).

The correct prediction was that the genetic material would be found to be what he called an aperiodic crystal. If one allows a polymer to be regarded as a kind of crystal, this is a good description of DNA.

The incorrect prediction was that the molecule would behave like a determinate crystal. This idea leads directly to strong interpretations of the Central Dogma that attribute faithful replication and determinate qualities to DNA alone. These strong interpretations permeate the language of modern biology, particularly in its popularizations ("we found the gene *for* X"), and even more so of sociology and economics, with immense implications for a possible rehabilitation of eugenics. Many examples can be found in the brilliant analysis by the sociologist Catherine Bliss (2).

Resolution of the Debate

Now, if the reader has borne with me this far, she may well be extremely puzzled. Surely DNA is a fantastically accurate replicator? Isn't the debate over—game, set, and match—even before it begins?

Well, yes. If you compare the DNA in a daughter cell with that of its parent cell, you will indeed find a copying error rate of less than one base pair in a complete genome. The error rate is around only 1 in 10^{10} base pairs, which is remarkably

accurate. But now suppose that we could compare the DNA sequence *immediately after being copied*. We would find an error rate of around 1 in 10^4, which in a genome of 3 billion base pairs would mean millions of errors. No eukaryotic cell could survive such an error rate. Schrodinger was therefore wrong about the molecule itself behaving like a determinate crystal. Crystal structure growth is indeed determinate, but that is the wrong metaphor for DNA. That is not how it grows and replicates. On its own, it would be left with millions of errors.

It replicates accurately only in a complete cell containing all the objective functionality that enable cells to be alive. Cells achieve this outcome through a very complex three-stage process in which the millions of errors are detected and corrected (https://en.wikipedia.org/wiki/DNA_replication). Those processes rely on an army of specialized proteins and on the lipid membranous structures for which there are no DNA sequences. Outside a living cell, DNA is inert, dead. The living functionality is crucial.

Causation and Stochasticity

So far in this brief editorial I have outlined the experimental evidence for the restoration of functionality and for an integrative physiological interpretation of the central ideas of modern biology and how to characterize life itself, its reproduction, and its evolution.

Now I want to finish by making a few necessary conceptual points.

1) There are different forms of causation (Ref. 15, p. 176–181). DNA is a passive cause. As Watson said to Crick when they first made their momentous discovery of the double helix: "Francis, it's a template" (https://www.dnalc.org/view/15474-RNA-s-role-in-the-cell-James-Watson.html). Active causation lies at the level of the cell, or of multicellular structures and organisms. This distinction in forms of causation is absolutely fundamental. It is why we don't regard viruses as really alive, and why DNA probably was not the origin of life (20).
2) Stochasticity, which is the origin of the massive copying error rate, is usually presented by evolutionary biologists as just the origin of variation, which indeed it is. The remaining errors after correction form one of the bases of evolutionary variation. But stochasticity is much more than that. It is also the origin of functionality and creativity in organisms. They achieve that by *harnessing* stochasticity (17). The harnessing process is a complete physiological control process that achieves creativity through finding new solutions to new environmental challenges, much as the immune system does.
3) As Karl Popper argued in a very significant, but little known, debate with Max Perutz, there is a fundamental difference between biology and chemistry (14). Biology necessarily requires the concept of function, of goals toward which a process tends to go. To slightly misquote JBS Haldane,[4] teleology might indeed be a great mistress, but she can be an even greater openly acknowledged wife.

Conclusions

Physiology was mistakenly sidelined from mainstream biology, including evolutionary biology, during the second half of the 20th century. The consequences are serious.

1) There is no way in which the Weismann Barrier can be regarded as "embodied by the Central Dogma of molecular biology," as widely thought. That completely confuses cellular and molecular level processes.
2) The results of GWAS do not reveal the secrets of life, nor have they delivered the many cures for complex diseases that society badly needs. The reason is that association studies do not reveal biological mechanisms. Physiology does. Worse still, "the more data, the more arbitrary, meaningless and useless (for future action) correlations will be found in them" is a necessary mathematical statement (3).
3) Nor does applying a highly restricted DNA sequence-based interpretation of evolutionary biology, and its latest manifestation in GWAS, to the social sciences augur well for society.
4) Stochasticity is not only the source of genetic variation, it is also the clay from which organisms actively seek solutions to environmental challenges. Causation operates from the cellular and higher levels. This is what enables cells and organisms to be alive and maintain their integrity.

For all these reasons, let the Central Debate replace the debate on the Central Dogma. The uni-directionality of sequence information transfer from DNA to proteins no more determines life than the QWERTY keyboard determines what I wrote in this article. ■

[4] The original statement attributed to Haldane is, "Teleology is like a mistress to a biologist: he cannot live without her but he's unwilling to be seen with her in public" (Mayr E. *Boston Studies in the Philosophy of Science*. Dordrecht, Holland: D. Reidel Publishing, 1974, vol. XIV, p. 91–117).

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Lost in translation

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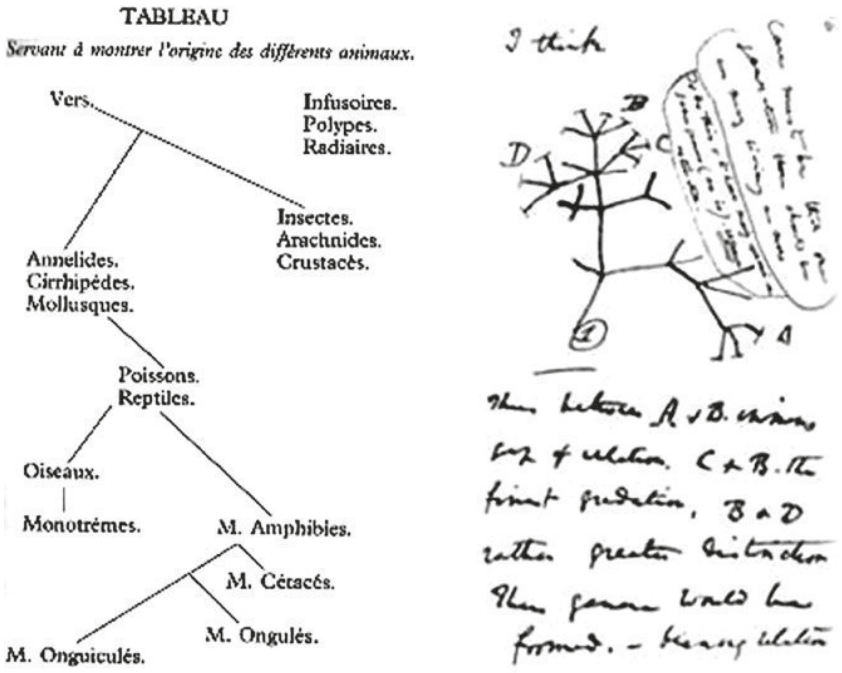
Eight years ago[1] Denis Noble dreamt that he was the Editor of PN and had received a letter from Lamarck, which he imagined he had translated into English for readers of PN. Much has happened since then so it is not surprising that Jean-Baptiste Lamarck has appeared again in Denis' dreams over the New Year 2018.

Jardin des Plantes, Paris, le 1 janvier 2018.

Monsieur le Rédacteur,
Puis-je profiter de votre Société la plus distinguée encore une fois...



Left: Lamarck's tree of life from the addition to *Philosophie Zoologique*, 1809. Right: Darwin's first sketch of a tree of life from his first notebook on transmutation of species (1837). Lamarck draws his tree from the root downwards. Darwin does so from the root upwards. But they clearly had the same idea.

May I use your most highly distinguished Society, and its eminent *Physiology News* magazine, once again to present my reflections on what has transpired since I last wrote a letter to you?

First I welcome the special issue of your *Journal of Physiology* devoted to *the integration of evolutionary biology with physiological science*.[2] I did not call myself a physiologist. After all, I had only just coined the word biology. But if your Society had existed in 1809 I would have loved to be a member, as was your great Charles Darwin at your foundation in 1876. We didn't call evolution 'evolution' in my day. 'Evolution' was the development of the embryo, itself a physiological (functional) process. Evolution in your modern sense was called transformationism. Charles Darwin praised me for championing the transformation of species against creationists like Cuvier.[3]

You rightly used the subtitle *physiology returns to centre stage* in the special issue. You physiologists understand the nature of function in biology. It is what distinguishes biology from physics. That is why I regarded biology as a separate discipline and introduced its name, *biologie* in my language, to emphasise that point. I also said that living organisms have innate tendencies, what I called 'le pouvoir de la vie'. Sadly, that phrase earned me the misunderstanding that I was a kind of vitalist. That is far from true. I vigorously opposed the vitalism of Bichat and other vitalist physiologists of my day.[4]

I note with pleasant surprise that your Society's bold initiative was followed two years later by your national academy, The Royal Society, which, in collaboration with The British Academy, organised a meeting on *New Trends in Evolutionary Biology*, now also published in a special journal issue.[5] Pleasant for me because after the withering attack on me at my funeral by Georges Cuvier[6] I imagined that my reputation could never recover.

Those two magnificent volumes of British journals have done much to reassure me that not all is lost. So, why am I writing this letter to you? There are still two other matters on which I would like to correct the historical record. These are the inheritance of acquired characteristics and the famous 'tree of life'.

When I am referred to at all in your evolutionary biology textbooks, I am usually a figure of ridicule. It is not widely understood that Charles Darwin agreed with the inheritance of acquired characteristics and

[1] Notes by Denis Noble (2010). *Physiology News* **78**, 31.

[2] The integration of evolutionary biology with physiological science(2014). *Journal of Physiology* **592**, 2237–2438.

[3] Preface to the fourth edition of *The Origin of Species* (1866).

[4] This is very clearly explained in André Pichot's introduction to a reprint of *Philosophie Zoologique*. Flammarion, Paris, 1994. 'Philosophy' was synonymous with 'science' in Lamarck's time. Hence the journal title 'Philosophical Transactions of the Royal Society'.

[5] New trends in evolutionary biology: biological, philosophical and social science perspectives (2017). *Interface Focus* **7**(5).

[6] http://www.victorianweb.org/science/science_texts/cuvier/cuvier_on_lamarck.htm

[7] This theory is formulated in Darwin's later book: *The Variation of Animals and Plants under Domestication* (1868).

[8] Spadafora (2017), Sperm-mediated transgenerational inheritance. *Frontiers in Microbiology* **8**, 2401. DOI: 10.3389/fmicb.2017.02401. eCollection 2017. Spadafora concludes: 'On the whole, this phenomenon is compatible with a Lamarckian-type view and closely resembles Darwinian pangenesis.'

[9] It is true that the bulk of *Philosophie Zoologique* fits the view he is expressing, but he made a late addition at the very end of the book that already contains the essence of the tree viewpoint. In the Flammarion reprint of *Philosophie Zoologique* there is a diagram on page 649 that must rank, as Gould says, as the first construction of an evolutionary tree. Lamarck even writes 'In its production of the different animals, nature has not fashioned a single and simple series.' (Gould's translation). See Fig. 1, left. Darwin's first tree sketch is shown on the right. I doubt whether Darwin knew of Lamarck's conversion to branching trees.

[10] 'In its production of the different animals, nature has not fashioned a single and simple series' (Gould's translation)

[11] Lamarck JB (1820). *Système analytique des connaissances positives de l'homme*. pp. 134–148.

he even formulated a theory for how it could work. He called the objects of transmission from the soma to the germline 'gemmules'.[7] I had a similar idea. I called them 'subtle fluids'. The modern discovery of transmission of RNAs through the germline could surely achieve what he and I postulated.[8]

This is beginning to be understood. But there is something else highly important on which Darwin and I agreed. This is his famous 'tree of life': the branching network of development of new species from previous ones. By contrast, I am represented as believing that evolution was a single 'ladder of life', from simple to more complex, following my idea of le pouvoir de la vie.

It is true that this was my view when I wrote *Philosophie Zoologique* in 1809.[9] But as I further studied worms (remember that I was Professor of worms and insects at the Jardin des Plantes) I came to the clear conclusion that a single ladder could not be true. To use my terminology, the 'internal' worms (such as tapeworms) and 'external' worms (such as earthworms) could not possibly be fitted into a single ladder of life. In my 1815 *Histoire naturelle des animaux sans vertèbres,*[10] and again in my last book, *Système analytique des connaissances positives de l'homme,* published in 1820, I corrected this mistake.[11] Your great, and sadly lamented, evolutionary biologist Stephen J Gould clearly outlined the history of my ideas. After doing so, he concluded:

'how can we view his [Lamarck's] slow acknowledgement of logical error, and his willingness to construct an entirely new and contrary explanation, as anything other than a heroic act, worthy of our greatest admiration and identifying Lamarck as one of the finest intellects in the history of biology?'[12]

Veuillez accepter, cher Monsieur le rédacteur, l'expression de mes sentiments les plus distingués,

Jean-Baptiste Pierre Antoine de Monet, Chevalier de la Marck

Lamarck repeatedly uses the word 'branch': 'The polyps ... seem to divide into three branches'; '... the crustaceans come from another branch separate from the arachnids'; '... the reptiles ... another branch seems to lead to the lizards, towards the mammals' (my translations).

[12] Gould, SJ (2000). A tree grows in Paris: Lamarck's division of worms and revision of nature. In *The lying stones of Marrakech.* Harmony Books, Chapter 6. I am very grateful to Jonathan Bard, (author of *The Principles of Evolution, Systems, Species and the History of Life,* Garland Science, 2016) for drawing my attention to Gould's scholarly and insightful essay. In a review of Bard's valuable book I wrote 'The book I needed as a student and young researcher.' I still think that.

International relations

David Eisner

President, The Physiological Society

I am sure that all readers would agree that science is an international activity and should not be constrained by national borders. This point has been thrown into strong relief by worries over the consequences of Brexit for European funding as well as restrictions on overseas scientists coming to work in the UK. Members of The Society's Policy and Communications Committee and staff have joined with the rest of the scientific community to try to influence decisions in this area.

The Physiological Society has also been thinking about its own international relations. The Society is, of course, truly international. Our journals have international Editorial Boards and readerships. Indeed, of the three journals, only *Experimental Physiology* currently has an Editor-in-Chief residing in the UK or Ireland. Meetings have always been international; typically, more than a third of those attending our annual main meetings come from overseas. As regards our membership, 30% live overseas. Readers will not be surprised to learn that the country with the greatest number of members, after the UK, is the USA. What may be less expected is that Nigeria is the third most represented country with 51 members, more than Ireland (47).

The Society has long participated in joint meetings with overseas societies. For many years, up to about 2007, there were typically one or two such joint meetings every year. The number of such meetings has declined in recent years, perhaps an unintended consequence of the move to having one main meeting a year. For many years, members of The Society have participated in the meetings of the International Union of Physiological Sciences (IUPS), which meets every four years. The Society is also a member of the Federation of European Physiological Societies (FEPS). This September in London, will be the first in a series of Europhysiology meetings organised together with the German and Scandinavian Physiological Societies, and FEPS. The next meetings in this series will be held in Berlin (2020) and Copenhagen (2022). The Europhysiology meetings in Berlin and Copenhagen will constitute the annual meetings of The Society in those years.

In 1935, The Society established the post of Foreign Secretary, occupied until 1945 by AV Hill, who at the time was concerned about the plight of scientists at the hands of the Nazis (see *Physiology News* 106). Hill was succeeded as Foreign Secretary by ED Adrian, GL Brown and AL Hodgkin. In 2001, the post was renamed as the more politically correct 'International Secretary'. I was actually the last International Secretary as the post was abolished in 2007 with the idea that all of the activities of The Society had an international dimension and there was therefore no need to have a single role.

Concern has been raised recently at Council that current arrangements do not make it easy for us to coordinate our international activities. Simple questions include: how do we decide which countries and societies to interact with? How do we coordinate the international activities covered by journals, meetings, policy, education and membership?

A major aim of The Society's new strategy is to enthuse the public, in particular 16- to 25-year-olds, with physiology. Much of what we do occurs in the UK and Ireland. I have, however, been very impressed by activities carried out by our members in areas as far flung as Pakistan, South Africa and Canada. Should The Society be helping its members more with their engagement internationally? If so, are there countries we should prioritise in order to make best use of finite funds?

The Society is often approached for financial help with meetings organised by other societies, particularly those in developing countries. What criteria should be used to decide which of these to prioritise? To what extent should The Society make use of IUPS and FEPS for international activities as opposed to setting up its own bilateral links?

To address these, and other, questions, we have set up the International Working Group chaired by Stefan Trapp with the remit of making recommendations as to the way forward. I would like to encourage readers to send their thoughts to either Stefan (**s.trapp@ucl.ac.uk**) or myself (**eisner@manchester.ac.uk**).

Harnessing stochasticity: How do organisms make choices?

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Choice in the behavior of organisms involves novelty, which may be unpredictable. Yet in retrospect, we can usually provide a rationale for the choice. A deterministic view of life cannot explain this. The solution to this paradox is that organisms can harness stochasticity through which they can generate many possible solutions to environmental challenges. They must then employ a comparator to find the solution that fits the challenge. What therefore is unpredictable in prospect can become comprehensible in retrospect. Harnessing stochastic and/or chaotic processes is essential to the ability of organisms to have agency and to make choices. https://doi.org/10.1063/1.5039668

Faced with unusual challenges in their environments, organisms have to make new choices to survive. The question addressed in this paper is how such choices can be creative and non-deterministic. We argue by analogy with the immune system, which faces a similar difficulty when a new antigen invades the organism, and for which it does not have the relevant DNA sequence to make an antibody with the correct shape. The immune system responds by rapidly mutating the variable part of the immunoglobulin sequence until, by chance, a cell evolves which does have the DNA sequence for an immunoglobulin with the correct shape. Stochasticity is therefore used to generate novelty. We speculate that by harnessing stochasticity in their nervous and other systems, organisms can similarly generate novel behavioral responses to meet the unusual challenge.

I. INTRODUCTION

How do organisms make choices? One very simple answer to this question would be that they do not. Following Descartes (1665),[1] the assumption would be that organisms are determinate machines. Despite their fiendish complexity, if we knew enough about the mechanisms involved, we would be able to predict their behavior to any arbitrary degree of accuracy.

Descartes actually excluded humans from this viewpoint, but that requires an assumption either that a *non-material entity* somehow intervenes in the case of humans, or that some *non-determinate (stochastic) material* process operates. Descartes chose the first option, which creates the difficulty that we have no way of representing how a material body could be so influenced. For example, would such an influence necessarily appear to be stochastic to scientific investigation, precisely because it would not be caused by any measurable physical events, and would have to appear to be stochastic in order to be indeterminate? Without making metaphysical assumptions beyond the possibility of scientific investigation, what we would find in this case simply collapses to the second possibility, at least insofaras we can investigate it objectively.[2]

In this article, we will conclude that stochastic material processes are involved. Moreover, there is no reason to suppose that such processes do not operate in organisms other than humans. Since humans evolved from other organisms, we should expect both of these conclusions.

Moreover, at the micro-level, we now know that the material universe is fundamentally stochastic, whether it be by virtue of random kinetic energy producing the form of stochasticity observed in the Brownian motion of molecules or by virtue of quantum mechanical behavior at the level of particles. Organisms must be affected by such stochasticity. Neither animals nor humans can be fully determinate. But that leaves open the question how stochasticity is involved or used in living processes.

In recent articles, we have addressed the following issues which can be seen to be introductory to the focus of the present article.

1. Can stochastic and/or chaotic processes be *used* in organisms, rather than organisms being arbitrarily subject to them, i.e., can such processes be harnessed so that they become part of the necessary functional repertoire of organisms? This issue was addressed in Noble[3(p.1)] and the answer is yes, organisms necessarily harness stochasticity.
2. Can we know whether organisms have agency, and does their behavior generate a form of directionality both in individual organisms, and at the level of populations so that the behavior can in turn influence the direction of evolution? This issue was addressed in Noble and Noble[4] and the answer again is yes, organisms do have agency. Harnessing stochasticity is an essential part of the means by which they do so. As we will show later in this article, a fully determinate process (meaning completely predictable) would not satisfy the conditions for agency.

Those articles leave open the question how the harnessing of stochasticity and the possession of agency may be represented in empirical (i.e., experimentally testable) terms. As a test of

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what we propose, we will also ask the question whether any such representation can show why we cannot predict what we call free choice, yet can often account for it in rational terms in retrospect.

II. DEFINITIONS

A. Agency

An agent acts, it does not just react in the way, for example, in which a billiard ball is caused by another ball to move. There are many levels of agency (Ref. 5, p. 32–40). Organisms are agents to the extent that they can interact socially with other organisms to choose particular forms of behavior in response to environmental challenges. Agency requires causal independence.[6] It also requires intentionality, i.e., the sense of purpose, in order to be causally effective as a driving force.[7]

B. Information

Inanimate objects can contain information. But it requires interpretation by an organism to become knowledge of what the information means. For example, rocks contain information, and that only becomes knowledge when organisms interpret it, e.g., to work out dates of events in the history of the earth. By this definition, DNA is also inanimate. It contains sequence information, but it does not contain knowledge. Until they are interpreted, DNA sequences are like uninterpreted hieroglyphics.

C. Interpreter

DNA information is interpreted by organisms. Outside a living cell, DNA is inert. A complete cell therefore is a minimal interpreter of DNA.

D. Knowledge

Knowledge about the world arises through organisms being creative in finding new solutions to environmental challenges. We can distinguish two types of knowledge:

E. Objective knowledge

This can be verified by those other than the organism that has the knowledge. In this sense, plants and bacteria have knowledge. Plants possess functional processes enabling them to use sunlight to create oxygen, and nutrients like sugars. We do not yet have that knowledge but wish we did! Note that this definition is not identical with Popper's use of "objective knowledge."[8]

F. Subjective knowledge

Organisms that "know that they know" have this kind of knowledge. They can communicate this kind of knowledge to others through behavior and language.

Note. Many philosophers do not attribute knowledge to organisms unless they are conscious, e.g., Anthony Kenny,[5] who refers to "capacity or ability" rather than "objective knowledge." We acknowledge the difference of usage of "knowledge" but do not think that the conclusions of our article depend very much on which view one takes. Here, we simply note that resolving this question would depend on one's view of animal consciousness; see, e.g., Ref. 9. In this article, we are not primarily concerned with this kind of knowledge, and we do not address questions of self-awareness and consciousness.

G. Rational choice

In this article, we refer to accounting for choice behavior in retrospect as being rational. What is meant is that it is possible to answer the question why an organism did what it did using the common sense meaning of rational, e.g., in terms of the organism's presumed goals. This does not mean that the organism's choice would be predicted by any particular version of Rational Choice Theory (https://en.wikipedia.org/wiki/Rational_choice_theory). Nor does it mean that the "rationality" does not contain an element of delusion. We will return to this question in the Discussion.

H. Stochasticity

Interpreted as the inability to predict, stochasticity is a level-dependent property. Thus, molecular level stochasticity is compatible with higher level predictability, as is obvious from the predictability of thermodynamics. Stochasticity is therefore a relativistic concept. Whether underlying stochasticity can influence the overall behavior of a system must depend on whether the higher level is organized to enable it to do so. Organisms are high-level systems. In this article, we show that molecular stochasticity does not only cancel itself out at higher levels, as in the case of thermodynamics, it also becomes used in goal-directed feedback control processes. Higher-level organization can make that possible.

I. Chaos

As many readers, particularly of this journal, will be aware, stochasticity and chaos are not identical. Chaotic sequences can be produced by determinate algorithms as first shown by Lorenz.[10,11] The difference is important because the variations in determinate chaos are constrained by an attractor, whereas genuine stochasticity is not. The difference can be made clear in phase plots. However, we doubt whether the difference between determinate chaos and stochasticity is relevant to the process we ascribe to choice behavior. If the attractor constraining a chaotic sequence is not itself an integral part of the organism's control networks, the variations will appear random to the choice process.

III. MULTI-LEVEL CAUSATION

An important basis for our paper is that organisms are open systems in which causation operates between multiple levels. That they are open systems is obvious: they exchange matter and energy with their environment and engage in social interactions with other organisms. Multi-level causation is not, however, universally accepted in biology. We follow the argument that causation *must* be multi-level. The demonstration that this is the case is mathematical. Even if we try to imagine that only molecular level mechanisms are causative,

we are faced with a fundamental difficulty when we try to solve the differential equations for those mechanisms. There is no solution unless we introduce boundary conditions that represent the causative action of higher levels and scales. This is the mathematical basis of the principle of biological relativity.[12,13] The principle states that there is no privileged level of causation. But it is important to note that the upward and downward forms of causation are not necessarily of the same form. Causation by setting the boundary conditions for lower level processes is more like a constraint on the forms of organisation that the lower level elements may take.[14] These causal interactions can occur between any of the levels of organization and are the reason why downward is causally effective. Indeed, in purposive behavior, it is primary since it will only be at the higher levels that the purposive organization may be evident.[4] This is the general causal basis for the choice process that we will now present.

IV. THE CHOICE PROCESS

For an empirically testable theory of choice to be possible, we need to know at which stages in the process experimental interventions could test its validity. At first sight, that may seem impossible. How can we specify a process that is necessarily *unpredictable* but which can be given an at least apparently *rational* justification once it has happened? Our previous work provides a clue to that problem. In Ref. 4, we analyzed agency by comparing it to the purposive behavior of the immune system. The immune system solves what we can best characterize as a template puzzle: given a new invader with an unknown chemical profile (shape of template), what is the best way to find the key (an anti-template, i.e., the antibody) to lock onto and neutralize the invader? The answer in the case of the immune system is one of the most remarkable forms of the harnessing of stochasticity. In response to the new environmental challenge, a feedback loop activates a massive increase in mutation rate in a highly targeted region of the immunoglobulin DNA sequence.[15]

The process of choice in organisms can be viewed as analogous to the immune system. The process can be represented as follows:

1. Influences from the environment (boundary conditions) and the organism's history (initial conditions) lead to defining the problem facing the organism. This will be the state of the organism in which the environmental challenge has occurred but not yet a solution in the organism's reactions. We conjecture that such a problem can be viewed as a puzzle analogous to the form of a template for which a match is needed. The configuration of these conditions might be a routine one, in which case what we normally characterize as a reflex response may be adequate. But it is precisely such responses that we would *not* characterize as involving a choice. We say that a choice occurs when there is no automatic reflex response possible. The challenge facing the organism then is what could fit the puzzle template?
2. Instead of an automatic response therefore, the organism must search amongst existing stored possible fits to the problem template. By analogy with the immune system, this is equivalent to finding that the DNA sequence for the correct immunoglobulin shape already exists. It is precisely when no such solution exists that hypermutation is triggered. We hypothesise that a comparable process occurs generally in choice situations in organisms.
3. In which case the organism can spin (i.e., activate) stochastic processes within itself to generate further possible new solutions. This is where novelty arises. These processes can be of any biological kind. For cognitive problems in organisms with highly developed nervous systems, these will be primarily neural. Note also two important characteristics of this stage of the process. First, the organism *triggers* the resort to stochasticity but no longer *controls* it, just as the immune system does not directly control which mutations occur. Second, the options at this stage are effectively infinite. In the case of the immune system, the number of possible sequences for the variable part of the immunoglobulin must be larger than the total number of particles in the universe. That is also true for the number of interactions between the 20 000 or so genes in a human.[16] Stochasticity and/or chaos in the nervous system must make even more options available.[17]
   Neural processes are extensively stochastic—at all functional levels, from the opening and closing of ion channels via action potential generation, spontaneously or through synaptic transmission in neuronal networks, up to cognitive functions including decision making (8 chapter 22).[18–22] As pointed out in Braun,[23] the reason may be found in the functional organization of living systems composed of a manifold of nonlinear feedback loops that often are adjusted to operate in the neighbourhood of bifurcations where it can essentially depend on random effects of what will happen next, e.g., whether an ion channel is opened or remain closed or whether an action potential is generated or not—what even may decide the choice between leaving the bar and going home or having another drink.
4. The organism returns to direct control at the next stage, which is to compare what is thrown up by the stochastic process with the problem template to determine what fits. "Template" and "fit" here are used metaphorically, in much the same sense in which a logical answer can be said to "fit" (i.e., answer to) the problem posed by a question. This is the essential choice process, needing a comparator. The comparator therefore forms part of what we call the interpreter (see definitions). This is the stage at which we can say that the organism knows that it has found a possible solution.
5. The final stage is to implement the discovered action to solve the problem.
   This is an idealized process, but it clearly helps one to explain an apparent paradox regarding the predictability or otherwise of what we call a free choice. Step 4 ensures that, in retrospect (and only in retrospect), the choice may be what in the case of humans we call rational. There may be a complete logic to why it was made. The logic lies in the fit between the problem template and the solution template. But step 3 ensures that the choice was

unpredictable since we cannot predict what stochasticity will throw up. So, free choice is both rational and novel. (See also Parallels in the work of Karl Popper below.)
This hypothetical process is open to empirical tests at all stages since it makes significant assumptions about what is actually happening within the organism. The kind of knowledge the organism has is what Popper characterised as objective knowledge (as distinct from subjective knowledge) and is fully open to observational test.

For example, the existence of stage 3 naturally explains why problems leading to the necessity for making a choice may lead to what we can call the puzzled state. Before stage 3, there is no solution in sight. Only after stage 3 might there be a solution that can lead to rational action. There will therefore be a period during which the organism does not know the solution. In the case of humans, we can communicate such states in language ("I havn't a clue"). Other organisms can communicate by behavior: frustration, depression, displacement activity, etc.

V. ACKNOWLEDGMENT OF PREVIOUS WORK

We are far from being the first to favor active agency as an explanation of the behavior of organisms and to favor the role of choice in the direction of evolutionary change. The arguments about the active role of organisms have their origins in a long tradition in which deterministic and non-deterministic views of life have been pitted against each other. As noted in our Introduction, the two threads were present in the same philosopher in the case of Descartes who in the seventeenth century struggled to reconcile his determinist interpretation of animal behavior with his conviction that this could not be true of humans. How else could he have written his great works? It would have taken a monkey billions of years to manage by chance to type just a single sentence of Descartes' work (Ref. 4, p. 1). (The relevant combinatorial mathematics is given in Ref. 16; see https://en.wikipedia.org/wiki/Weasel_program.)

The existence of creativity shouts out loud and clear that the universe cannot be simply deterministic, and since the early 20th century revolutions in physics, we have the proof that it is not. Yet, this revolution had surprisingly little effect on biology, which continued with deterministic interpretations of life and its evolution throughout the century. It was thought that indeterminacy at microphysical scales could hardly be relevant to processes at physiological scales. The proof that it is relevant came with the discovery of the hypermutation mechanism in the immune system. As we have shown in previous articles, the harnessing of stochasticity at a molecular level is precisely what enables organisms to be creative. The immune system serves as a model, which can be generalized (Ref. 4, p. 4–5). Given the nature of the universe, uncertainty is inevitable. Choice necessarily involves dealing with uncertainty. Low-level stochasticity is the clay from which high-level novelty can develop.

We wish to credit two more recent predecessors for major influences on our ideas: Patrick Bateson and Karl Popper. Patrick Bateson's work on the active role of behavior in evolution[24–28] was pursued throughout his career and has been summarized in a book published just before his death in 2017.[27] He was a careful historian as well as a great biological scientist. He documented the development of the ideas of active agency through from Darwin, through Spalding and Baldwin to his phrase the "adaptability driver" to describe the active nature of organism agency.[26] His phrase captures the directionality of agency in organisms.

VI. PARALLELS IN THE WORK OF KARL POPPER

Amongst fore-runners of the ideas explored in this article is the outstanding work of Karl Popper. In 1986, Popper gave a lecture to The Royal Society in London in which he laid out his "New Interpretation of Darwinism."[29] In that lecture, he distinguished between "passive Darwinism" and what he called "active Darwinism." His "passive Darwinism" is more or less identical with classical neo-Darwinism: the theory that random genetic variation and natural selection are entirely sufficient (*allmacht* in Weismann's words)[30] to explain evolution. Popper wrote: "I shall attempt to turn the tables completely on passive Darwinism … I shall claim that the *only* creative element in evolution is the activity of living organisms."[29(p.119)] "Active Darwinism" is therefore equivalent to the theory that organisms have agency and make choices, which is the main theme of our paper. Those choices include choosing niches (niche selection theory) and which other organisms they interact with (including sexual selection), and more recently, the discovery of aversion to cheating behavior in populations of dogs[31] and monkeys.[32]

Popper regarded the "metaphor of 'natural selection'" as "a theory of error elimination"[29(p.120)] rather than being creative of novelty itself. He saw it as a filter eliminating errors. To understand this point, we should remember that Darwin contrasted natural selection with artificial selection, which is clearly choices made by organisms (the selective breeders). When Darwin realised that sexual selection is more like artificial selection, he therefore faced a problem. Sexual selection is clearly an *activity* of organisms determining their evolution. The problem is that this blurs the distinction he was drawing. Sexual selection is therefore a form of active Darwinism to use Popper's terminology. Specifically, he wrote "sexual selection is a refutation of natural selection."[29(p.128)]

Popper saw that complete determinism was incompatible with viewing organisms as agents making choices. He would therefore have seen the importance of the role of stochasticity in our paper. In *The Open Universe*, Popper demonstrated that indeterminism is a necessary but not sufficient condition for emergence and openness.[29(p.70)]

In the same exposition of Popper's ideas leading up to his Royal Society lecture, Niemann[29] presents some other points that correspond well to the ideas of our paper. Summing up Popper, he repeated that "all life is problem solving. Acquiring new knowledge is always purposeful activity."[29(p.90)] He insisted that "in all cases the activity comes from outside of the DNA. The former 'centre of life' is rather a dead place."[29(p.96)] That it is the cell that divides, not only the DNA.[29(p.98)] And that it is "The cell … also managing the genome."[29(p.101)] This insight resembles that of Barbara

McClintock, the discoverer of natural genetic engineering[33] in saying that "the genome is an organ of the cell."[34]

Finally, there is his point that "influences (on action) [are] traceable in hindsight… we are unpredictable but not irrational" (Ref. 29, p. 110). Popper therefore arrived at many of the points we are making here.

It would therefore be surprising if he had not also seen the obvious implication, which is that organisms harness stochasticity; otherwise, choice behavior would not be possible. We are grateful to Hans-Joachim Niemann for directing us to Popper sources preceding his Royal Society lecture where he does clearly draw the correct conclusion. Some of the relevant texts occur in his dialogue with John Eccles *The Self and Its Brain*.[35] Popper writes "New ideas [*in statu nascendi*] have a striking similarity to genetic mutations" and continues "describing 'the process with respect to new ideas and to free will decisions' (Ref. 35, p. 540). As randomly produced proposals followed by selection based on standards coming from the world" (cf. Ref. 36, Secs. 31–33). Popper arriving at this conclusion is all the more remarkable for the fact that it required him to abandon his earlier (1973) conclusion that "indeterminism is not enough."[37,38]

The main difference is that while he envisaged "the cell… also managing the genome," (Ref. 29, p. 101). He does not seem to have arrived at the details of the comparison with hypermutation in the immune system. Perhaps, this is attributable to the fact that the discovery of some of the detailed molecular mechanisms of somatic hypermutation occurred in 1999 after his death in 1994.[39,40] There may also have been a puzzle regarding the molecular mechanism of hypermutation. Increasing the natural mutation rate by a factor of up to 10^6 must have seemed implausible. But this is also roughly the order of magnitude difference between the natural mutation rate in DNA copying before and after repair by cellular editing mechanisms. Mismatch DNA repair is indeed suppressed during somatic hypermutation.[41]

VII. DISCUSSION

Our main conclusion is that it is possible to construct an account of choice behavior using stochastic processes by analogy with the way in which the immune system harnesses stochasticity to discover novel solutions to new challenges. There are several predictions and implications.

A. Psychological experiments on primates

One of the implications is that it could be important in investigations of choice behavior in animals to include tests for signs of delay or other behavioral signs attributable to stage 3 in our choice process. These could include hesitation (time taken to decide), displacement activity, or other signs of puzzlement. Just as an example, we could take from many good and interesting studies of animal choice; a study of risk-taking behavior in primates[42] was successful in showing varying degrees of risk-taking in the different primate species but did not include any parameter that would answer this question. Most studies on choice in animal behavior seem to be assuming that animals behave as though they solve a calculation of probability. Thus, in the cited paper, we find: "Any agent, in order to successfully navigate a world of possibilities, needs to strike the right balance between these factors, utilizing mechanisms that when confronted with risky choices, lead to decisions, which optimally combine the probability of receiving a reward multiplied by the amount of the reward." Animals may not actually be "calculating" in quite the way this quote implies. If we are correct, no calculation or its equivalent, using, e.g., forms of Rational Choice Theory, could represent all of what is happening. That is particularly true when extrapolation to human behavior is involved. To quote the same source: "Based on our findings, we propose that decision-making in the great apes provides a promising context for the interpretation of decision-making in humans, the fifth great ape species." We agree with this conclusion, but note that it will be particularly important to consider the role of stochasticity in both animals and humans.

Krupenye *et al*. have in any case shown that humans and animals display departures from Rational Choice Theory which they characterize as biases in choice behavior dependent on whether decisions involve losses or gains.[43] The involvement of stochastic processes does not of course exclude biases.

Rosati and Hare have shown that chimpanzees and bonobos can distinguish between risk and ambiguity in choices presented to them.[44] They write "Importantly, apes' divergent preferences for risk and ambiguity diminished with time: although apes chose the risky option more frequently than the ambiguous option in the first session; by session two they showed no difference. One possibility is thus that the apes are able to rapidly incorporate new information about previously ambiguous options into their decision strategies: after choosing the ambiguity option and receiving some feedback about what it provided, they may have treated the ambiguity and risk option as equivalent because the functional outcome was the same." The stochastic choice process we describe here would account for this form of learning. By analogy with the immune system model, once a novel challenge has been met, it becomes part of the standard repertoire.

Santos and Rosati have written a valuable review of this field.[45] They write "we now know that human choice is often not as rational as one might expect." We see two ways in which this statement can be interpreted. First, within the context of our Choice Process, there is obviously no guarantee that a stochastic process will throw up a fully rational solution. Partial success is what would be expected most of the time. The same is true of the immune system. All it needs to do is to come up with a "good enough" template match. It does not have to be the perfect match. If a key fits the lock, it does not really matter whether it is an exact fit.

Second, that leaves the question how it happens that, nevertheless, most of the time, we and others can give a "good enough" rational explanation of a choice, at least in retrospect. That seems to be true however partial the "fit" seems to be to the problem. A possible solution to that problem could be what Santos and Rosati call the endowment effect. Animals and humans privilege retaining what they already own. Could the same effect operate in the case of decisions? Do we and perhaps other animals "own" decisions. It seems plausible at least.

B. Observations on primates in the wild

Observations of primates in their natural environments have extended our knowledge of choice behavior in ways that enable us to obtain important insights into *subjective* knowledge.

That organisms may know that others have subjective knowledge is itself an important factor in objective knowledge and is part of situational logic or behavioral cognition. Such knowledge necessarily carries with it a great deal of uncertainty. An animal must predict that the other knows and how they might act on such presumed knowledge. This is manifest in both human and non-human animal behavior.

Spinning the wheel as a creative process therefore occurs not solely at a physiological level, but also at a social and cultural level. The evolution of language allows sophisticated and abstract problem solving. Language allows a cultural spinning of the wheel. Thus, chimpanzees use communication that distinguishes private from public interaction. What they know that others may not know is a part of their objective knowledge. Chimpanzees employ signals with a sensitivity to the public/private nature of information, by adjusting their use of signal types according to social context and by taking into account potential out-of-sight audiences.[46]

The written and recorded word, together with artistic representation, allows problem solving across many generations—a repository of social wheel spinning, and to "see" the world in different ways. Solutions to problems can differ from group to group depending on context and cultural history. This is evident in the use of tools by chimpanzees to crack nuts. The use of stones to crack nuts has to be "introduced" to the group and is learned by others in the group. Furthermore, the stones are modified to better crack the nuts. Tools may be shared or hidden and kept for later use. This demonstrates creative decision making in practice.

C. Observations on *Drosophila* short-term memory mutants

A further prediction is that choice behavior should depend on the processes of plasticity since the ability to store and retrieve the results of stochastic variation requires such plasticity. Tang and Guo[47] and van Swinderen[48,49] showed that choice behavior in *Drosophila* is strongly affected by mutations that lead to defective short-term memory. The behavior that remains is then rigid optomotor responses. As van Swinderen expresses it, "a strong and non-distractable optomotor response, as seen in the *dnc* and *rut* mutants, may reflect failure of an interacting attention-like mechanism designed to periodically alternate among competing percepts of variable salience." Alternating between competing outcomes of stochastic processes is precisely what must be involved in the choice process.

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[1] In his Treatise on the formation of the foetus, Descartes wrote: "If one had a proper knowledge of all the parts of the semen of some species of animal in particular, for example of man, one might be able to deduce the whole form and configuration of each of its members from this alone, by means of entirely mathematical and certain arguments, the complete figure and the conformation of its members."

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[23] H. A. Braun, *Der Zufall in der Neurobiologie - von Ionenkanälen zur Frage des freien Willens. Zufall in der belebten Natur* (Verlag Roman Kovar, Hennef, 2018), pp. 109–137; (chance in neurobiology—from ion channels to the question of free will).

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Is the whole different from the sum of its parts? A proposed procedure for measuring divergence from additivity

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ABSTRACT

The behaviour of many small systems, such as a family or a football team, epitomizes the famous Gestalt slogan of a "Whole which is different from the sum of its parts". However, and in the context of these small cognitive systems, conceptualizing and measuring the extent and the way in which a whole is different from the sum of its parts is a non-trivial challenge. One possible direction for addressing this challenge involves measuring the extent in which the entropy of the whole is non-additive, meaning different from the sum of the entropy of its parts. However, measuring divergence from additivity is far from trivial. In this short paper, we propose a simple procedure for measuring divergence from additivity and illustrate the procedure by analysing the behaviour of a soccer team.

KEYWORDS

General systems approach; multiscale system; modelling; category theory; non-additivity

Introduction

Studying biological, social and cognitive systems alike, it has been long realized that these systems may be modelled as hierarchies (e.g. Bateson 2000; Miller 1965; Watzlawick, Weakland, and Fisch 2011), or more accurately as "recursive-hierarchies" (Harries-Jones 1995, 2016) where ensembles of components form wholes that are different in their properties and behaviour from the simple sum of their parts.

The Gestalt idea of a whole which is different from the sum of its parts, seems to be diametrically opposed to one of the basic properties of statistical entropy which is *additivity*. Therefore, a good opening stance for understanding wholes characterized by this Gestalt like property, is to discuss them through the concept of additivity.

Statistical entropy, additivity and non-additivity

In the context of statistical entropy, additivity means that the entropy of a system, composed of two probabilistically independent subsystems, is the sum of the entropy of the subsystems. Moreover, in classical physical systems the entropy is supposed to be *extensive* meaning that the entropy is a property proportional to the system's size. For example, when we double the size of a given system, the entropy should be doubled too.

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In contrast, non-extensive systems, or as Tsallis (2009a) describes them "non-additive" systems, rely or connect to a non-additive form of entropy (Tsallis 2009a, 2859), that should be used when the system that we analyse is characterized by strong correlations between its microstates which is the case with biological, social and cognitive systems.

For example, human language involves strong correlations between its words/microstates (Cartwright 2014) as expressed in multi-words expressions such as "hotdog". It is much more probable to get the word "dog" after the word "hot" than vice versa and therefore the probability of the microstate "hotdog" is significantly higher than the probability of the microstate "doghot". This idea can be illustrated in the context of social systems as well. Let's take a family system as an example. A married couple is a system composed out of two sub-systems; person A and person B. When analysing the behaviour of a married couple, we conceptualize it as constituting a different unit of analysis. The behaviour of the married couple may be considered as the result of merging two subsystems and it clearly expresses non-additivity as there are certain constraints imposed on the behaviour of the couple due to interactions/correlations between the two people. In other words, when two individuals are bounded by marriage and certain forms of interactions, their degrees of freedom, at least in certain respects, are reduced, which mean that the entropy of the family system may be lower than the one of its composing parts: The husband and the wife. In addition, when increasing the size of this family system by including for instance the wife's mother, the constraints do not proportionally increase (or decrease) or to put it in other words, the system's degrees of freedom do not proportionally decrease. In sum, from the perspective of additivity, the result of adding the mother in law to form a novel triadic whole (i.e. husband-wife-mother) may probably be *sub-additive* as the entropy of the emerging triad is smaller than the sum of its members' entropies. Think for example about a single person. Theoretically, and we must emphasize "theoretically", she has the full freedom to choose her course of behaviour among potentially infinite and equally-probable options. For example, she can use her free time for listening to music, boxing, visiting museums, watching football matches etc. Therefore, the entropy associated with her behaviour is *potentially* maximal when she is theoretically modelled as an isolated system that has full and absolute freedom of choice. In practice, her behaviour is limited of course, such as in the case where under cultural norms of gender she is directed to listen to music rather than to watch football. However, when entangled to a spouse, her degrees of freedom are probably reduced further and when entangled to a wider family system her degrees of freedom might be reduced further in such a way that the constraints imposed on the woman, probably increase.

In sum, the system's degrees of freedom are not proportional to the increase in the size of the system and when "merging" two people within the same family system the entropy of the family system is non-additive. One may even argue that the unique irreducible behaviour of certain social systems, as insightfully observed by some of the pioneers of systems theory (e.g. Watzlawick, Weakland, and Fisch 2011) may be explained by their non-additivity here interpreted as the *nonlinear function associating the system's granularity level and its components' degrees of freedom*. This point must be emphasized and remembered from now on as whenever we use the term divergence from additivity, we use it in the above sense of a non-linear change in the system's degrees of freedom as a function of scalability.

We have opened our discussion by using two concepts from physics. However, their use in the context of biological and specifically social and cognitive systems isn't trivial and straightforward. Therefore, we must clarify that from now on when we use the concepts of additivity and non-additivity, *we use them in a "borrowed sense"* to discuss the way in which the constraints imposed on the components of the system change when we scale-up, or zoom-up, from the behaviour of micro-states (or objects) to higher level configurations. We may now return to our discussion, with this broad conceptual understanding in mind.

The idea of divergence from additivity, as illustrated above in the context of a family system, is applicable to biological systems as well. Indeed, there is a clear gap between the "hierarchical organization of matter in physical science and the irreducibility of emergent behaviour in biology" (Strohman 2000, 575), and biological systems are characterized by non-additivity that must be taken into account. Strohman (2000) attributes this emergent behaviour to dynamics existing at the mesoscopic level and this theorization is supported by Tsallis theorization (Tsallis 2009b) attributing the nonextensive property to this level of organization as well.

The non-additivity is implicitly evident in the systems biology approach, as proposed by Noble (e.g. Bard et al. 2013; Noble 2010, 2012; Kohl et al. 2010), where it is acknowledged that "Biological functions are *integrated* at many different levels" (Noble 2010, 1130, our emphasis). According to one of Noble's ten principles of systems biology, biological functionality is multi-level, meaning that the system should be approached as composed of sub-systems that are layered in the sense that they form different "*logical types*" as evident in many living systems (Neuman 2002); When scaling up in the system's hierarchy, and components form macro level systems, some information is lost and some new information is formed (Neuman 2011) and therefore the system is both non-additive and non-extensive.

As implied by Noble's principle, in multi scale biological systems there is no privileged scale of causality (Noble 2011) and one may study such systems by conceptualizing the way *micro level information is integrated on a higher scale forming new information which in its turn forms constraints recursively determining the behaviour at a micro-scale*. Recalling our previous family dynamics example, when increasing the size of the family system to include the mother-in-law, a new bigger system is formed at the macro level that may impose new constraints on the behaviour of the married couple and the individuals constituting this unit, in such a way that re-directs its behaviour. This situation illustrates the way in which shifting to a higher level of the system involves the formation of new constraints on the behaviour of the lower level as argued by Noble. The top-down causality as discussed by Noble and others (e.g. Emmeche, Køppe and Stjernfelt 2000), may be therefore explained through higher level constraints formed through the interactions of the micro level states.

Tsallis entropy and divergence from additivity

While the multi-levels principle of the systems approach seems to be justified *Prima facie*, the way in which divergence from additivity may be measured, as a part of multiscale modelling whether in studying biological systems (e.g. Hunter et al. 2006) or small scale social systems, such as a family system, is far from being a trivial challenge and the gap between the "hierarchical organization of matter in physical science and the irreducibility of emergent behaviour in biology" is difficult to bridge. A possible solution for the modelling of non-additivity may come from parametrized entropy in which Tsallis non-additive entropy

is a specific instance. We don't believe this is the case but present it as it naturally follows our previous discussion.

Tsallis (2014) proposed a new measure of entropy defined as follows:

$$q\,(pi) = \frac{1}{q-1}(1 - \sum pi^q) \tag{1}$$

where the parameter q, known as the entropy/Tsallis index, is a measure of the correlations between the system's microstates. The Tsallis index may be considered as a biasing parameter that privileged common events/microstates when $q > 1$ and rare events when $q < 1$. When q approaches 1, it means that the correlations are weak or none and the Tsallis entropy is reduced to Shannon entropy. In this context, and given two *independent* systems A and B, the Tsallis entropy of the joined system is:

$$Sq\,(A, B) = Sq(A) + Sq(B) + (1 - q)Sq(A)Sq(B) \tag{2}$$

where the parameter $|1 - q|$ is a *measure of divergence from additivity*.

While the classical statistical concept of entropy is generally far from optimal in modelling social and biological systems, Tsallis entropy, in which the classical Shannon sense of entropy is only a specific instance (where $q = 1$), seems to open a wide new spectrum of modelling social, biological and cognitive systems.

In this context, the idea that we may measure divergence from additivity may be highly important for understanding the scaling behaviour of a system as discussed above. More specifically, if the parameter q is a measure of divergence from additivity then we may measure it in order to better understand *the way in which constraints scale up in multiscale systems*. Indeed, the association between the Gestalt behaviour and Tsallis entropy has been already made (Tsallis et al. 2003). As argued by Tsallis et al. (2003), $Sq(A + B) > Sq(A) + Sq(B)$ if $q < 1$ and $Sq(A + B) < Sq(A) + Sq(B)$ if $q > 1$. Therefore, when merging two subsystems to form a new "whole", it seems that we have at least a hint whether the ensemble is sub or super additive. However, there are several problems with this idea such as (1) the identification of the joint probability distribution of A and B, which is necessary for the above computation, (2) the scaling of this methodology to systems composed on more than two sub-systems and (3) the fact that it is not clear both theoretically and practically how to *identify* the appropriate q index as a measure of divergence from additivity, specifically in situations where we attempt to measure the ensemble of more than two systems. In situations where we have no knowledge of the system's dynamics, q has to be obtained from fitting experimental data (Cartwright 2014). The way q is obtained is not a solved issue although fitting the parameter for empirical data is definitely possible (e.g. Neuman et al. 2018; Ramírez-Reyes et al. 2016) and in this context the identification of the q value may be considered as a problem of optimization. Given these difficulties, an alternative way of measuring the way wholes diverge from the simple sum of their parts, is presented in the next section.

Measuring divergence from additivity

For introducing the proposed measure of divergence from additivity, we use a simple example of a soccer team. This worked-out example, is then followed by a more general

procedure for measuring the divergence from additivity of two interacting objects. This procedure can be trivially scaled up to the measurement of systems composed of more than two objects/sub-systems.

In a soccer team, the players pass the ball to each other forming a network of passes. The passes are actually interactions between the players and each player's interactions with the others may be represented by the distribution of his ball-passes here represented as a *vector* (i.e. array) of passes. To simply our presentation, let's take an imaginary soccer team comprised out of three players only: {A, B, C}. The partition of this set to sub-sets of two players forms three microstates where two different players are considered together: {A, B}, {A, C}, {B, C}. In the case of four players, we similarly have six microstates of two players and four microstates of three players. Choosing *r* players among n players follows the next equation:

$$\frac{n!}{r!(n-r)!} \tag{3}$$

and the macro-state of the system at layer *r* may be considered in terms of all micro-states of the set partitions of r players; We may consider a system of two players as a macro state of size 2, a system of three players as a macro state of size 3 and so on. For simplicity, we focus our example on the divergence from additivity of two players only. When we examine the behaviour of two players as an ensemble, or a system composed out of two sub-systems, we are actually ignoring their individuality. Representing the soccer team as a directed graph where players A and B are two nodes, we may simply merge these two nodes when considering the behaviour of the ensemble (i.e. the microstate) A + B. In this case, the vector describing A + B includes the sum of passes both A and B have delivered to each of the other players. This ensemble *isn't the joint probability distribution* of their passes to the other players but simply the vector of the sum of their passes. Hence this whole is similar to the type of abstraction as discussed in the modelling of biological systems (Fisher, Piterman, and Vardi 2011). Along the same line, when considering the ensemble of three players A + B+C, we merge them, the vector of the whole A + B+C is the sum of their passes to each of the other players, and so on. The formation of A + B through the sum of their vectors is not an inherent property of the proposed methodology and it was selected for more practical reasons. More sophisticated ways of representing such constructs appear in the literature (Neuman, Neuman, and Cohen 2017) and may be determined ad hoc for the specific context of the study.

Let's continue introducing our idea with a simple toy example. Assume that we have a team of five football players. The behaviour of each player is represented as a vector (i.e. array) of passes to the other players. See Table 1 for an example.

We can see that player A passes the ball three times to player B, seven times to player C and so on. The behaviour of player A can be represented by the vector of his ball-passes to players B, C, D and E:

$$\mathrm{A} = [3, 7, 2, 5]$$

If we would like to represent the behaviour of the pair of players A + B then we merge their ball-passes and represent their passes to players C, D and E using the following vector:

$$\mathrm{A} + \mathrm{B} = [13, 4, 6]$$

Table 1. Ball passes from players A–E (rows) to the other players.

	A	B	C	D	E
A		3	7	2	5
B	1		6	2	1
C	9	7		4	8
D	5	5	5		3
E	8	3	0	1	

that when normalized can be represented as:

$$\mathrm{A} + \mathrm{B} = [0.57, 0.17, 0.26]$$

The behaviour of footballs player, whether considered as individual micro-states or as higher order states such as A + B, may therefore be represented by the entropy of their ball-passes. This is an important point. We propose to measure the behaviour of the system's states at different scales by using the entropy of the behaviour. In this context, we may examine the scaling behaviour of the players by measuring the extent in which we may approximate the entropy of a given microstate at scale N (e.g. A + B) through the entropy of its micro-states that exist on a lower scale. For example, let's approximate the ball-passes distribution of A + B using the ball-passes distribution of A. The normalized distribution of A's ball passes to C, D and E is:

$$\mathrm{A} = [0.15, 0.14, 0.36]$$

The normalized distribution of ball-passes from A + B to the *same* players is:

$$\mathrm{A} + \mathrm{B} = [0.57, 0.17, 0.26]$$

For measuring the divergence of A + B from A, we may use the Kullback-Leibler Divergence, also known as Relative entropy:

$$D_{KL}(P||Q) = \sum p(i) \log \frac{p(i)}{q(i)} \tag{4}$$

where in our case, the P distribution always stands for the examined micro-state, in our case A + B, and the Q distribution always stands for its direct sub-set, in our case A.

The relative entropy has been chosen as it resonates with the idea of statistical entropy and other measures such as cross-entropy or others may work as well. The relative entropy measure, may be considered as a measure of information gain when one revised his beliefs from the prior probability distribution Q to the posterior probability distribution P. However, in our case, the proposal is that understanding the scaling behaviour of a microstate such as A + B, requires contextualization, both through the probability distribution of its sub-sets (e.g. A and B) and the probability distribution of the sets in which it is contained

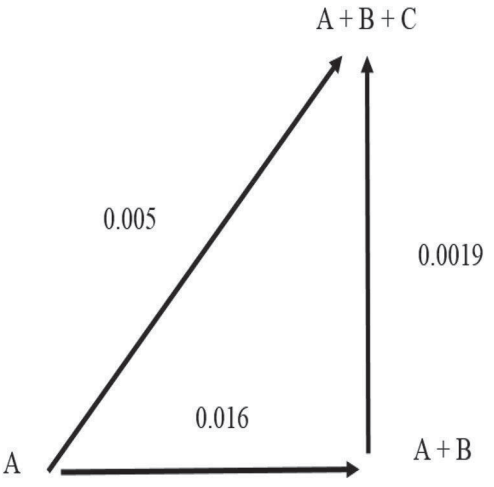

Figure 1. Information gain when revising beliefs from A

(e.g. A + B+C). In other words, to understand the scaling behaviour of A + B, we need to measure the behaviour of A, B and A + B+C (for instance).

Let's assume, we examine the scaling behaviour of two players Messi and Vidal. Each player has his own unique pattern of ball-passes. However, we may ask what happens if we ignore these individual patterns of behaviour and consider the couple Messi + Vidal as a new integrative unit of analysis. In a case no scaling is evident, then the information gained by revising our beliefs from the behaviour of A (e.g. Messi) to the behaviour of some A + B+C (e.g. Messi + Vidal + Suarez) should be equal to the information gain of revising our beliefs from the probability distribution of A to A + B (e.g. Messi + Vidal) and from A + B to A + B+C. The justification for this proposal is as follows: If no scaling effect is evident, then *factoring* from A to A + B+C through the *intermediate* level of A + B has no significance whatsoever in terms of information gain. This idea can be explained through the following graph using Table 1 data of ball-passes and where arrows point from the prior probability distribution of a state to the posterior probability distribution of a state and the arrow's value represents the relative entropy measure (Figure 1).

Following this idea, and in the specific case of A + B, we may measure its divergence from additivity by measuring the extent in which the information gain of the path from A to A + B to A + B+C is different from the information gain of the direct path from A to A + B+C, and this idea symmetrically holds for the comparison of the paths from B to A + B to A + B+C and the path from B to A + B+C. This logic is grounded in an abstract structure of Category Theory, an idea fully explained and elaborated in the appendix. Therefore, our proposed measure of divergence from additivity for the specific case of A + B may be described as follows:

Let 1 be $D_{KL}(\mathrm{A}+\mathrm{B}+\mathrm{C}||\mathrm{A}+\mathrm{B}) + D_{KL}(\mathrm{A}+\mathrm{B}||\mathrm{A}) - D_{KL}(\mathrm{A}+\mathrm{B}+\mathrm{C}||\mathrm{A})$

Let 2 be $D_{KL}(\mathrm{A}+\mathrm{B}+\mathrm{C}||\mathrm{A}+\mathrm{B}) + D_{KL}(\mathrm{A}+\mathrm{B}||\mathrm{B}) - D_{KL}(\mathrm{A}+\mathrm{B}+\mathrm{C}||\mathrm{B})$

Then the proposed measure for divergence from additivity is defined as:

$$\mathrm{DivgCom} = \mathrm{MEAN}\ (1, 2) \tag{5}$$

And the general procedure for measuring divergence from additivity for a two-elements micro-state is:

Given a set $M^1 = \{A, B, C \ldots\}$ define M^2 to be the set of all dual sets

i.e. $M^2 = \{\{A, B\}, \{A, C\}, \{B, C\} \ldots\}$

And similarly, $M^3 = \{\{A, B, C\}, \{A, B, D\}, \{A, C, D\} \ldots\}$

For each $m_i \in M^2$ (i.e. {A, B}):

Select the subset $m_j \in M^3$ where $m_i \in m_j$ (i.e. {A, B, C}, {A, B, D} ...)

For each element in m_j calculate:

$$\mathrm{ABS}(\mathrm{DKL}(A + B, A) + \mathrm{DKL}(A + B + C, A + B) - \mathrm{DKL}(A + B + C, A))$$

Sum and average all the results of m_j

Sum and average all the results of m_i

where the operation *ABS* stands for the absolute value of the expression.

Measuring divergence from additivity in the context of soccer

After, we have understood the general logic of the above procedure, let's see how it was implemented in the context of real world data. For illustrating our measure, we have used the English Premier League (2015–2016)[1] and analysed the data of Leicester City F.C. that won the league in this season.

First, we have identified and selected the ten players who made the most passes over the season. Next, we have applied our procedure and for each of the 45 pair combinations of these ten players produced the DivgCom score.

If DivgCom validly measures divergence from additivity then it should be correlated with the difference between the Shannon entropy of A + B (i.e. S(A + B)) and its simple sum: S(A) + S(B), here symbolized as DivgAdd. That is, we measure the Shannon's entropy of A's ball-passes vector, the Shannon's entropy of B's ball-passes vector, and the Shannon's entropy of A + B ball-passes vector. DivAdd is then the simple difference between the Shannon's entropy of the higher-level system A + B and the sum of the entropies of the lower level units A and B. Given the summation of each two player's vectors, it is expected that the entropy of the ensemble A + B will be smaller than the summed entropy of A and B. Therefore, our only interest is in the correlation between the absolute value of DivgCom and the absolute magnitude of the difference between S(A) + S(B) and S(A + B), as a way of establishing the validity of DivgCom.

For ease of interpretation, we have used a reversed ranking of DivgCom in such a way that the highest score was ranked "1" and the lowest "45". In this way, a higher score of divergence from commuting will be interpreted as a higher score of divergence from additivity. The same procedure has been applied to DivgADD and both scores has been subject to a logarithmic transformation.

The Pearson correlation between DivgCom and DivgAdd was found to be statistically significant ($r = 0.696, p < .001$). The higher was our divergence measure, the higher was the divergence from additivity as hypothesized. As can be seen in Figure 2, a linear regression model clearly fits the data, and the linear regression was found to be statistically significant ($F(1, 44) = 39.556, p < .001$), with $R^2 = 0.485$.

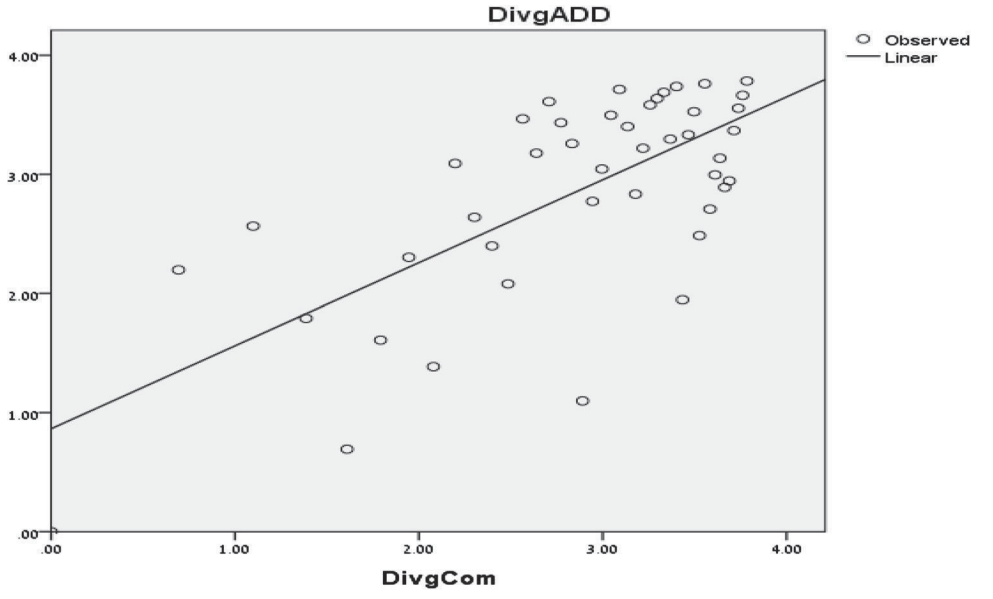

Figure 2. A linear curve fitting for DivgADD based on DivCom.

However, there are at least two possible rebutting arguments against the significance of this correlation. First, it may be argued that this result is confounded with the distance between two interacting players as the variance of divergence from additivity may be trivially explained through the distance between the players in a pair.

To address this potential criticism, we have ranked on a five-point scale the distance between each player and the goal keeper. The goal keeper holds the first line of defence and he was scored "0". Huth and Morgan are located at the second line of defence and therefore they scored "1" and so on. Next, we computed the absolute difference in distance between the players within each pair. For example, the pair {Vardy, Huth} scored "4" as Vardy scored "5" and Huth scored "1". This scored have been ranked and log transformed.

It was found that DivgCom is negatively correlated with the distance between the players composing the pair ($r = -0.340, p = .024$); the shorter is the distance between the players, the higher is the divergence from additivity or said differently, shorter range interactions between players are characterized by a higher level of non-additivity. These results may be explained by the fact that two closer players have a wider space of players than more distant players to which they may pass the ball. However, even when controlling for the players' distance, the correlation between DivgCom and DivgAdd was still statistically significant ($r = 0.657, p < .001$).

A second criticism against the correlation between DivgCom and DivgAdd may be concerned with the number of passes produced by each player. There is a significant gap between the number of passes made by each player with the lead of Drinkwater ($N = 1206$) and Kante ($N = 904$) and with Vardy ($N = 289$) and Schmeichel ($N = 329$) at the bottom. Drinkwater is the player who has both delivered most passes and got the highest number of passes. Therefore, it is likely that when measuring the divergence from additivity of each pair, the divergence will be biased by the pair's passes to a "hub" like Drinkwater. Ranking the players according to their passes, we have identified in each pair the player

with the lower rank of passes and scored the pair according to this value. Correlating this log transformed rank score with DivgCom was found to be statistically significant ($r = .589, p < .001$); the higher was the number of passes the higher was DivgCom. However, even when controlling this new measure, the correlation between DivgCom and DivgAdd remained statistically significant ($r = .50, p < .001$). In sum, and in the very limited context of this illustrative example, it seems that DivgCom provides a valid measure for analysing divergence from additivity. It goes without saying that as we have analysed the behaviour of a single soccer team in a single season, and that in the current study we have no pretensions whatsoever to validate the measure but just to illustrate it in a single and limited case.

Discussion

From the early days and in various contexts, the pioneers of general systems theory have been fascinated by the way in which biological, social and cognitive systems form emerging wholes that cannot be trivially reduced to their components (e.g. Bateson 2000). The same ideas echo today is the systems approach to biology and in various attempt to model the synergy evident in various context from human language to the neural system. The current short paper resonates with this old venture by first discussing non-additivity in the context of entropy, arguing that the insightful Tsallis entropy doesn't provide a simple way of measuring non-additivity specifically in the context of complex biological, social and cognitive systems. Next, we have proposed a procedure for measuring divergence from additivity which is theoretically grounded in the category theory concept of the co-product (see appendix). Using category theory for clarifying general properties of systems may be traced back to the work of Ross (1957) and others, but unfortunately and to exclude rare cases (e.g. Neuman 2018), this approach has been quite marginal. We use the abstract concept of the co-product only as a starting point but interpret it in a way which may be applied to the measurement of complex systems. The procedure proposed in this paper, concerns the object of the product in terms of vectors and the relations between the objects in terms of the Kullback-Leibler divergence measure. This unique combination, that has been illustrated in the context of soccer, is only a first sketch of a methodology in progress and as such it should be judged. However, the measure we propose has the benefits of being theoretically meaningful, easy to compute and easy to scale up to larger system than dyads. These benefits distinguish the measure from some proposals for measuring information synergy (e.g. Griffith 2014; Timme et al. 2014). Future studies should further develop this measure and its practical applications. To conclude, we would like to emphasize the importance of our contribution by returning to the family dynamics example. Pre-theoretically, one may easily realize that the behaviour of a married couple cannot be described as the simple sum of each of the partners' behaviour. Is there an easy way to conceptualize and measure this scaling effect? One may hardly find answers to this question, specifically in the context of small systems, and therefore our contribution is justified only if by presenting a possible direction for studying wholes different from the sum of their parts.

Note

1. Data have been purchased from Perform group (www.performgroup.com)

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Appendix

Modelling divergence from additivity: the category theory perspective

Category Theory as an abstract mathematical language (e.g. Lawvere and Schanuel 2009), has been shown to have a great promise for modelling relational systems (e.g. Neuman 2018) even since the early work of Ashby (Klir 2009). In this context, one of category theory basic structures – the co-product – may be of relevance for addressing our challenge of developing a procedure for measuring divergence from additivity.

The co-product, or *sum* of objects, is defined as follows: a co-product of C-objects A and B is an C-object A + B together with a pair (i_A: A $\rightarrow$ A + B, i_B: B $\rightarrow$ A + B) of C-arrows such that for any pair of C-arrows of the form f: A $\rightarrow$ C, g: B $\rightarrow$ C there is exactly one arrow $[f, g]$: A + B $\rightarrow$ C that makes the diagram in Figure 3 commute in such a way that $[f, g] \circ i_A = f$ and $[f, g] \circ i_B = g$. $[f, g]$ is called the co-product arrow of f and g with respect to injections i_A and i_B (Goldblatt 1979, 54).

Like its dual notion – the product, the co-product is a way of seeing the general object ("the best of its type") from the perspective of the *particular* objects. In this sense, it is the *least specific* object to which the objects admit morphisms.

The idea that A + B is an abstraction of A and B (i.e. "the least specific") as seen from the perspective of its *particular objects*, combined with the idea that the above diagram involves commuting paths, may be used for measuring divergence from additivity as A + B is actually a structure formed through the merge of A and B. For presenting this idea, we will re-use the context of a soccer team.

Now, in the context of soccer, we may measure the extent in which the ensemble is a co-product or the simple sum of its constituents by measuring the extent in which the *co-product diagram commutes*. Here the A object is represented through the vector of passes from A to the other players, B is represented through the vector of passes from B to the other players, A + B is represented as the vector of the sum of passes from A and B to the other players and the C object is every triad of players, such as A + B+C, in which the pair A + B is a subset. In the above diagram, the arrows represent the values of the Kullback-Leibler divergence. Now, if A + B is the simple sum of its objects then the co-product diagram should commute in a way to be shortly explained. Let's use Table 1 for illustration. First, we would like to understand how two players A and B behave when they are ensembled together to form the whole "A + B". To address this challenge, we use the ball-passes distributions of A + B+C, A + B, A, and B. A + B+C is a triad in which the pair A + B is a subset. Figure 4 illustrates this procedure.

Each object is represented by the vector of its ball-passes to the other players. If A + B is the sum of A and B, then this diagram should theoretically commute, and the path A $\rightarrow$ A + B $\rightarrow$ A + B+C should be equal to the path from A $\rightarrow$ A + B+C, and the same holds for the path B $\rightarrow$ A + B-> A + B+C and the path B $\rightarrow$ A + B+C. If the D_{KL} from A to A + B+C is equal to the sum (or the average in other possible formulations) of the D_{KL} of A from A + B and from A + B to A + B+C then it means that the efforts we need in order to update our beliefs when shifting from the distribution of A that exist on level 1, to the distribution of A + B+C, that exist on level 3, are bigger than the efforts we need in order to update the shift from macro level 1 (i.e. A) to level 2 (i.e. A + B) and

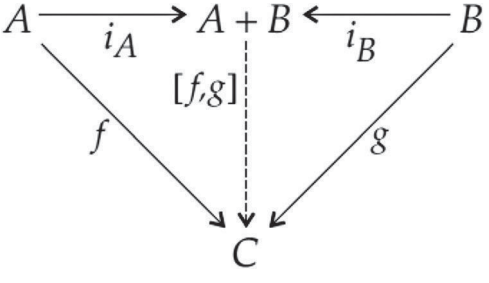

Figure 3. The co-product.

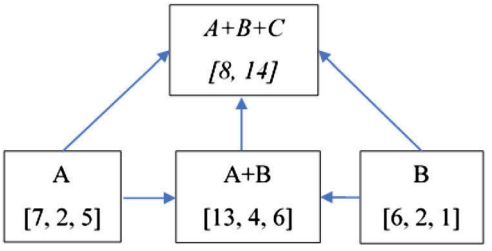

Figure 4. Divergence from additivity.

from level 2 (i.e. A + B) to level 3 (i.e. A + B+C). Therefore, the "scaling factor" between the levels of the system is different and the system is *non-additive in the broad conceptual sense as described above*.

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Current Opinion in Systems Biology

Systemic integration of different inheritance systems

Eva Jablonka[1] and Denis Noble[2]

Abstract

The between-generations transmission of phenotypic variations is based on networks operating at different levels—genetic, epigenetic, behavioral and symbolic. Since each level involves a network of interactions, integrating such networks of networks may seem hopelessly complex. We suggest that the problem can be drastically simplified if analysis starts from a description of the heritable trait of interest as an attractor in a developmental landscape constructed by networks of inputs at underlying and overlying levels of organization. On this basis, further studies quantifying the different inputs that contribute to the between-generational re-construction of the trait can be made and enable the development of a systemic, dynamic and predictive model of inheritance.

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Canalization, Developmental systems theory (DST), Heritably varying trait (HVT), Inclusive inheritance, Inheritance systems, Landscape model, Price equation, Waddington.

Introduction

There are two broad ways to think about the inheritance of phenotypes. The first and most familiar is the exclusive genetic approach. With the exception of the inheritance of cultural variations, the inheritance of phenotypes is described in terms of genetic variations on the assumption that variations in heritable phenotypic traits (**heritably varying traits: HVTs**; all bold, key concepts, are defined in Box 1) can be fully mapped to DNA sequence variations. This approach has been challenged, since today it is clear that inheritance includes the transmission of variations that are not based solely on differences in DNA base sequences: two individuals with identical DNA sequences can pass different developmentally acquired variations to their descendants, sometimes for many generations. The second approach is described by **developmental systems theory (DST)** [1], in which **heredity is** seen as a complex system of interacting **inheritance systems** at different levels (genetic, epigenetic, behavioral and symbolic) [2]. We adopt this multi-level approach in this paper and argue that it is the HVT — embedded within the life-cycle of the individual — that should be the unit of analysis [3] rather than any of the specific inputs into it (e.g., the gene, the epiallele, the "meme"). We use two hypothetical examples to illustrate how inputs at different levels contribute to the inheritance of a phenotypic trait — a food preference.[1] We then describe a systems biology approach to **inclusive inheritance** based on Waddington's epigenetic landscape model, and point to methodologies that quantify and tease apart some of the heritable inputs into a trait's development and inheritance.

Two examples

Imagine two types of organisms, a butterfly and a human, that display heritably variant food preferences, such that some individuals of the same species show a preference for food type X1 rather than for other equally nutritious food X2, while others prefer X2. In the butterfly case we discover that genetic, epigenetic and ecological factors contribute to these food preferences: some individuals have genetic variants that make them slightly prefer food X1, while others are indifferent, or prefer X2. The butterflies' preferences are also influenced by the food consumed by their mother: the larvae of a female that drinks the sap of plant X1, on which she also lays her eggs, prefer to eat the leaves of that X1 plant (rather than food-plant X2), which, in turn positively affects their adult sap preferences (and the correlated egg-laying preferences); this maternal effect can override the effect of the genes that make them prefer X2. Moreover, when eggs of X1 mothers are transferred for several consecutive generations exclusively to X1 plants, the frequency of X1-preferring

[1] A review of the extensive literature on food preferences in insects and humans is beyond the scope of this short review. We therefore used hypothetical examples to make the non-controversial point that there are different interacting inputs into the development of multigenerationally-stable food preferences.

Box 1. Definitions of key concepts used in the text

Attractor A point or a trajectory towards which a dynamical system evolves over time as a result of multi-level constraints. Heart rhythms, circadian rhythms, hormonal rhythms, cycles of deprivation, are examples of attractors.

Canalization The adjustment of developmental pathways so as to bring about a uniform developmental result (a particular phenotypic trait or capacity) in spite of genetic and environmental variations.

Developmental Systems Theory (DST) A dynamic systems approach integrating all the developmental resources that contribute to the life cycle of an individual, without *a priori* privileging any of them. From this viewpoint, the unit of evolution is the whole life cycle [1].

Epigenetic landscape A visual model, suggested by C.H. Waddington, which describes the dynamics of embryological development and its underlying genetic network of interactions [9].

Epigenotype The total developmental system of interrelated developmental pathways through which the adult form, physiology or behavior of an organism is realized. A more restricted definition is: the actual pattern of gene activity in a specialized cell type.

Heredity The regular resemblance between parents and offspring, which involves the acquisition of developmental resources and their subsequent retention and transfer. Transfer can be vertical (from parents to offspring), horizontal (among same-generation individuals), and oblique (between generations, but not via conventional biological parents). "Parent" in the broad sense is seen as the sender of information and the receiver as the "offspring". **Hereditary transmission of information** requires that a receiver interprets (or processes) an input from a sender who was previously a receiver, so that the processing leads to the reconstruction of a similar organization-state to that in the sender; variations in the sender's state lead to similar variations in the receiver [2].

Heritably Varying Trait (HVT) A characteristic of an organism resulting from the interactions between internal initial conditions and the external environment, which can take several variant forms and can be transmitted between "parent" and "offspring" entities.

Inheritance system An evolved set of factors, processes and mechanisms that lead to the developmental reconstruction of variations transmitted from ancestors to descendants, and result in similarity between them. The system underlying the replication of DNA variations – the genetic inheritance system – is one of several inheritance systems. Others include the epigenetic, behavioral and symbolic systems [2].

Inclusive inheritance is inheritance that includes all relevant factors (at all levels) that contribute to the transmissibility of a variation.

Plasticity The ability of a single organisms to generate variant forms of morphology, physiology and/or behavior in response to different environmental circumstances. Usually it is described in terms of the ability of a single genotype to generate several different phenotypes. Canalization cannot occur without plasticity, because keeping a trajectory of developmental change constant requires flexibility at underlying or overlying levels.

Principle of biological relativity There is, *a priori,* no privileged level of causality in biological systems. Multi-level causation with feedbacks between all the levels is an important feature of all biological organisms.

Scales and levels Organisms exist at many different scales. They also manifest different levels of organization. Scale is a neutral dimensional category. A level, in contrast, is defined by its organization, which constitutes the way in which properties at the organized level constrain the lower-level elements.

butterflies is further increased, suggesting grand- and great-grand-parental effects based on epigenetic mechanisms. Another contributing factor is the ecological stability of the environment to which the butterflies contribute by their activities. Since the butterflies act as pollinators, they increase the abundance of the plants of choice, and since the microorganisms that live on the feeder-plants contribute to the break-down of the sap in the insects, their consumption contributes to the positive reinforcement the insects experience as they consume it. Thus, the butterflies construct the ecological niche in which they live in ways that contribute to the intergenerational stability of the observed preference in their lineage.

Now take a human example. Imagine a community with a culinary tradition in which very spicy food is strongly preferred. (Originally, the preference may have conferred an advnatage because the spices helped to preserve food when the climate was warmer, but this is not reflected in genetic differences between spice preferring and non-preferring extant communities). Assume that in this society the tradition of preparing and consuming spicy dishes has been stable for many generations. People cultivate the plants from which the spices are made, and the economic activities of selling, buying and preparing spicy food are practiced in the community. Pregnant women transmit traces of food to their offspring through the placenta and later through

milk, priming their offsprings' food preferences, and young children, who are exposed to and encouraged to consume spicy food during childhood, acquire from their mothers the microbiome that facilitates the digestion of spicy food, and end up developing a lifelong preference for it. Eating extremely spicy food gives an individual social prestige, as does preparing tasty spicy dishes, and spicy food has become a marker that identifies individuals as members of this particular cultural group. The spicy food is therefore related to a whole network of factors, processes and social activities, which are mutually reinforcing [4,5].

These hypothetical cases show that the ways in which food preferences are reconstructed in the two species are different. The reconstruction in each case requires a multi-level systems biology approach, which is similar in principle to the multi-level analyses of physiological and morphological traits. For example, an analysis of a functioning heart includes processes all the way up and down between molecular and whole organ levels [6]. Both the effects of low-level components on the system's overall behavior (bottom-up causation) and the constraints by higher levels, which become represented in the initial and boundary conditions of the system (top-down causation), can be described by differential equations. The central feature of this form of systems biology is that networks of causal interactions occur at all levels and between levels, and there is no *a-priori* privileged level of causation. In other words, the **principle of biological relativity** [7] is manifest not only for developmental/ontogenetic systems but also for inheritance systems, which involve, in addition to ontogenetic stabilizing processes, additional processes that lead to between-generation persistence.

A detailed study of developmental-inheritance networks can tell us under what conditions the transmissibility of a focal trait is likely to change. But how can we capture the complex dynamics of such a multi-cause system? Although equations representing the degree to which network interactions are additive or super-additive have been developed [8], the complexity of a multi-level inheritance system seems to preclude detailed description in terms of differential equations. The use of Waddington's visual model may be a step towards an understanding of the dynamics of such a system that needs to be analyzed not only at different levels but also at different temporal scales.

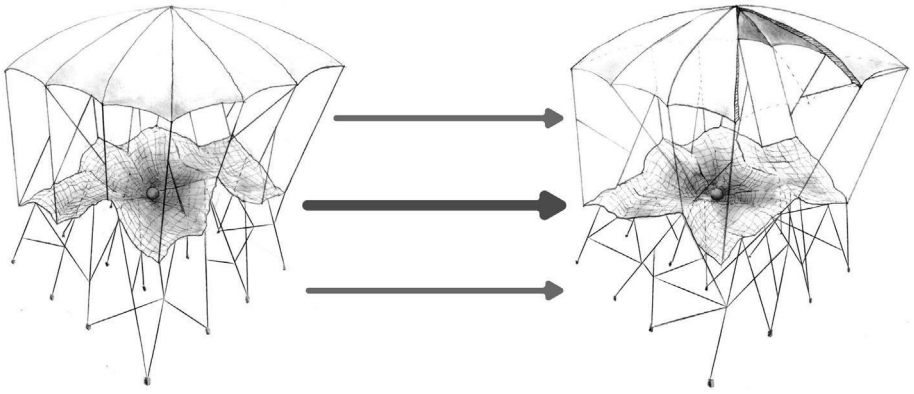
A visual model of systemic developmental integration

Waddington's **epigenetic landscape** is a model of a complex, multi-level, developmental system. It describes ontogenetic development as a ball rolling down a tilted landscape, with many hills and branching valleys descending from a high plateau, which represents the initial state of the developmental system. This landscape is itself a network of interactions, with lateral regulatory connections among different factors and processes. Underlying the landscape and contributing to its dynamic architecture are networks of interacting genes and their products, which respond to the developmental environment (itself a network at this level of organization) and lead to a functional, usually species-typical developmental end state [9]. Waddington called these underlying regulatory networks the **epigenotype**.

Waddington's visual model was translated into dynamical systems theory terms that could accommodate genetic assimilation and accommodation processes, ontogenetic plasticity and evolvability [10]. However, non-genetic inheritance has not been considered in these terms, although the developmentally-plastic cellular network includes the epigenetic factors and mechanisms of which we have become aware over the last 40 years – dedicated regulatory and cell-heredity mechanisms such as DNA methylation, histone modifications, and RNA regulatory factors, which contribute not only to the stability of the developmental network during the individual's ontogeny, but also to its transmission between generations [2]. This is not surprising: the relationship between the **canalization** of development and epigenetic transgenerational inheritance was not explicit in Waddington's model, and non-genetic inheritance is not central to analyses of development in terms of systems' models [11]. Although Waddington discussed the likelihood that environmental factors (e.g., pollution) and the related high-level networks (e.g., social/political networks driven by big companies and government policies) will impinge on the development of the trait (functional heart, with a normal cardiac rhythm), this was not shown in his original illustrations of the landscape model. Our discussion uses Waddington's framework to discuss the developmental aspects of non-genetic inheritance, and therefore goes beyond current discussions.

Figure 1 illustrates our attempt to include factors at different levels of organization in the depiction of an HVT, a beating heart with a typical rhythm (indicated by the ball in the valley) embedded within an epigenetic landscape that is part of the overall developmental system of the organism. It shows both the constructive effects of the underlying epigenotype and the constructive effects of the overlying ecological/social niche (the network emanating from the umbrella that represents the encompassing environment). In the case we describe, the fidelity of transgenerational transmission of the physiological trajectory leading to the heart's regular beating (in the middle of the figure) is more stable than the fidelity or transmission of the relevant cellular and social networks. It represents, for example, the fact that a genetic factor that may be normally important for the construction of the heart can be modified (by genetic or drug manipulation) while the rhythm of heart-beating remains almost unaffected

Figure 1



The canalized construction and persistence of an HVT. A part of the complex system of interactions that form the epigenetic landscape. The ball in the middle of the landscape depicts the trait at the level of interest (e.g., a typical cardiac heart rhythm) at a particular developmental (e.g., postnatal) stage. The bottom network shows the epigenotype, with black dots representing genes; the guy ropes emanating from them represent the interacting products of the genes and the epigenetic factors that form the network's architecture. The upper umbrella and the network emanating from it represent the ecological/cultural/social conditions, with the ropes representing processes such as geographical conditions (e.g., elevation), institutional opportunities, social norms, etc. Both the upper and lower networks construct, anchor, stabilize and support the epigenetic (middle) landscape, which is itself a network (indicated by its grid structure). Following injurious changes in external conditions (broken umbrella), both the top and bottom network self-organize and rearrange in a way that maintains the architecture of the epigenetic landscape and the functioning of heart. (Note the greater changes to the top and bottom networks compared with the relative stability of the middle epigenetic network; the changes in the right hand side of the bottom network are compensatory changes at the cellular level.) The darkness and thickness of the arrows represents level of similarity (darker-thicker – higher similarity, lighter-thinner – lower similarity) following the injury. (Reproduced by permission of Adi Mark, all rights reserved to the artist).

[12], or that different social conditions (e.g., class differences that are correlated with the quality of nutrition) lead to similar physiological effects. The heart rhythm is a strong **attractor** (a relatively stable dynamic state towards which development proceeds in spite of some variations in the starting underlying and overlying conditions), which is reconstructed every generation. Importantly, for the canalized trait to be an HVT there should be *several* variant canalized attractor-states (for an example, see Ref. [13]).

The focal landscape can be described at any level: it can be social, ecological, cellular, or, as it was originally described by Waddington morphological/physiological. Going back to the food preference of butterflies, we can uncover the ecological inputs at the higher level that contribute to the intergenerational stability of a particular food preference that is described at the behavioral level, as well as the genetic and epigenetic inputs at the lower level. The model for the human case is more complex, although social-symbolic inputs may make some of the inputs at the lower level (e.g., those depending on genetic variants) irrelevant, in the sense of rendering them selectively neutral. In both cases the scientist has to not only identify "hubs" — factors and processes that have multiple connections — but also pinpoint the positive and negative feedback relations between factors at different levels that increase the stability of the trait. These feedbacks underlie the dynamic stability of the landscape and of the attractor state. Importantly, the changes in the landscape can be heritable and persist across generations, even when there is no relevant change in DNA [for examples see Ref. [2]). In terms of dynamical system theory, one may say that the change in the initial conditions that initiated the landscape change has become internalized through the operation of non-genetic inheritance systems.

Measuring transmissibility: composing and decomposing inclusive inheritance How can the persistence of a particular HVT be measured in a complex system like that depicted in Figure 1, in which persistence (which may include persistence in offspring, following reproduction) is seen as a facet of development, and multiple inheritance systems are involved in the trait's transmission? Day and Bonduriansky [14,15] and Uller and Helanterä [16,17] use the Price equation to measure the change in a trait that is the product of different heritable inputs. The Price equation describes, in very general terms, the change in the average value of a trait between generations, which is the consequence of selection (the covariance of the trait with reproductive success or its proxy) and transmissibility bias (the bias introduced by processes such as mutation, epimutation, recombination, environmental induction, drift, developmental biases, etc.) (see Okasha [18] and Day and Bonduriansky [15] for excellent, clear, expositions).

$$\Delta z_{av} = \mathrm{Cov}(w/w_{av}, z) + E((w/w_{av})\Delta z) \quad (1)$$

The individual's character value is z, its number of offspring is w [or more generally its fitness], and w_{av} the average fitness in the parental population; the difference between the character values of the individual and

its offspring is Δz; the subscript av denotes average; Cov and E denote covariance and expectation; $\Delta \bar{z}_{av} = \bar{z}' - \bar{z}$, where $\bar{z}'$ and $\bar{z}$ are the average values of the trait in the offspring and parent generations.

The first covariance term on the right hand side of Eq. 1 describes the change in the trait value as a result of selection or the fitness of the trait (w), while the second (expectation) term on the right describes the effect of transmission bias on the change in the trait (which is unrelated to the direct selection for the trait), both weighted by the average parental fitness w_{av}. The fidelity of inheritance captured by the transmission-bias term in the Price equation and can be expressed in terms of the change in the trait value between generations that remains after subtracting the effects of selection:

$$E\left(\left(w/w_{av}\right)\Delta z\right) = \Delta z_{av} - Cov\left(w/w_{av}, z\right) \qquad (2)$$

All the inheritance systems' inputs that contribute to the trait's transmissibility are therefore collapsed into one general term. When transmission is perfect (there is no transmission bias) the change in the trait value can be fully explained by selection alone. This, of course, is an idealization. Since there are many factors that contribute to the overall change in the trait value, the estimation of the transmission bias term can be considered as only a first step towards evaluating the contribution of different hereditary inputs to the HVT. The change in the value of the HVTunit of analysis (Δz_{av}) is not, typically, the entire life cycle: the focus is on a particular heritably varying trait (HVT), which is the outcome of recursive, transgenerational, developmental processes, which tend to reoccur with each life cycle. However, it is possible to compare different heritably variable developmental cycles too. The Price equation can be applied at any chosen scale, and for any type of selection. The "trait" can be genotypic (a particular DNA sequence composition) or phenotypic (epigenetic-cellular, organismal-physiological, behavioral-cultural), and multiple "parents" (e.g., cultural models) can provide heritable inputs.

The Price equation is dynamically insufficient because it requires that Δz_{av} is computed anew every generation. Nevertheless, in spite of this limitation, it can provide a general picture of change in a trait's value because it allows the inclusion of both selection and fidelity parameters at genetic, epigenetic and behavioral-cultural level, as well as the inclusion of selection that is based on sampling from a repertoire of variation without the need for replication (a type of selection called sample selection by Price) [19] The equation can therefore provide a good guide for further research. For example, the expansion of the Price equation to include the fitness and fidelity parameters that specify particular inheritance systems (e.g., genetic, epigenetic and cultural; see Refs. [14,15] for expansions of the original Price equation) can be compared to the overall phenotypic change in the trait value. When the overall Δz_{av} estimate differs from that of the value found using the parameters in the expanded equation, nonlinear interactions between inheritance systems can be assumed. An HVT may be, for example, more heritably stable than any of the inputs that contribute to it, including genetic inputs: the genetic networks[2] that underlies the same HVT in parents and offspring may be substantially different because of meiotic reshuffling and syngamy. Hence, such results suggest that the HVT is canalized, and canalization mechanisms should therefore be the focus of further research. This requires different, dynamic models that use differential equation to show how the dynamics of the system change over time. Such formulations are extensively use in systems biology, and should inspire equivalent formulations in evolutionary biology, along the lines suggested by Jaeger and Monk [10].

Novel variations in an HVT can be induced by genetic (mutational, recombinational) or non-genetic inputs, and can be thought of as changes in the form and depth of the trait-attractor, additions or deletions of attractors, and changes in the trajectories leading to existing attractors. Such variations are the outcomes of **phenotypic accommodation** — processes of developmental reorganization mediated through general biological properties such as mechanical flexibility, the multiplicity of partially-overlapping regulatory elements, and ontogenetic processes of exploration and selective stabilization. The novel variants result from the **developmental plasticity** of the system, which responds to environmental and genetic-mutational challenges by system-reorganization. Following the challenge, the organism responds by recruiting coping mechanisms that include developmental exploration and improvisation, with adaptive genetic changes following rather than preceding the accommodated phenotypic adaptations [20,21]. Novel, and adaptive HVTs have to become developmentally canalized to have high phenotypic transmission fidelity. Whatever the fidelity of their transmission, the heritable inputs into an HVT may be favored or suppressed by natural selection and can affect evolutionary change in populations [22].

Since the transmissibility of an HVT is variable and is likely to evolve, both the transmissibility and the covariance of the trait value with fitness can be decomposed into genetic, epigenetic, and cultural inputs and transmissibilities, as Day and Bonduriansky have shown by using the Price equation [14]. Such decomposition can also be made when there is more than one level of selection (for example, selection of both individuals and

[2] By genetic networks we mean networks defined by the DNA sequence variations of the contributing genetic elements (with the idealized assumption that everything else is equal). From a developmental perspective it is more accurate to refer to cellular networks (which include all inputs at the cell-level).

groups [18]). The overall transmissibility can be equal to, or greater or smaller than the sum of the transmissibilities of each inheritance system, and when this is the case it indicates that the relationships between the systems are non-additive. Comparative studies of organisms in different conditions could provide information about the way in which the transmissibility bias can evolve.

Uller and Helanterä [17] point to two additional approaches to inclusive inheritance that decompose the inheritable inputs in ways that take a statistical rather than a mechanistic approach. The first is based on quantitative models, with phenotypic heritable variance decomposed into distinct (though interacting) genetic, non-genetic (e.g., epigenetic, cultural), and niche-constructed environmental variances that, when combined, account for heritable phenotypic variance [23,24].

A second approach treats inheritance systems as special types of communication systems that lead to the transmission of information (which is measured as the number of decodable variant states in a source). Information can be decomposed into variations that are transmitted through different channels between senders and receivers. Since information is substrate neutral, different types of informational inputs can be distinguished, added, subtracted, and so on [25,26].

In real biological systems there are interactions between changes in selection and in transmission biases. For example, the developmental effects of organisms' behavior on their selective environment can alter both selection and transmission bias. Selection regimes are not independent of the organism's actions but are the outcome of the niche-constructing activities of individuals and communities, with ecological legacies being transmitted between generations [27]. Under some conditions, when the constructed ecological legacies are similar between generations, they confer stability on the developmental reconstruction of the HVT, and the fidelity of transmission is increased (the pollinating activities of butterflies on their food is an example of such an effect). When a change in stability is beneficial, genetic, epigenetic or cultural accommodation may occur [2,20]. Therefore, the interactions between selection and transmission bias needs to be built into an extended Price equation.

As we see it, the quantitative and informational models, which are statistical, provide data that qualify the overall transmission bias described by the Price equation, and specify what type of heritable variation is being selected. They can therefore lead to mechanistic insights. For example, extended models of quantitative genetics describing the quantitative contribution of epigenetic variations must include parameters such as induction coefficients and fidelity of transmission following reset [23] and therefore point to the mechanisms of developmental reprogramming ("forgetting") that occurs during gametogenesis and early embryogenesis. Models that include such parameters can also point to the potential synergetic effects of different epigenetic mechanisms in the reconstruction of an epigenetic mark [28,29]. Such considerations can contribute to a more informed systemic integration of the different inheritance systems within an explicit, detailed model of the type depicted in Figure 1. It can also open up new questions about the mechanisms involved in transmissibility, which underlie the developmental construction of HVTs, and unravel the diverse and subtle effects of development on evolution [30]. This developmental systems approach also highlights the shared adaptive dynamics of learning and Darwinian evolution [31], leads to an extension of the concept of heredity, and is the basis of an updated, extended and integrated evolutionary theory [2,6,15,17,24,32,33].

Conclusions and future directions

- From a developmental systems perspective, the inheritance of phenotypes can be described in terms of developmental re-production. Heritably varying traits (HVTs), which are embedded within the individual's life cycle, are the products of integrated inputs contributed by inheritance systems of different types and at different levels. The level at which the HVT (e.g., the cardiac rhythm of a heart) is described determines which inputs, at which levels, are relevant for its analysis.
- Interactions among different levels of organization can be described using an extended Waddingtonian landscape model. A particular HVT is the outcome of self-sustaining processes that construct an attractor. The HVT at the focal level (e.g., physiological level) can be far more stable than the elements constructing it.
- An integrated view of heredity is captured by extensions of the Price equation. In spite of the integrated nature of the HVT, it is possible to estimate the relative contribution of different inheritance systems by decomposing the inputs into genetic, epigenetic and behavioral/cultural facets, and estimating their contribution to the HVT's heritability. Similarly, it is possible to describe the contribution of different inheritance systems in terms of information.
- To make the approach more predictive, differential equation models, such as those used in dynamical systems theory, should be developed. This would overcome the limitations of algebraic models, which describe a system at equilibrium.
- The interactions between hereditary variants at each level and for each inheritance system can affect evolutionary change (e.g., the interactions between genetic and epigenetic variants may bias the generation of

selectable genetic changes). The evolution of transmissibility is an important focus for future research.

Conflict of interest statement

None.

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Exosomes, Gemmules, Pangenesis and Darwin

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Abstract.

Charles Darwin accepted Lamarck's ideas on the inheritance of acquired characteristics. He also developed a theory for the mechanism by which changes in the soma might be transmitted to the germline cells, sperm and eggs. He supposed that tiny particles, called gemmules, can move through the body, presumably via the body fluids. To all intents and purposes he was proposing a theory of particle transmission between cells that resembles the exosomes discovered in our time. Exosomes are involved in transgenerational epigenetics, which would help to explain many observed maternal and paternal transgenerational effects in health and disease. Recent research shows that exosomes cross the Weismann Barrier and can convey their cargo via sperm, so the mechanism of transgenerationl transmission of effects does exist.

Keywords: exosomes in transgenerational inheritance, gemmules, Charles Darwin, pangenesis, inheritance of acquired characteristics.

1. Introduction

This chapter takes the form of a historical note. I will first explain why the recent discovery of exosomes and their importance is strongly compatible with some of the ideas of Charles Darwin as expressed in his books and correspondence. I will then explain why those ideas were deliberately removed from standard evolutionary biology as it developed after Charles Darwin's death in 1882, and through to the formulation of the neo-Darwinist Modern Synthesis during the first half of the twentieth century. Finally, I will comment on current trends in Evolutionary Biology that can be viewed as returning to a modern form of Darwin's more nuanced multi-mechanism position. I will then briefly review modern work showing that the mechanisms for transgenerational transmission via sperm exist and are functional.

2. The Darwinist view

If Charles Darwin were alive today, what would he think of the discovery of exosomes and their importance? What would he say about this book? To answer these questions

we need to understand Darwin's character as a scientist and thinker, and how he reacted to his own doubts about his theories.

2.1 *The Origin of Species*

Darwin was a cautious and slow thinker. When he published his ground-breaking work *The Origin of Species* in 1859 [1], the book was finished in a hurry, partly because Alfred Russel Wallace was hot on his heels with important information supporting the same ideas from his work in the Malay Archipelago. The ideas that Darwin developed into the theory of evolution by natural selection had previously slowly matured through three decades since his famous voyage on *The Beagle* in 1831-1836. For all that period of time he allowed his ideas to mature before publishing them, and even then he was not sure he had fully arrived at his goal.

After finishing *The Origin of Species* Darwin wrote to the geologist Charles Lyell:

> "I suppose that I am a very slow thinker, for you would be surprised at the number of years it took me to see clearly what some of the problems were, which had to be solved — such as the necessity of the principle of divergence of character—the extinction of intermediate varieties on a continuous area with graduated conditions — the double problem of sterile first crosses & sterile hybrids, &c &c —".

One consequence of the sudden rush to publish is that Darwin did not fully acknowledge his predecessors until the third edition of his book. His predecessors even included his grandfather, Erasmus Darwin. So Charles Darwin knew that he was by no means the first to propose a theory of the transformation of species, as evolution was called in his time. When he did get around to acknowledging his predecessors, in the third (1861) edition of his book, he referred to no fewer than 30 of them. He was generous in his selection. Even Aristotle was listed. In the fourth edition (1866) the list had grown to 38. Prominent in this list was Jean-Baptiste Lamarck, of whom Darwin wrote:

> "this justly celebrated naturalist….who upholds the doctrine that all species, including man, are descended from other species."

He had good reason to praise Lamarck. Half a century earlier in his *Philosophie Zoologique* [2] published in 1809, exactly 50 years before Darwin's *Origin*, Lamarck had laid out the reasons for transformationism, which he had to defend against severe critics amongst his scientific colleagues, just as Darwin would have to do. Darwin was greatly helped in this task by Thomas Henry Huxley, 'Darwin's bulldog', who acted as the public face of Darwinism, while Darwin himself worked quietly away at his country home. Lamarck had to fight his battles in the intellectually challenging Parisian culture largely alone against powerful opponents like Georges Cuvier, who wrote an obituary oration that systematically trashed Lamarck's reputation.[1] That oration was read at Lamarck's pauper burial and it was to reverberate like a death knell across time. It was written from a highly biased perspective. Cuvier proposed a form of creationism in which new species were separately created following global catastrophes, and he was

[1] http://www.victorianweb.org/science/science_texts/cuvier/cuvier_on_lamarck.htm

strongly opposed to Lamarck's more radical ideas of evolution as a gradual transformation from one species to another.

A further example of Darwin's caution is found in a letter that he sent to the explorer and natural historian Moritz Wagner in 1876:

> "In my opinion, the greatest error which I have committed, has not been allowing sufficient weight to the direct action of the environment, i.e. food, climate, etc., independently of natural selection."

It is significant that this was written later than the preface to the fourth edition of *The Origin of Species*. His doubts seem to have increased with time, not the reverse. Note that he refers to the *direct* action of the environment, *independently* of natural selection. This is extraordinary, given the strong bias in favour of attributing everything to natural selection in the neo-Darwinist synthesis. This is one, but by no means the only, reason that it would be wrong to view Darwin as a neo-Darwinist.

It is often thought that, apart from both favouring gradual transformation of species, Lamarck and Darwin were poles apart on the question of the processes involved. There are two key defining aspects. First, Lamarck is usually represented as favouring a 'Ladder of Life', a single continuum of increasing complexity, while Darwin is famous for his sketch of the 'Tree of Life', a branching process of differentiation radiating from a common origin (Figure 1). Second, Lamarck is famous (or infamous) for espousing the inheritance of acquired characteristics, while Darwin is famous for his theory of natural selection, a process which, in the neo-Darwinist view, no longer requires the inheritance of acquired characteristics.

2.2 *Trees of Life*

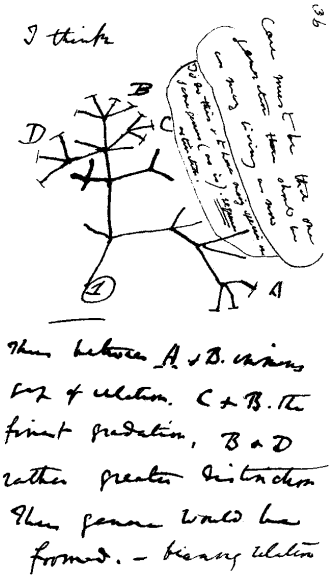

Figure 1. Darwin's first sketch of the evolutionary Tree of Life, from his "B" notebook begun in 1837.

The historical facts do not support this common misunderstanding of the difference between the Lamarckian and Darwinian positions.

First, consider who developed the Ladders and Trees of Life. It is true that, when Lamarck wrote his great work, *Zoologie Philosophique*, published in 1809, he described his idea of a Ladder of Life, moving upwards to increasing complexity through a tendency that he called "le Pouvoir de la Vie". Translated as "the Force of Life" this has been misinterpreted to mean that Lamarck was a vitalist favouring the idea of a "vital force". This is far from the truth. In his introduction to the Flammarion (1994) reprint, the French historian of genetics, André Pichot, wrote [3]:

> "Lamarck's claim that … there is a radical difference between living beings and inanimate objects might lead people to think that he was a vitalist. But he is not. On the contrary, his biology is a mechanistic reply to the physiological vitalism of Bichat, which was then the dominant theory" (my translation of Pichot's French).

But even more importantly, towards the end of the book, in a not very widely known addendum, Lamarck completely changed his mind. His research on worms led him inexorably to the conclusion that what he called 'internal worms' (e.g. tapeworms) and external worms (e.g. earthworms) could not be part of a single ladder of life. Once he had realised that, many other facts fell into place. The outcome is shown in Figure 2: he constructed an absolutely clear Tree of Life, to the extent that was possible from scientific knowledge in 1809. This discovery is 28 years before Darwin's 1837 notebook. Darwin cannot have known of this Table in Lamarck's book.

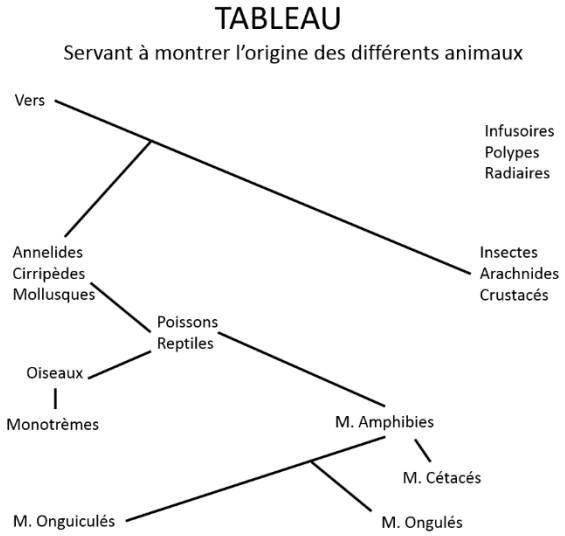

Figure 2. Lamarck's Tree of Life, Redrawn from the addendum to *Philosophie Zoologique*, 1809.

Not only did Lamarck change his theory while writing his 1809 book, he explicitly repeated the branching Tree of Life theory in two later books published in 1815 [4] and 1820 [5]. The 1809 addendum was not therefore just a token passing whim.

Reviewing this aspect of Lamarck's research Stephen J Gould wrote:

> "How can we view his [Lamarck's] slow acknowledgement of logical error, and his willingness to construct an entirely new and contrary explanation, as anything other than a heroic act, worthy of our greatest admiration and identifying Lamarck as one of the finest intellects in the history of biology?" [6]

On the Tree of Life theory Lamarck unambiguously predated Darwin. Now let's turn to the other widely-assumed difference.

2.3 *The inheritance of acquired characteristics*

Why did Darwin write to Moritz Wagner (see above) that he (Darwin) had not given "sufficient weight to the direct action of the environment, i.e. food, climate, etc., independently of natural selection." What influence could the environment exert "independently of natural selection" if it was not to be a form of the inheritance of acquired characteristics? And so, indeed, we find the evidence in *The Origin of Species* itself. In his introduction to Harvard's 1964 republication of *The Origin of Species*, Ernst Mayr wrote:

> "Curiously few evolutionists have noted that, in addition to natural selection, Darwin admits use and disuse as an important evolutionary mechanism. In this he is perfectly clear. For instance,... on page 137 he says that the reduced size of the eyes in moles and other burrowing mammals is 'probably due to gradual reduction from disuse, but aided perhaps by natural selection'. In the case of cave animals, when speaking of the loss of eyes he says, 'I attribute their loss wholly to disuse' (p. 137). On page 455 he begins unequivocally, 'At whatever period of life disuse or selection reduces an organ...' The importance he gives to use or disuse is indicated by the frequency with which he invokes this agent of evolution in the Origin. I find references on pages 11, 43, 134, 135, 136, 137, 447, 454, 455, 472, 479, and 480." [7]

As with Lamarck's Tree of Life in his 1809 book, we can ask whether this was just a passing phase in Darwin's thinking. Many evolutionary biologists have thought that it must have been. This idea is also completely incorrect. Darwin did much more than refer to use and disuse as a mechanism in his 1859 book. In fact he developed it into a theory for how it might happen.

The problem that Darwin saw is that it is not obvious how such a process could happen in multicellular organisms possessing a separate germ line. If changes in the soma occur as a consequence of adaptation to the environment, there is no reason why this should change the germ line cells unless the adaptation is somehow transmitted to them. How for example could an adaptation in a sense organ like the eye, forming part of the soma, have an effect on sperm or egg cells far away in the reproductive organs containing the germ cells? Something would have to transmit the information to the germ cells so that it could be inherited by the next generation. To deal with this problem Darwin invented the idea of gemmules, little particles that he supposed to travel (presumably through the blood stream) to carry the relevant influences. The gemmule theory of what is

usually called pangenesis is found in Darwin's 1868 book *The Variation of Animals and Plants under Domestication*.[8]

Readers of this book on exosomes do not have to go far to identify what might correspond to Darwin's gemmules.

I think therefore that we may answer the question at the beginning of this section of my chapter: Darwin would surely be thrilled to see his idea mirrored by modern discoveries in this way. Of course, discovering exosomes does not itself prove that they are used in the way Darwin envisaged. That question is dealt with in other chapters in this book that present and discuss the recent discoveries on exosomes and, in particular, chapters **** addressing their possible role in inheritance.

3. The Neo-Darwinist (Modern Synthesis) view.

Darwin's work was done with no knowledge of genetics. Mendel's work on the genetics of hybridization in peas was not rediscovered until the late nineteenth century, when the first experiments to test the inheritance of acquired characteristics were also done.

3.1 *Early experiments to test inheritance of acquired characteristics*

August Weismann was the first to do this in 1890 [9]. He decided to do so not by exposing animals, and particularly their embryos, to different environments, but rather by treating them surgically. This fact is crucial.

The experiments consisted in amputating the tails of mice and then observing whether this had any effect on the progeny. The answer was a clear 'no'. Since this work forms a foundation stone of Neo-Darwinism it is important to ask whether it really answers the relevant question. Even on the older versions of Lamarckism, as expressed by Lamarck himself, this is a curiously inappropriate way of testing the idea. The idea is that Lamarckian inheritance may occur in a *functional* interaction between the organisms *and their environment*, through use and disuse of the organism's structures and functions, not whether the *non-functional results of surgery* can be inherited.

Furthermore, Darwin must have known already that such inheritance did not occur from the work of animal breeders. Tail amputation in dogs for aesthetic reasons does not result in stunted tails in the offspring, no matter how many generations are bred from the animals. To put the question in a more modern form, it is whether the germ-line is or is not isolated from environmental influences. The relevant way to do a tail-cutting experiment or any other experiment to answer that question would be to change the environment in a way that makes tail-lessness a functional advantage. Quite apart from the obvious question why a surgical change should be inherited, even a standard Lamarckian would notice that the environment, apart from the surgery, is not different. Furthermore, even if there were environments that would favour tail-lessness the experiment would not test for that.

The work of Conrad Waddington in the 1950s showed the more successful way forward for such experiments, which is to investigate environmental effects on embryos. His

classic 1957 book *The Strategy of the Genes* [10] contains his experimental work showing that acquired characteristics can be assimilated into the genome after just a dozen or so generations of exposing embryos to environmental change.

Nevertheless, the tail-cutting experiment convinced Weismann and others that Lamarckism is impossible. The Weismann Barrier, i.e. the isolation of the germ cells from the soma, then became a cornerstone of the development of Neo-Darwinism into the Modern Synthesis. To quote Weismann directly:

> "In my opinion this [the hereditary substance] can only be the substance of the germ cells; and this substance transfers its hereditary tendencies from generation to generation, at first unchanged, and always uninfluenced in any corresponding manner, by that which happens during the life of the individual which bears it. If these views . . . be correct, all our ideas upon the transformation of species by means of exercise (use and disuse), as proposed by Lamarck, and accepted in some cases by Darwin, entirely collapses." (1883 Lecture *On Inheritance*)

Weismann was therefore fully aware of Darwin's acceptance of the inheritance of acquired characteristics as part of evolutionary theory.

It is curious, to say the least, that such a distinguished and widely praised scientist should have put up such an inappropriate Straw Man to knock down, and even more curious that so many other eminent scientists should have accepted it as the basis of a major evolutionary theory. No major publishable science today could be based on such flimsy and inappropriate evidence. Why did Weismann and his successors do that?

In Weismann's case, part of the answer lies in the little-known fact that he fully acknowledged that his experiments disproved only the inheritance of surgical mutilation. He performed these experiments because he was aware of 'Lamarckian' claims that such mutilations could be inherited. The Straw Man had already been set up by over-imaginative Lamarckians, who had claimed, for example, that repeated circumcision in the generations of populations that practice this particular surgery, could lead to babies born without foreskins. He well knew the limitations of his experiments and that their crudeness was responding to a similarly crude and incorrect alternative.

The answer to this puzzle is that Weismann was *already* convinced of the correctness of his other assumption, the randomness of variations. In the same 1883 lecture (before his tail-cutting experiments) he rejected the inheritance of acquired characteristics and proposed alternative explanations for the use and disuse examples Darwin gave in the *Origin*. But showing that there are alternative explanations for Darwin's examples does not prove that they are the correct explanations. So far as I am aware, the tail-cutting experiments Weismann performed are the only direct experimental tests.

3.2 *Randomness of mutations*

Weismann is also credited with the idea, which he developed in his *Essays upon Heredity* [11] as early as 1889, that changes in the germ line cells were largely random, which,

like the Weismann Barrier, also became a kind of dogma. He was therefore responsible for the two main assumptions of the Modern Synthesis and it is not surprising that he is often judged to be the most important evolutionary biology thinker forming the link between Darwin and the formulation of the Modern Synthesis in the 1930s and 1940s. Ernst Mayr, author of the magisterial 1982 book *The Growth of Biological Thought* [12], described him as "one of the great biologists of all time."

3.3 *Is the Weismann Barrier now "embodied by the Central Dogma of Molecular Biology"?*

We now know that the Weismann Barrier is not absolute. It can be bypassed in many ways. This is how maternal and paternal effects are transmitted across generations [13] and it is now an important question to what extent exosomes may mediate such transmission. Evolutionary biologists have therefore known for many years that a theory based on absolute isolation of the germline cannot be correct.

So, why was neo-Darwinism not abandoned when these effects became known during the later part of the twentieth century? After all, the main reason for distinguishing neo-Darwinism from Darwin's own theories was the exclusion of the inheritance of acquired characteristics. This was the very essence of the distinction, and it was also supported by Alfred Russel Wallace who, like Weismann, took a hard line on this issue.

The issue was never debated in Darwin's lifetime since Darwin died in 1882, just a year before Weismann's seminal 1883 lecture.

This realisation that the cellular barrier is permeable led to a subtle shift in the Barrier concept. Instead of defending the supposed absolute nature of the Weismann Barrier, some neo-Darwinist theorists proposed that it had become embodied in the Central Dogma of Molecular Biology, which is the fact that sequences of nucleotides can specify sequences of amino acids, but change in sequences of amino acids cannot be used to determine changes in nucleotide sequences.

The idea that the Weismann Barrier is now "embodied by the central dogma" is widespread. It even appears on the Simple Wikipedia entry on the central dogma: "The dogma is a modern version of the Weismann barrier"[2].

The statement on the main Wikipedia page is more circumspect: "This [the Weismann Barrier] does not predict the central dogma, but does anticipate its gene-centric view of life, albeit in non-molecular terms." The grossly misleading statement on Simple Wikipedia is repeated on a website designed for schoolchildren.[3]

This confusion is unfortunate. The difference between germ line cells and the genome is fundamental. The cell contains much more than its genome. In most of life on earth, it is the complete organism. Moreover, it can be shown that the information content of the rest of the cell matches that of the genome [14]. So how did the idea that the barrier could be embodied in the dogma come about?

[2] https://simple.wikipedia.org/wiki/Central_dogma_of_molecular_biology
[3] https://wiki.kidzsearch.com/wiki/Central_dogma_of_molecular_biology

3.4 *Shifting definition of a gene*

This development is explained by a shift in the definition of a gene. When Johannsen first introduced the word in 1909, it was defined as an inheritable phenotype characteristic [15]. This was also essentially Mendel's concept. Their concept of a gene would therefore have included anything that went through the germline *cells*. Johannsen made this clear when he explained that the gene could be anything (*ein etwas*) in the organism that was responsible for inheritance of the characteristic. Had he known of them, RNAs, and epigenetic marks on DNAs and histones, would have been included, as would cellular structures that make the replication and inheritance possible. This is clearly not the modern molecular biological definition of a gene, which is restricted to a DNA sequence forming a template for a protein [16, 17].

This major shift in definition was not known at the time the Modern Synthesis was formulated. DNA was not even known to be the genetic material, so it is not surprising that the shift in definition did not matter to those who formulated the Modern Synthesis. The great advances in, for example, the mathematical theories of population genetics [18] worked perfectly well with the gene being defined as a phenotype characteristic. In fact, for most applications of genetics to the social sciences, such as economics and sociology, retaining the phenotype definition is important and even necessary.

But, to return to the definition of a gene, the difference between phenotype and genotype definitions matters enormously to versions of neo-Darwinism, such as selfish gene theory, based on distinguishing the replicator (regarded as DNA) from the vehicle (the phenotype) [19].

There are two fatal difficulties in the *selfish gene* version of neo-Darwinism. The first is that, from a physiological viewpoint, it doesn't lead to a testable prediction. The problem is that the central definition of selfish gene theory is not independent of the only experimental test of the theory, which is whether genes, defined as DNA sequences, are in fact selfish, i.e., whether their frequency in the gene pool increases [20]. The second difficulty is that DNA can't be regarded as a replicator separate from the cell [16]. The cell, and specifically its living physiological functionality, is what enables DNA to be replicated faithfully.

This difficulty leads to the next error in the development toward the Central Dogma, since the fact that the cell, not its DNA, is the real replicator is fundamental. This is the core issue so I will now explain how the development toward the Central Dogma got this part of the story wrong.

When the Central Dogma was first formulated, Watson and Crick acknowledged their indebtedness to Schrödinger's famous book *What is Life?* [21]. In that book, Schrödinger made two predictions, one of which was spectacularly successful, the other is necessarily incorrect [22](p. 176–181).

The correct prediction was that the genetic material would be found to be what he called an aperiodic crystal. If one allows a polymer to be regarded as a kind of crystal, this is a good description of DNA.

The incorrect prediction was that the molecule would behave like a determinate crystal. This idea leads directly to strong interpretations of the Central Dogma that attribute faithful replication and determinate qualities to DNA alone.

If you compare the DNA in a daughter cell with that of its parent cell, you will indeed find a copying error rate of less than one base pair in a complete genome. The error rate is around only 1 in 10^{10} base pairs, which is remarkably accurate. But now suppose that we could compare the DNA sequence *immediately after being copied*. We would find an error rate of around 1 in 10^4, which in a genome of 3 billion base pairs would mean millions of errors. No eukaryotic cell could survive such an error rate. Schrodinger was therefore wrong about the molecule itself behaving like a determinate crystal. Crystal structure growth is indeed determinate, but that is the wrong metaphor for DNA. That is not how it grows and replicates. On its own, it would be left with millions of errors.

It replicates accurately only in a complete cell containing all the objective functionality that enable cells to be alive. Cells achieve this accurate outcome in DNA copying through a very complex three-stage process in which the millions of errors are detected and corrected. The famed 'immortality' of the genome is actually a function of the complete cell, not of the genome alone.

Moreover the fact that the error-correcting process is under control by the cell, not by the genome itself, leads to the genome being much more than a "ready-only" database. Varying the efficiency of error correction, and allowing insertions and deletions, enables organisms to perform what James Shapiro calls Natural Genetic Engineering [23-25].

4. The New Trends view

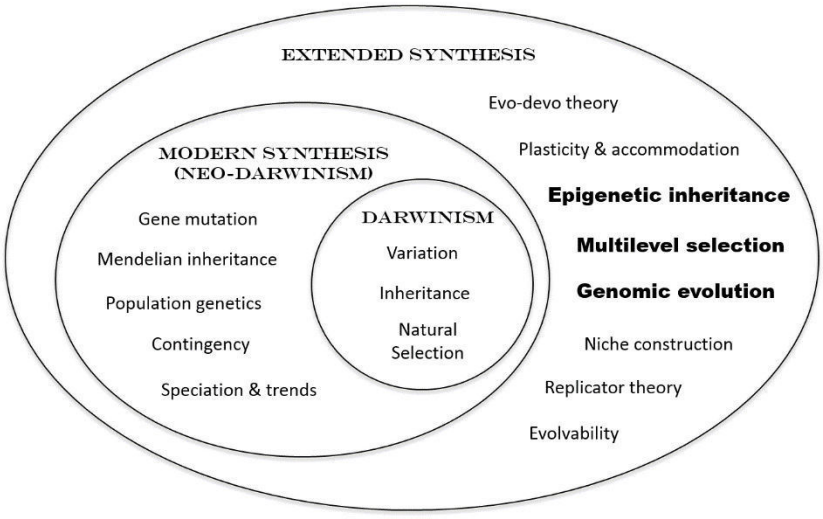

Figure 3. The Extended Evolutionary Synthesis. Each extension includes all that went before. Based on Pigliucci & Mueller [26]

There is now considerable debate about whether neo-Darwinism needs fine-tuning, extending, or perhaps abandoned altogether. Before I try to answer that question I want to acknowledge the fact that the neo-Darwinist modern Synthesis was very useful. Whole fields of mathematical biology, such as population genetics, would probably not have flourished in the twentieth century without the modern synthesis as a framework. But, I also think that we have reached a watershed in relation to the issue of the utility of the neo-Darwinist modern synthesis. As I have argued in detail elsewhere [22], there are too many experimental breaks with the original theory as formulated by Weismann & Wallace. The time has come to see that evolutionary biology would progress faster if we used a different framework to develop a more inclusive theory, as illustrated in figures 3 and 4. Figure 3 shows the Extended Evolutionary Synthesis, EES (Pigliucci & Mueller, [26]).

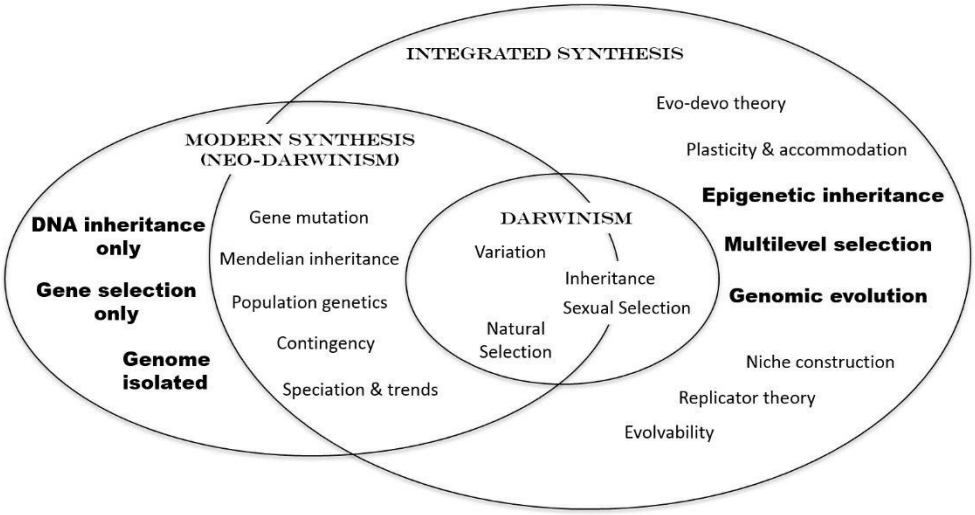

Figure 4. The Integrated Evolutionary Synthesis. Darwinism no longer completely contained within neo-Darwinism, but is contained within the integrated synthesis. From Noble 2017 [27]

Figure 4 shows the version of this diagram that I think better represents the conclusions of this chapter. There are several important differences. First, it represents the fact that Darwin's view of inheritance included the inheritance of acquired characteristics, which was excluded by neo-Darwinism. Darwin's concept of inheritance is therefore shown as being partly outside the neo-Darwinist modern synthesis. So also is his ideas on sexual selection [28]. Second, it represents the features of the extended synthesis (highlighted in bold in both figures 3 and 4) that lie outside the range of neo-Darwinism as defined by Weismann and Wallace. The features of that theory that were excluded are shown as corresponding bold-face items. The highlighted items on the far left correspond with the highlighted items at the far right. Sexual selection is also included as overlapping Darwinism and Integrated Synthesis (see ref [29]).

As Figure 4 makes clear, the integrated synthesis flows more naturally as extensions of Darwin's ideas than as extensions of neo-Darwinism. I believe that a key field of experimental facts that will enable us to more accurately decide on a more inclusive theory is that of exosome research, i.e. the focus of this book. The field is at the very frontier of questions concerning the fundamentals of modern biology, which is why this

book will command widespread attention. I therefore finish this chapter with a brief review of modern work on exosomes and transmission of DNA and microRNA by sperm cells.

5. Modern work on trans-generational role of exosomes and microRNAs

The reason for the great interest in this possible role for exosomes is that any transmission of exosome RNAs, DNAs, protein transcription factors, etc. via the germline would cross the Weismann Barrier. Furthermore, the transmission of a *pattern* of such molecules will represent, at least in part, the state of genome regulation by the cells from which the exosomes come. Smith and Spadafora reviewed this field in 2005 and concluded:

> "It is now well established that spermatozoa can play a role in transgenesis in virtually all species. ……..the underlying mechanism of SMGT [Sperm Mediated Gene Transfer] is best viewed essentially as a retrotransposition-mediated process. Inasmuch as sperm cells are vectors of exogenous genetic information, there is little doubt that they have the potential to cause both genetic and phenotypic modification in individuals of a variety of species and are worthy of further methodological investigation for optimal use in biotechnology." [30]

A more recent review by Spadafora reinforces this conclusion:

> "Epigenetics is increasingly regarded as a potential contributing factor to evolution. Building on apparently unrelated results, here I propose that RNA-containing nanovesicles, predominantly small regulatory RNAs, are released from somatic tissues in the bloodstream, cross the Weismann barrier, reach the epididymis, and are eventually taken up by spermatozoa; henceforth the information is delivered to oocytes at fertilization."[31]

Lavitrano et al [32] have demonstrated that SMGT can be used to generate transgenic pigs, leading to the possible development of multigene transgenic animals suitable for organ transplantation to humans. Similar methods had previously been demonstrated in mice [33]. In humans, the expression profiles of RNAs in exosomes from seminal fluid can be used as a marker for mechanisms of male infertility [34, 35].

That the microRNAs transmitted by exosomes can be functional when transferred between different species has been shown by Valadi et al, [36] who found that "After transfer of mouse exosomal RNA to human mast cells, new mouse proteins were found in the recipient cells, indicating that transferred exosomal mRNA can be translated after entering another cell."

This is a rapidly developing field. We have probably not heard the last from Darwin's gemmules idea. Nor have the echoes of Lamarck died away.

Acknowledgements. Parts of this chapter draw on some of my recent publications, which are included in the references.

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A-mergence of biological systems

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Summary

We argue that (1) emergent phenomena are real and important, (2) for many of these, causality in their development and maintenance is necessarily circular, (3) the circularity occurs between levels of organization, (4) although the forms of causation can be different at different levels, there is no privileged level of causation a priori: the forms and roles of causation are open to experimental investigation, (5) the upward and downward forms of causation do not occur in sequence, they occur in parallel, i.e. simultaneously, (6) there is therefore no privileged direction of emergence, the upper levels constrain the events at the lower levels just as much as the lower levels are necessary for those upper level constraints to exist, (7) to emphasise this point, we introduce the concept of a-mergence, which expresses the lack of causal directionality. We illustrate these points with a major test case: Schrödinger's distinction between physics and biology in which he proposed that physics is the generation of order from molecular disorder, while biology is the generation of order from molecular order. This characterization of biology is physically impossible. Modern biology has confirmed both that this is impossible and that, on the contrary, organisms harness stochasticity at low levels to generate their functionality. This example shows in fine detail why higher level causality can, in many cases, be seen to be more important than lower level processes. The chapter highlights a number of further examples where a-mergence seems to be a more appropriate way of describing what is happening than emergence.

(1) *Emergent phenomena are real and important*

Biological reductionism can be seen to have originated with Descartes in the seventeenth century, while relying heavily on Newtonian mechanics later in the century, and in later centuries on the mathematical genius of Pierre-Simon Laplace. Descartes laid the foundation by arguing that animals could be regarded as machines in some way comparable to the ingenious hydrostatic robots that had become popular amongst the aristocracy in their gardens. Newtonian mechanics cemented the foundation with the laws of mechanical motion, and Laplace systematised the ideas with his famous statement that a supreme intelligence could use mathematics to predict the future completely, and retrodict the past as well. Everything that has or will happen would be clear to such a being. Descartes even foresaw one of the central ideas of Neo-Darwinism:

> "If one had a proper knowledge of all the parts of the semen of some species of animal in particular, for example of man, one might be able to deduce the whole form and configuration of each of its members from this alone, by means of entirely mathematical and certain arguments, the complete figure and the conformation of its members." (*On the formation of the fetus*)[1]

which is essentially the idea that there is a complete mathematical 'program' there in the semen, prefiguring Jacob and Monod's 'genetic program'. Complete because he writes "from this alone". The causation, on this view, is entirely one way.

It is therefore significant that the first clear statement of the opposite view can be traced back to Descartes' main philosophical opponent. In 1665, just two years after the foundation of The Royal Society, Benedict de Spinoza, working in Holland, was in extensive correspondence with the first Secretary of that Society, Henry Oldenburg, working in London.

Oldenburg had just returned from meeting Spinoza in Holland and had been fascinated by his discussions with him on "the principles of the Cartesian and Baconian philosophies". Spinoza was opposed to the dualism of mind and body espoused by Descartes. This was necessary in Descartes' view of animals as automata since he wished to exclude humans from this view and so attributed their free will to a separate substance, the soul, which could interact with the body. Spinoza was in the process of seeking to publish his great work (The Ethics: *Ethica ordine geometrico demonstrata*) in which he proposes an alternative philosophy. Spinoza did not publish in *Philosophical Transactions*, but this correspondence includes an important letter from Spinoza which could form a text for the systems approach and the concept of Biological Relativity (Noble 2012, Noble 2016). The original letter in Latin is still kept in the Royal Society library. He writes: "every part of nature agrees with the whole, and is associated with all other parts" and "by the association of parts, then, I merely mean that the laws or nature of one part adapt themselves to the laws or nature

[1] The French text reads « Si on connoissoit quelles sont toutes les parties de la semence de quelque espece d'Animal en particulier, par exemple de l'homme, on pourroit déduire de la seul, par des raisons entierement Mathematiques et certaines, toute la figure & conformation de ses membres ; » (de la formation du fœtus, para LXVI p 146)

of another part, so as to cause the least possible inconsistency." He realised therefore some of the problems faced in trying to understand what, today, we would characterise as an open system. An open system is one that freely exchanges energy and matter with its surroundings. By definition, in a closed system each part must be influenced only by rules governing the behaviour of the parts within it. If those parts behave deterministically then the whole must also do so. But when parts of wholes are considered as sub-systems, they are necessarily open in the context of the whole. As we will explain in diagram 1 below, even the equations used to describe the behavior of parts require initial and boundary conditions provided from outside the system. Thus, biological systems are open in relation to their environment.

Spinoza therefore appreciated the difficulty in working from knowledge of minute components to an understanding of the whole:

> "Let us imagine, with your permission, a little worm, living in the blood, able to distinguish by sight the particles of blood, lymph etc, and to reflect on the manner in which each particle, on meeting with another particle, either is repulsed, or communicates a portion of its own motion. This little worm would live in the blood, in the same way as we live in a part of the universe, and would consider each particle of blood, not as a part, but as a whole. He would be unable to determine, how all the parts are modified by the general nature of blood, and are compelled by it to adapt themselves, so as to stand in a fixed relation to one another"[2]

This paragraph could stand even today as a succinct statement of one of the main ideas of Biological Relativity. He doesn't use a mathematical medium to express his idea, but this could be so expressed as the aim to understand how the initial and boundary conditions of a system constrain the parts to produce a particular solution to the differential equations describing their motions. We need then to move to the complete system (with whatever boundary we choose to use to define that) in order even to understand the behavior of the parts.

The essence of Spinoza's argument, to use modern language, is that organisms are open systems. This must be so since they can survive only by exchanging matter and energy with their environment. If an organism, or a part of an organism, is treated as a closed system by experimentally preventing those exchanges, it will become dead. The great majority of biochemical and molecular biological experiments are performed on dead and dying organisms, or their parts, such as cells and molecules. To understand how they operate as complete organisms it is completely necessary to take into account the exchanges of matter and energy with their environment. It is through those interactions that organisms can be alive.

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[2] The Latin text is « Concipiamus iam, si placet, vermiculum in hoc fluido, nempe in sanguine, vivere, qui visu ad discernendas particulas lymphae et chyli etc. valeret, et ratione ad observandum, quomodo unaquaequae particula ex alterius occursu vel resilit, vel partem sui motus alteri communicat. Ille quidem in sanguine, ut nos in hoc universi parte viveret, et unamquamque sanguinis particulam ut totum non vero ut partem consideraret nec scire posset, quomodo partes omnes ab universali natura sanguinis moderantur, et invicem prout universalis natura sanguinis exigit accomodari coguntur ut certa ratione inter se consentiant. »

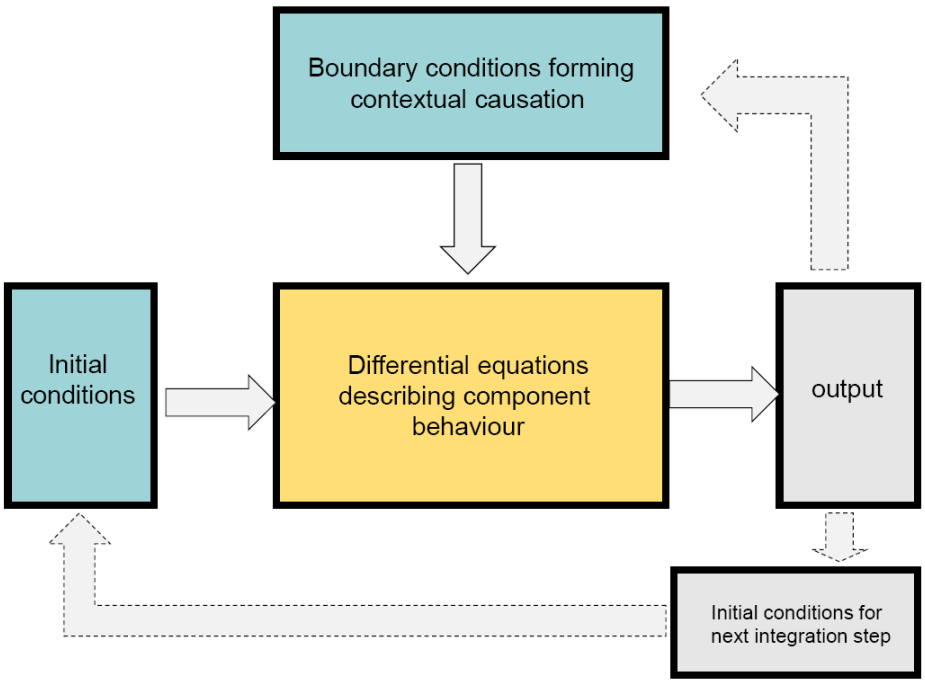

Figure 1 Diagram of causal sequences involved in integrating differential equation models. Description in text. (from Noble(Noble 2012)).

(2) *Causality in the development and maintenance of emergent processes is necessarily circular*

Since the environmental influences arise from a higher level, circular causality must occur, downwards as well as upwards. 'Down' and 'up' here are metaphors and should be treated carefully. The essential point is the more neutral statement: there is no privileged scale of causality, which is the a priori principle of Biological Relativity. One of the consequences of the relativistic view is that genes, defined as DNA sequences, cease to be represented as active causes. They are templates and are passive causes, used when needed to make more proteins or RNAs. Active causation resides in the networks which include many components for which there are no DNA templates. It is the interactive relationships of those dynamic networks which determine what happens. No single component or single mechanism can do so.

This view of organisms can be formalized mathematically as shown in Figure 1. Many models of biological systems consist of differential equations for the kinetics of each component. These equations cannot give a solution (the output) without setting the initial conditions (the state of the components at the time at which the simulation begins) and the boundary conditions. The boundary conditions define what constraints are imposed on the system by its environment and can therefore be considered as a form of contextual causation from a higher scale. This diagram is highly simplified to represent what we actually solve mathematically. In reality, boundary conditions are also involved in determining initial conditions and the output parameters can also influence the boundary conditions, while they in turn are also the initial conditions for a further period of integration of the equations. The arrows are not really unidirectional. The dotted arrows complete the diagram to show that the output contributes to the boundary conditions (although not uniquely), and determines the initial conditions for the next integration step.

Several important conclusions follow from this analysis. First, the equations used in modeling biology cannot even be solved if we do not specify the boundary and initial conditions. Second, those conditions necessarily require causal information about the environment of the system we are modeling. Third, this conclusion is true irrespective of whether we consider the world to be determinate. Even a Laplacian determinist would have to accept this. Recall that Spinoza also was a determinist. We can of course introduce stochasticity into the modeling to produce a non-determinate model. In fact this is necessary to formulate the complete principles of biological relativity (Noble 2016)(chapter 6), but this does not change the essential need for input from the environment of any open system.

(3) *The circularity occurs between levels of organization*

Consider as a concrete example the regularity of the normal heartbeat and how it is disturbed in life-threatening arrhythmias. The normal heartbeat is an attractor caused by a circular form of causality in which both the cell potential and the individual proteins are entrained by their interaction. Once the rhythm begins it can continue indefinitely. Even large perturbations in the individual proteins or their genes can be resisted (Noble 2011). This is precisely what is meant by an attractor. If you represent the parameters as a multidimensional space, there are large volumes within this space representing possible parameter sets, from which the system will automatically move towards the attractor.

Now consider what happens when a different kind of attractor is established. This happens in the heart when abnormal spiral waves of excitation arise at the level of the whole heart. The individual molecules in each cell are now constrained to dance to a different and more chaotic rhythm. Viewed from the level of the individual molecules both of these influences from the higher levels of the cell or the whole organ will seem inexplicable. The molecules are like boats tossed around in a storm beyond their own control. Yet, the storm also depends on their activity. Indeed it can be modeled using the equations for that activity (Carro, Rodríguez et al. 2011). But as explained in the previous section, those equations will necessarily represent the circularity between the causal levels. Each of the three views, the molecular, the cellular and the organ, are valid, but only from the higher levels can we provide a full account of what is happening, including the lower levels whose behavior is in need of explanation. .

(4) *Although the forms of causation can be different at different levels, there is no privileged level of causation a priori: the forms and roles of causation are open to experimental investigation.*

The principle that there is no privileged scale of causality can easily be misinterpreted. It is important now to introduce some clarifications.

First, we must distinguish between its conceptual status and its practical implications. It is an *a priori* statement, i.e. a statement about what we should or should not assume in advance of doing the experiments. We should not assume that causation *necessarily* resides at a particular, e.g. molecular, scale. That is the mistake made by naïve reductionism in biology. The reduction to molecular level events is treated as a

methodological necessity, whereas it should emerge, if at all, from the experiments themselves. Before we do those experiments, we cannot know which parts of a system are involved in its behaviour, nor attribute any privileged position to them.
But that does not mean that all scales must be involved in any given example. The circles of causal networks may span particular ranges of scales, which may be more or less limited in extent. And there may be particular levels that act as important hubs. Those facts are for us to discover as empirical observations. For example, many biologists regard the cell as a central level of integration in much of biology. That conclusion is a result of extensive experiments on cells showing their functional integrity and that many physiological functions cannot be ascribed to entities lower than the cell. Cells contain the main metabolic networks, circadian and various other rhythm networks, cell cycle networks, and so on. Moreover the great majority of living organisms are single cells.

The genome also has a unique position. But it is not the one most often ascribed to it as a program dictating life. As the American cell biochemist Franklin Harold puts it in his book *In Search of Cell History* "The genome is not the cell's command center but a highly privileged databank, something like a recipe or a musical score, yet for the purpose of parsing evolution, genes have a rightful claim to center stage."(Harold 2014). Parsing is the analysis of strings of symbols, usually with guidance from some rules of grammar. In the case of DNA, the start and stop sequences and those for binding transcription factors, amongst other features, provide those guidelines. Analysis of this kind has indeed been exceptionally useful in the inter-species DNA sequence comparisons that now form the basis of much of our understanding of evolutionary history.

The genome sequences are therefore comparable to a formal cause[3] in Aristotle's classification of the forms of causation, while the causation from the networks operating at higher levels than the genome can be regarded as an efficient cause (Noble 2016)(pp. 176-181). The sequences are a formal cause since they form templates to enable ribosomes to construct the proteins specified by those sequences, while those proteins form part of the dynamic networks that form the efficient cause[4] necessary for the attractor to exist. This distinction is particularly clear in the example of cardiac rhythm discussed above. The attractor doesn't even require the involvement of DNA or RNA sequences until the cell requires more proteins to be made. Some other rhythmic attractors do involve DNA sequences in the cycle. Circadian rhythm is a good example. One form of the attractor includes feedback from the level of the protein involved to inhibit the formation of the protein (Hardin, Hall et al. 1990, Foster and Kreitzman 2004). But even in this case, the genome is not completely necessary. So-called 'clock' genes in mice can be knocked out without affecting circadian rhythm (Debruyne, Noton et al. 2006).

[3] A formal cause exists when it is the geometrical arrangement of something that influences the outcome. It is the formal arrangement of nucleotides in sequences that gives the genome the power to determine amino acid sequences in proteins and nucleotide sequences in RNAs.
[4] An efficient cause exists when it is the motion of something that affects the outcome, as in billiard balls colliding. The billiard balls may all have the same form, but their movements are different.

Each feature of organisms at the various levels may therefore have unique causal properties. The principle of Biological Relativity should not be taken to require that all forms of causation involved are equivalent.

(5) *The upward and downward forms of causation do not occur in sequence, they occur in parallel, i.e. simultaneously*

It is important to understand that the processes represented in Figure 1 all occur as a process. It is merely a convenience of representation that the integration step is represented as coming after setting the initial conditions, which then precedes the formation of the output. In a computer program representing the sequence, we do indeed write the code in precisely this sequence. But this is a mathematical fiction arising from the fact that we solve the equations in finite steps. Differential equations themselves do not express finite steps. On the contrary, the differential symbol 'd/dt' represents a vanishingly small step. In reality also, all the processes represented in the equations proceed simultaneously. Our 'difference' equations actually solved by the computer are simply approximations. The test we use for whether they are accurate enough is precisely to reduce the integral step length until the solution converges to an arbitrarily high degree of accuracy. In principle, for infinitely good accuracy, we would have to reduce the step length to zero, which is exactly what differential equations themselves represent. In this respect the equations are better representations of what we are modeling than any particular computer simulation. In the rare cases in non-linear differential equation models where we can solve the equations analytically (Hunter, McNaughton et al. 1975, Jack, Noble et al. 1975), the solution is revealed as a complete solution as a function of time, with no sequence of causation. This is an important reason for which we will introduce the concept of *a*-mergence at the end of this chapter.

(6) *There is therefore no privileged direction of emergence, the upper levels constrain the events at the lower levels just as much as the lower levels are necessary for those upper level constraints to exist.*

It follows that it is simply a matter of convenience that we often talk of the higher level functions arising *from* the interactions of the components. It would be just as correct to say that the constraints on the lower level components arise *from* the existence of the higher level function. Best of all, we should conclude that they necessarily co-arise.

(7) *To emphasise this point, we introduce the concept of a-mergence, which expresses the lack of causal directionality.*

We have developed our argument with examples from cardiac and circadian rhythms. We will now illustrate all these points with a central test case: Schrödinger's distinction between physics and biology in which he proposed that physics is the generation of order from molecular disorder, while biology is the generation of order from molecular order (Schrödinger 1944). This is a central test case because, as both Watson and Crick acknowledged, the formulation of the central dogma of molecular biology was greatly influenced by Schrödinger's ideas. It is also hard to think of a more concrete example where the directionality of causation is widely accepted to be

one way. The Central Dogma has been interpreted to mean that the genome sequences cause the organism but are not themselves affected by the organism. This view has been taken to deny the existence of emergent properties, and it is implicit in versions of evolutionary theory that equate the Central Dogma to the Weismann Barrier or, at least, claim that the Weismann Barrier is now 'embodied by' the Central Dogma. We develop this final section of our argument in four stages.

(a) *It is a mistake to interpret Crick's statement to mean one-way causation*. The relevant statement is:

> "The central dogma of molecular biology deals with the detailed residue-by-residue transfer of sequential information. It states that *such information* cannot be transferred back *from protein* to either protein or nucleic acid."(Crick 1970)

We have italicised '*such information'* and '*from protein*' since it is evident that the statement does not say that *no* information can pass from the *organism* to the genome. In fact, it is obvious that it must do so to produce many different patterns of gene expression, which enable many different phenotypes (e.g many different cell types in the same body) to be generated from the same genome.

This information from organisms is conveyed to their genomes by patterns of transcription factors, genome marking, histone marking, and many RNAs, which in turn control the patterns of gene expression. These controls are exerted through preferential targeted binding to the genome or histone proteins. For example, methylation of cytosines preferentially occurs at CpG sites. Binding to histones preferentially occurs at the histone tails. Even though these are the targeted molecular mechanisms by which the functional control is exerted, there is no guarantee that the functionality will be evident at the molecular level. It would require many correlations between the *patterns* of binding and the functional processes at a higher level to identify the functionality involved. Without that correlation the binding patterns will appear random.[5] Yet it is those patterns that control the expression of individual genes. Those patterns are phenotypes, not themselves genotypes. A good example comes from the study of the evolution of hemoglobins in many avian species to adapt to altitude. Natarajan et al show that "predictable convergence in haemoglobin function has unpredictable molecular underpinnings" (Natarajan, Hoffmann et al. 2016).

That first point establishes that the same genotype can be used to create an effectively unlimited number of phenotypes (Feytmans, Noble et al. 2005). That demonstration does not, however, exhaust the role of the phenotype in determining the functioning of the genome. Not only is it true that the same genotype can be used to generate

[5] The stochasticity is therefore epistemological. In principle, once the higher level constraints are known a bottom-up computation becomes conceivable. However, given the effectively unlimited combinations and the associated requirement for unlimited computer power, it is extremely unlikely that such computations could be successful. And they almost certainly could not be performed without the insights provided by the higher level functional information. As we emphasized earlier, the initial and boundary conditions are essential for the computation to be performed.

many different phenotypes, *it is the phenotype that enables it to be the same (or even a different) genotype*.

(b) *It is the phenotype that enables the genome to be the same genotype.*

If correct, this statement would completely reverse the direction of causality assumed in reductionist explanations of living organisms. How, then, was the currently accepted one way genotype —› phenotype explanation ever thought to be correct? The answer lies in Schrödinger's book *What is Life?*(Schrödinger 1944). That book makes one spectacularly correct prediction and a second necessarily incorrect prediction. The correct prediction was that the genetic material would be found to be what he called a non-periodic crystal. Remember that this was in 1942 before it had been shown that genetic information is found in DNA sequences. If one thinks of a linear polymer as a crystal that does not endlessly repeat itself, then non-periodic (or a-periodic) crystal is quite a good description of what molecular biology subsequently discovered. Remember too that the book was written at a time when X-ray crystallography had come into use to 'read' the molecular structure of organic molecules. This enabled Dorothy Hodgkin to determine the structure of cholesterol in 1937, penicillin in 1946, and vitamin B12 in 1956. These were spectacular achievements. What was more natural than to conclude that if the genetic material is a form of crystal it could also be 'read' in a determinate way? That was indeed the conclusion Schrödinger drew in his book.

But he was too good a physicist not to notice, initially at least, that this conclusion was 'ridiculous':

> "We seem to arrive at the ridiculous conclusion that the clue to understanding of life is that it is based on a pure mechanism, a 'clock-work'…."

'Ridiculous', because how could biological molecules not show the extensive stochasticity that would arise from their possession of kinetic energy?[6] That was precisely why he had, earlier in his book, concluded that physics was the generation of order, e.g. the laws of thermodynamics, from disorder, i.e. molecular level stochasticity.

But he had difficulty harmonizing the two insights. Confusingly, he wrote:

> "The conclusion is not ridiculous and is, in my opinion, not entirely wrong, but it has to be taken 'with a very big grain of salt'".

He then explains the 'big grain of salt' by stating that even clock-work is, 'after all statistical' (p. 103).

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[6] The only way known to modern physics would be for the molecules to form a Bose-Einstein condensate. But molecules can only do this at extremely low temperatures near absolute zero (Whitfield, J. (2003). "Molecules form new state of matter." Nature **doi:10.1038/news031110-16**. Nevertheless, some biologists have speculated along these lines (Ho, M.-w. (2008). The Rainbow and the Worm. The physics of organisms. London, World Scientific Publishing.). Whether or not this happens, it is not needed as an explanation since we already know that the stochasticity is present, even in copying DNA.

Schrödinger clearly realised that something is far from right but was struggling to identify what it might be.

(c) *It is the phenotype that enabled the genome to be a different genotype.*

We would now say that the molecules involved (DNA) *are* subject to frequent statistical variations (copying errors, chemical and radiation damage, etc.), which are then corrected by the cell's protein and lipid machinery that enables DNA to become a highly reproducible molecule. This is a three-stage process that reduces the copy error rate from 1 in 10^4 to around 1 in 10^{10}, which is an astonishing degree of accuracy. In a genome of 3 billion base pairs this works out as less than 1 error in copying a complete genome, compared to millions of errors without error correction. The order at the molecular scale is therefore actually created *by the system as a whole*, including lipid components that are not encoded by DNA sequences. This requires energy, of course, which Schrödinger called negative entropy. Perhaps therefore this is what Schrödinger was struggling towards, but we can only see this clearly in retrospect. He could not have known how much the genetic molecular material experiences stochasticity and is constrained to be highly reproducible *by the organism itself*. The order at the molecular (DNA) level is actually imposed by higher level constraints. If we ever do synthesise from scratch the complete genome of a living organism, we would have to give it this cellular environment in which to function accurately. Otherwise, any genome longer than about 10,000 bases would fail to be preserved reliably at the first copying process.

The Central Dogma was originally formulated by Crick in 1958 (Crick 1958) in a very hard form: DNA —› RNA —› protein. This formulation would have completely protected the genome from alteration of its sequence by the organism. No changes in proteins or their relative expression patterns could conceivably have altered a genome that was isolated in such a way. By 1970 however, the Dogma had to be modified after the discovery of reverse transcriptase (Temin and Mizutani 1970) to become DNA ‹—› RNA —› protein, and even to become:

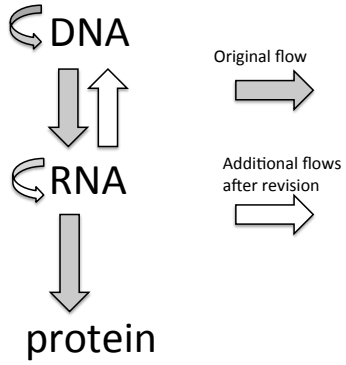

Figure 2. The central Dogma of Molecular Biology

Reverse transcription enables the white upward arrow to occur to allow RNAs to be back-transcribed into DNA, while the upper curved arrow enables DNA sequences to be pasted directly into the genome. The two together completely counter the Central Dogma since they enable sequences of any length to be moved around the genome,

either directly as DNA or indirectly via RNA. Way back in the 1930s and 1940s Barbara McClintock had shown that such transfers do occur in plants in response to environmental stress. This was why, on winning the Nobel prize for mobile genetic elements in 1983, she referred to the genome as a

> "highly sensitive organ of the cell, monitoring genomic activities and correcting common errors, sensing the unusual and unexpected events, and responding to them, often by restructuring the genome." (McClintock 1984)

Did this happen in the evolution of genomes? The answer is yes, it must have done. The evidence comes from the comparative sequencing of genomes from many different species ranging from yeast to man reported in the 2001 *Nature* report on the first full draft of the human genome sequence (International Human Genome Mapping Consortium 2001). The gene sequences for both transcription factor proteins and chromatins show precisely this kind of massive genome re-organisation (Shapiro 2011). Add Shapiro 2014.

This process has also been recorded in real time experiments performed on bacteria evolving in a nutrient medium that does not provide what was an essential metabolite. By periodically allowing conjugation with bacteria that metabolise the new chemical and gradually removing the usual essential metabolite the bacteria succeeded in weaning themselves completely off their usual nutrient. Sequence analysis showed that conjugation had allowed the shuffling of gene domains during the periodic conjugations. Significantly, the authors entitle their article with reference to "directed evolution" (REF Crameri et al 1998)). In a recent article we have shown why this kind of process should be characterised as "directed" since it arises from circular causation that represents a form of organism intelligence (REF). Hosseini et al (2016) have confirmed such findings, which they characterise as "phenotypic innovation through recombination".

(d) *It is the phenotype that <u>enables</u> the genome to be a different genotype*

Notice the small difference in tense compared to statement (c). In this section we ask whether the phenotype can be observed to alter the genome in real time observations on the evolution of cells and organisms.

It is in fact well-known already that cells can harness stochasticity to generate specific function since cells of the immune system show the phenomenon of highly targeted somatic hyper-mutation. Figure 3 summarizes what we know. Faced with a new antigen challenge, the mutation rate in the variable part of the genome can be accelerated by as much as 1 million times. So far as we know, the mutations occur randomly. But the location in the genome is certainly not random. The functionality in this case lies in the precise targeting of the relevant part of the genome. The mechanism is directed, because the binding of the antigen to the antibody itself activates the proliferation process.

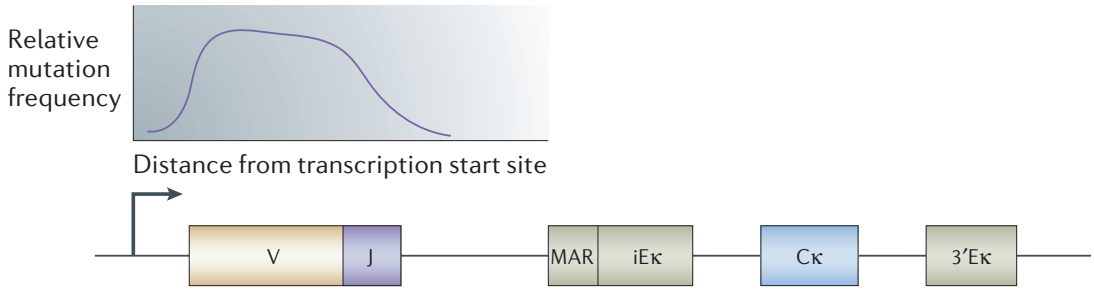

Figure 3. Schematic diagram of gene-specific targeted hyper-mutation in immunoglobulin gene loci. The mutation rate is greatly increased only in the variable part of the genome, which is a ~1.5 kilobase region in each of the three immunoglobulin loci. In this figure, the graph above the rearranged variable (V) and joining (J) gene segments that form the variable region of Igκ depicts the mutation domain in the κ-light chain (Igκ) locus. 3′Eκ, Igκ 3′ enhancer; Cκ, Igκ constant; iEκ, Igκ intronic enhancer; MAR, matrix attachment region (Odegard and Schatz 2006).

This example from the immune system shows that functionally significant targeted hyper-mutation can occur in the lifetime of an individual organism. There is no reason why this kind of mechanism should not be used in evolutionary change, and it is.

A well-known functionally-driven form of genome change is the response to starvation in bacteria. Starvation can increase the targeted reorganizations of the genome by five orders of magnitude, i.e. by a factor of over 100,000. This is one of the mechanisms by which bacteria can evolve very rapidly and in a functional way in response to environmental stress. It would be important to determine whether such targeted reorganization occurs in experiments on conjugating bacteria discussed above. The question would be whether bacteria sensing deprivation trigger higher frequencies of conjugation and shuffling of domains. This “sensing” of the environment, as in the immune system, is precisely what constitutes the feedback necessary for the process to be characterized as directed (see Noble & Noble 2017 for the relevant definitions of agency and directionality in the evolution of organisms and their populations).

A similar targeting of location where genomic change can occur has been found in experiments on genetically modified fruit flies. One of the common ways in which genetic modification is achieved is to use a particular kind of mobile genetic element that can move around the genome using a cut-and-paste mechanism that does not require an RNA intermediate. Most often the insertions occur in a random way. But when DNA sequences from certain regulatory regions are used, they get inserted preferentially near the gene from which the sequence was derived (Bender. W and Hudson 2000). This process targets the changes in a way that is clearly not random with respect to possible function.

There are many more examples in bacterial evolution (Noble 2017).

Conclusions

It will be evident that we have a concern about the word 'emergence'. The 'e' naturally leads to us asking "what emerges *from* what?" Our position makes it obvious that this is often the wrong question. Of course, there may be "emergence" in the sense that, *on a temporal time scale*, atoms emerged from fundamental particles, stars emerged from condensations of matter, life emerged from the formation of suitable planets, and so on. But at each stage a new level of organization takes over. Once an attractor has formed, the components are constrained *by the attractor itself*. The direction of causality then changes. The term 'a-mergence' tries to make that clear. There is no privileged level of causality, in the sense that all levels can be causal. But it is clear from what we have written that the nature of causality changes with the level, and that the higher levels can be said to be directed functionally, and in that sense they are privileged.

Moreover we doubt whether any directionality of causation should be assumed, whether sequential or in parallel. Once an attractor has formed, the best description would be to say that this condition of the system is followed by that condition. There is no need to isolate any components, at any level, as the primary cause. The condition is the a-mergent state and this condition is causative. Moreover, the organization of the state is precisely what defines the level at which it can be said to occur. Thus we refer to atomic, molecular, cellular, tissue, organ, organism, niche, habitat etc, each with a dynamic of causative, functional (goal-directed) organization.

Does the concept of goal-directed processes lead to a better understanding? It is difficult to understand causality without knowing this logic. We understand 'the function' of a thermostat better by understanding that it operates TO maintain temperature within a certain range - it is the logic of the thermostatic system. We understand better the function of baroreceptors in the circulatory system by knowing they are part of a system to maintain pressure within a given range and facilitate blood flow round the body - it is the logic of the system. Knowing or understanding the logic of a system is as important as any reductionist detail about the system. It is an organisational logic - no one part of the system has primacy in that logic. How each part behaves is influenced by its arrangement within the system. It is the situational logic of the system.

The thoughts and discussion we have had in writing this chapter are part of the dynamic organisation at a social level, where action can be identified not only as purposive but also intentional. It is only at this level that behaviour can be described, for example as 'selfish' or 'altruistic', as only at this level can there be reasoned choice, and reasoned logic, or where we can talk of motivation and emotion, hopes, desires, fears and anxieties.

Science by method will hold variables constant to study the effect of changing a given one – clamping a voltage for example. What we know is that this is artificial and establishes an artificially fixed sequence of events. The a-mergent state is in continuous flux, but biological processes maintain such states within functional range. Life as an a-mergent state is in this sense autopoietic, or self-maintaining (Maturana and Varela 1980, Luisi 2016)

The conclusions we have drawn in this chapter are firmly based on experimental findings on the ways in which organisms harness stochasticity to generate functionality. Our reinterpretation of Schrödinger's ideas to produce a conclusion diametrically opposite to the one he himself drew, and which has dominated biological science ever since, is clearly based on experimental findings on the mechanism of reproducibility of DNA copying in whole cells, which could not occur without the integrative activity of cells as a whole. While the cardiac rhythm and targeted hypermutation examples are also firmly based on experimental findings, requiring the integrative action of whole cells.

Our conclusions also strongly support the philosophical approaches developed by, for example, Nancy Cartwright and John Dupré. In his book *The Disorder of Things* ((Dupré 1993), p 101) Dupré writes "…causal completeness at one particular level is wholly incredible. By contrast with even the weakest versions of reductionism, the pluralism I have in mind precludes the privileging of any particular level." This statement accurately reflects the metaphysical position adopted in our work, and its empirical basis. Dupré's work focuses on biology. Cartwright ((Cartwright 1999), p 31) comes to very similar conclusions in her study of causality in physics and economics: "….nature is governed in different domains by different systems of laws not necessarily related to each other in any systematic or uniform way; by a patchwork of laws." This nicely expresses our analysis that the constraints exerted by higher level systems on lower level components depend on the rules being followed by the system, not the highly stochastic behavior of the molecular components.

Modern philosophers of science arrived at these conclusions at least twenty years ago on the basis of careful recognition of the significance of experimental work already achieved at that time. But the silo-isation of disciplines has meant that there has been very little cross fertilization back from these philosophical works and the scientific community. The veritable flood of experimental work now appearing (Noble 2017) that makes the conclusions even more convincing may, we hope, now have its impact in the strategy of experimental biological science. It is high time to escape the limited metaphysical straightjackets of purely gene-centric interpretations.

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REVIEW
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Biological Relativity Requires Circular Causality but Not Symmetry of Causation: So, Where, What and When Are the Boundaries?

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Since the Principle of Biological Relativity was formulated and developed there have been many implementations in a wide range of biological fields. The purpose of this article is to assess the status of the applications of the principle and to clarify some misunderstandings. The principle requires circular causality between levels of organization. But the forms of causality are also necessarily different. They contribute in asymmetric ways. Upward causation can be represented by the differential or similar equations describing the mechanics of lower level processes. Downward causation is then best represented as determining initial and boundary conditions. The questions tackled in this article are: (1) where and when do these boundaries exist? and (2) how do they convey the influences between levels? We show that not all boundary conditions arise from higher-level organization. It is important to distinguish those that do from those that don't. Both forms play functional roles in organisms, particularly in their responses to novel challenges. The forms of causation also change according to the levels concerned. These principles are illustrated with specific examples.

Keywords: biological relativity, downward causation, circular causality, entangled causation, boundaries in physiology

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INTRODUCTION

The principle of Biological Relativity is that, *a priori*, i.e., before performing the relevant experiments, there is no privileged level of causality (Noble, 2012). In multi-scale networks of interactions, as found everywhere in organisms, any parts of a network at any level might affect every other part.

The principle is based on mathematical approaches to understanding biological processes. While the differential (or equivalent) equations represent the dynamics of the components of the system, the initial and boundary conditions represent the historical and contextual (environmental) factors without which no specific solutions to the equations would be possible.

The principle has found many applications in physiology and in other fields of biology. This is not surprising since the mathematical point being made is a necessary one, regardless of whether the components are molecular (genes, proteins, and metabolites), networks (at all levels), cells, tissues, organs, or any other kind of component. Moreover, in practice the principle has been applied many times in physiology even before it was formulated as a mathematical principle. All forms of feedback

between levels in biological systems inherently assume the principle. It can therefore be seen as formalizing an idea that has been inherent in physiology, at least since Claude Bernard in the 19th century (Bernard, 1878, 1984; Noble, 2008, 2013), and Walter Cannon in the 20th century (Cannon, 1932) formulated the ideas of homeostasis. Nevertheless, the principle is not limited to the usual interpretations of homeostasis as linear circularity. The regulatory systems in organisms do much more than act like sophisticated thermostats. There are no fixed set-points. There are sets of set-points each of which can vary as the organism seeks to maintain itself. Buiatti and Longo (2013) express this point by using the word homeorhesis in place of homeostasis:

> "Biological objects are, as discussed by Waddington, "homeorhetic," as opposed to homeo-static, in the sense that, during their cycles, they keep changing. Moreover, their onto-phylogenetic path is largely unpredictable, though preserving, as long as possible, the internal coherence of an organism and its relations to the ecosystem. It is unpredictable because of the random effects at each level and of the bio-resonance effects between different levels."

As our article will make clear, the various levels communicate both randomness and order between each other. We agree therefore with Rosen in *Life Itself* (Rosen, 1991, 2000), that it is the *organization of the organism itself* that constrains the component parts, not the other way round. That organization forms the basis of active agency in organisms (Noble and Noble, 2017; Noble, 2018). One of the aims of this article is to interpret the principle of biological relativity in a more radical way.

The principle also raises many other questions. The aim of this paper is to formulate those questions and attempt to resolve them. Foremost amongst those are questions concerning what is meant by a boundary.

As physiologists we might think that question has an obvious answer. Cells have membranes, tissues have surfaces, organs have shapes with anatomical boundaries, the organism has its outer structure, skin. But where are such boundaries of the great systems of the body, the immune, nervous, circulatory, digestive, respiratory, reproductive, and hormonal systems? Merely to ask the question shows that the answer is not obvious. Anatomy is not necessarily the best basis for defining a functional boundary. To varying degrees, the boundaries used in models are somewhat arbitrary. And even when we can identify an anatomical boundary it is not necessarily the mathematical computational boundary.

As an example of the kind of problem we will address consider the problem faced in modeling the electrophysiology of the heart during the 1980s when processes involving changes in ion concentrations were added to the existing equations for the gating of ionic channels (McAllister et al., 1975). Prior to the DiFrancesco-Noble equations (DiFrancesco and Noble, 1985) this had not been done in any systematic way. Yet it was necessary to incorporate changes in K^+ concentration in intercellular spaces to understand how these could make a non-specific cation channel conducting both Na^+ and K^+ behave like a pure K^+ channel. The new model was completely successful in achieving this aim. But that was not possible without changing the boundaries of the model. One of us explained this boundary problem in 2012:

> "The obvious next step was to develop the McAllister–Noble–Tsien model of 1975 to replace i_{K2} by i_f. But that was much easier said than done. It took a full 5 years of development. This was because it was not just a matter of replacing one ionic channel mechanism by another. It also involved modeling global ion concentration changes for the first time in an electrophysiological model of the heart, including the intracellular calcium signaling. Dario and I did that because it was necessary to explore fully what we had discovered. We did not know then that we would be creating the seminal model from which virtually all subsequent cardiac cell models would be developed. There are now over a hundred such models for various parts of the heart and many different species[1]."
>
> Extending biological models is often like tumbling a row of dominoes. Once one has fallen, many others do too. The reason is that all models are necessarily partial representations of reality. The influence of the parts that are not modeled must either be assumed to be negligible or to be represented, invisibly as it were, in the assumed boundary conditions and other fixed parameters of the model. Once one of those boundaries is removed, by extending out to a different boundary, other boundaries become deformed too. In this case, modeling external potassium changes required modeling of the influence of those changes not only on the ion channels already in the model, but also on exchange mechanisms, like Na-K-ATPase (sodium pump) and the Na-Ca exchanger. That, in turn, required the model to extend to modeling internal sodium concentration changes, which in turn required modeling of intracellular calcium changes, which then required modeling of the sarcoplasmic reticulum uptake and release mechanisms. For a year or two it was hard to know where to stop and where to stake out the new boundaries" (Noble et al., 2012) (Page 58).

Even more difficult is the fact that physiological boundaries can be dynamic. When and why they occur are also important questions since it is at boundaries that many of the vital functional processes occur. Recall that the nervous system develops from the embryonic "boundary," the ectoderm, and in single cell organisms the surface membrane can be regarded as its nervous system. Organisms are open systems, so their boundaries are necessarily where much of the action occurs.

DEFINITIONS

Biological Relativity

Biological relativity is the principle that there is, *a priori*, no privileged level of causation. The necessary mathematical basis of the principle was first proposed in 2012 (Noble, 2012) when it was categorized as a "theory." It is better viewed as a principle since it expresses the conceptual point that there is no empirical justification for privileging any particular level.

Upward Causation

Upward causation is the set of processes by which the lower elements in a system interact and produce changes at higher levels. In differential equation models these processes are

[1] www.cellml.org

described by the dynamics represented by the differential equations themselves.

Downward Causation

Downward causation is the set of constraints imposed by the higher levels on the dynamics at lower levels through determining many of the initial and boundary conditions. El-Hani and Queiroz (2005) use the term, "Downward Determination", but they agree that what is involved is something that "can be understood in terms of constraints that the condition of belonging to a system-token of a given kind imposes on the behavior of the components." The sense of cause we are using includes that of determination. We agree that there are different kinds of causation (Noble, 2016) (pp 176–181). Mossio et al. (2013) also emphasize the role of higher level constraints when they refer to "emergent causal powers exerted as constraints, and we claim that biological systems crucially differ from other natural systems in that they realize a closure of constraints."

Initial Conditions

Initial conditions are the initial values of each dynamic element at lower levels. They are determined by the history of development of the system, including stochastic variation as well as previous states of the system. The upward and downward forms of causation interact (**Figure 1**).

Boundary Conditions

Boundary conditions are the conditions attributable to interaction with the environment. In partial differential equation models these conditions are represented by the state of the spatial boundary of the system. In ordinary differential equation simplifications in which spatial changes are assumed to be instantaneous these conditions are represented by the constant coefficients at any moment in time.

Structure

Structure is also a condition that could be regarded as initial or boundary according to the modeling chosen.

Conditioned Causation

Conditioned causation is a state of a system where it would be misleading to attribute causation to any particular element.

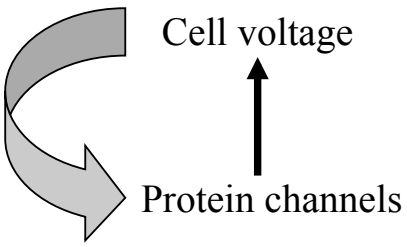

FIGURE 1 | An example of circular causality in physiology. The Hodgkin cycle represents the fact that global cell properties, such as electric potential, control molecular level properties, such as ion channel proteins, which in turn determine changes in cell properties.

MAIN SECTIONS

How Do Upward and Downward Forms of Causation Differ?

The existence of both upward and downward forms of causation is often represented as circular causality. While obviously correct in the sense that both forms exist and, in many ways, must influence each other, such diagrams hide the fact that there is an important difference. The upward and downward forms are necessarily different, just as the initial and boundary conditions of differential equation models are clearly not the differential equations themselves.

It is also important to distinguish conceptual questions about how we see things from what nature does. Nature is a continuum on which we impose somewhat arbitrary boundaries which are dependent on the models we use to understand nature. This point should be borne in mind throughout this article.

Upward Causation

Lower levels influence higher levels through the dynamic changes represented by the differential equations. These will result in global changes, for example in concentrations of ions, metabolites, proteins in cells, tissues and organs and these may in turn trigger further changes at any or all of the higher levels.

As an example, consider the processes involved in calcium movements in the various kinds of muscle in an athlete during vigorous exercise. Too much intracellular free calcium may cause maintained serious problems in the athlete's heart, skeletal muscles or smooth muscles. Any of these, such as a sudden heart attack, may cause severe pain, in turn leading the athlete to collapse. Then the influences become wider and wider as the team coach and physiotherapist enter the scene, which further leads to social interactions. This is an example of unintended effects at a lower level triggering many other events at higher and higher levels.

Downward Causation

Now let's consider how the athlete became an athlete in the first place. He spent hours a day training. This was his decision. It wasn't a decision of the calcium ions in his muscles, nor of the gene sequences in his DNA. Molecules and ions are not causes in that sense (Noble, 2016). It was a high-level choice that he made (Noble and Noble, 2018) and it resulted in many changes in his musculoskeletal, respiratory and cardiovascular systems, all becoming more powerful. Many of these changes came about through exercise influencing gene expression of the proteins in muscles, the lungs and the cardiovascular system. This in turn changes the innumerable boundary and initial conditions under which all the muscles in the athlete's body behave. The changes influence how much muscular, breathing and cardiovascular capacity the athlete has. Although the differential equations for each of his muscle fibers will still be much the same, the changed initial and boundary conditions now ensure that the athlete can do the same or even more vigorous exercise without experiencing disabling fatigue and cramp. This is an undeniable physical effect at the molecular level arising from the athlete's choice of lifestyle.

It doesn't alter the laws of molecular behavior. It alters the solution to the equations for those laws.

Identical Twin Athletes

At this point a rigorous genetic reductionist (Comfort, 2018; Plomin, 2018) might want to argue that no downward causation was involved. The athlete was simply born with the right genes to develop as an athlete. While that must be true – someone suffering from a genetic disease like muscular dystrophy, for example, could not do what the athlete does – it is far from being the complete story. Studies of identical twins who chose very different kinds of sports and exercise training show that very clearly. **Figure 2** is taken from such a study (Keul et al., 1981). The runner and the weightlifter showed completely different effects on their body physique. Bathgate et al. (2018) have recently published a more extensive study of many differences in muscle and cardiovascular health and performance in monozygotic twins. They conclude that "the cardiovascular and skeletal muscle systems exhibit greater plasticity than previously thought." Furthermore they have identified precisely which RNA levels of control are changed by the lifestyle choices.

Genome-Wide Association Studies

Genome sequence studies have failed to find just a single or a very few genes that are strongly correlated with athletic performance. A literature search on publications in the period 1997–2014 showed at least 120 genes show correlations with athletic performance, many of the correlations being very small (Ahmetov and Fedotovskaya, 2015). That number of correlated genes is likely to grow as even more extensive GWAS results appear. So much so that some GWAS scientists have come to the conclusion that virtually the whole genome may be correlated with most phenotypes, the so-called omnigenic hypothesis (Boyle et al., 2017). A study of 1520 endurance athletes and 2760 controls "did not identify a panel of genomic variants common to these elite endurance athlete groups" (Rankinen et al., 2016), and see their earlier studies (Rankinen et al., 2000, 2005). One recent study comparing the impact of genes and environment concluded "that the traditional argument of nature versus nurture is no longer relevant, as it has been clearly established that both are important factors in the road to becoming an elite athlete." (Yan et al., 2016) In a review of elite athletic performance Joyner and Coyle concluded "finding genetic markers that are strongly predictive of either success in endurance athletic performance or somehow preclude it is likely to be a daunting task because of the many cultural and environmental factors that contribute to success in sport, the many physiological factors that interact as determinants of performance, and the heroic nature of the training required" (Joyner and Coyle, 2008).

Epigenetic Control

The main reason for the failure to explain athletic performance from genetics alone is that the genome is controlled by the organism and its life-style experiences through extensive epigenetic control.

As an example, athletes have lower heart rates than non-athletes, which was once attributed to greater vagal tone. The changes have now been traced to microRNAs that downregulate expression of the HCN gene, so that the depolarizing current (I_f) produced in the sinus node cells is reduced by as much as 50% (D'Souza et al., 2017). Moreover, that changes in autonomic tone could not be the explanation was shown as long ago as 1967, but the authors could not at that time identify the mechanism (Sutton et al., 1967). The advent of modern techniques for identifying epigenetic control has transformed this field of study.

The interface between DNA and epigenetic control is therefore another important boundary. It is one of the means by which the organism controls its genome as a "highly sensitive organ of the cell" (McClintock, 1984). This boundary was first identified by Waddington (1957), who was the

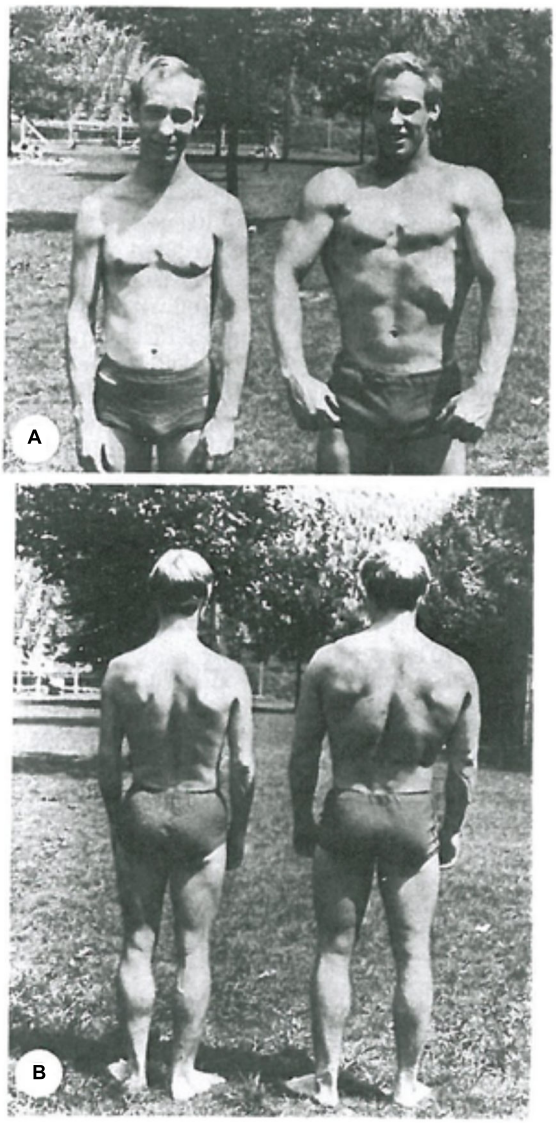

FIGURE 2 | Identical twins. **(A)** Long-distance endurance runner. **(B)** Weightlifter. Notice the highly developed calf muscles in the runner and the contrast with the highly developed arm and chest muscles of the weightlifter. Reproduced with permission from the publisher of Keul et al. (1981).

originator of the term epigenetics. Since then many forms of epigenetic control have been discovered. This control is so effective in transmitting the adaptive properties of the networks that most gene knock-outs have very little effect. The exceptions are, of course, the rare genetic diseases, such as cystic fibrosis, where the networks do not have sufficient plasticity to cope with a knock-out. But, in general, plasticity is common. In yeast, for example, 80% of gene knock-outs are silent in the sense that they produce no phenotypic effect when the yeast is well-nourished (Hillenmeyer et al., 2008). That result has been broadly confirmed by Galardini et al. (2018) who have shown the extent to which the effect of a gene deletion depends on the genetic background. They conclude that "interpretation of the impact of genetic variants on the phenotypes of individuals would likely need detailed gene-phenotype information in more genetic backgrounds than that of a model individual." We would add that the phenotype background must also be relevant. The boundary between regulatory networks and DNA is necessarily a two-way boundary. The regulatory networks can filter genetic changes, acting as what we have characterized as a "cloud" at the boundary (Noble and Noble, 2017; Galardini et al., 2018).

The downward forms of causation represented by the choices made by the individual organism and the influences of its environment must therefore be widespread and necessary.

Open Systems and Their Boundaries

One reason why boundaries are important is that all organisms are open systems. The interaction with the environment is an essential process of being alive. It is across the boundary between the organism and its environment that all the exchanges of energy and matter occur. The same principle applies within the organism. There are boundaries between cell components, between cells, tissues, organs, . . .all the way up. Downward causation can be seen to be traversing a cascade of boundaries. Each level of organization provides the boundary and initial conditions for solutions to the dynamic equations for the level below.

Are All Forms of Downward Causation Functional?

So far, we have established why downward causation is effective and that its necessary effectiveness is mathematically demonstrable. Now let's look at those initial and boundary conditions more carefully. When we inspect the most complete of the mathematical models of skeletal, cardiac and smooth muscles we can identify more than 100 constants in the equations (DiFrancesco and Noble, 1985; Yang et al., 2003; Shorten et al., 2007). Each of those, alone or in combination, reflects an initial or boundary condition. So, there are at least that many parameters that might be sensitive to causative action from higher levels. These parameters are determined by the state of the boundaries between higher and lower levels. In reality there will be many more. The model is just a partial abstraction of reality.

Could all parameter changes in the initial and boundary conditions be attributable to downward causation? There are several reasons why that cannot be true.

The lowest boundary: molecular stochasticity

As Robert Brown showed in 1827, fine particles suspended in water show stochastic movement which was eventually shown by Einstein to be produced by random bombardment by individual water molecules. The molecules in cells are an aqueous suspension and must also be subject to Brownian motion. Water, and all molecules, will also be subject to quantum mechanical randomness. On some interpretations of quantum mechanics, all objects are subject to such randomness (Becker, 2018), although it becomes negligible at a large enough scale.

This is a boundary *within* the system. In a sense it is a boundary between levels or scales. Later in this article we will discuss how organisms use this and other boundaries between levels. But here it is sufficient to note that the boundary is fuzzy. There is no precise cut-off scale at which molecular stochasticity, whether quantal or not, becomes negligible. This is a major issue in the interpretation of quantum mechanics (Becker, 2018), but it need not detain us here. We note that it is a good example of a boundary that cannot be given a precise anatomical location. In a sense the boundary is everywhere. It is a boundary between levels of organization.

Functional and non-functional initial and boundary conditions

Influences on a system from its environment and higher scales can be of at least two kinds. Some will be contingent and even apparently random. These will provide opportunities for novelty in the organism's behavior, in much the same way as we have described in related articles (Noble and Noble, 2017, 2018). Stochasticity can be used by organisms to generate novelty. That can happen whatever the origin of the stochasticity, whether molecular within the organism or environmental without the organism.

But what is usually meant by downward causation are influences that arise from the regulatory *organization* at higher levels. Organization is what defines a level as distinct from a scale. Cellular organization defines the level of cells, organ organization defines the level of organs, and so on through the levels.

What do we mean here by organization? What precisely is homeostasis? Yet again, the common diagrams of upward and downward causation can be misleading. Regulatory processes in the body are rarely simple feedback loops maintaining a specific parameter, like blood pressure or temperature, constant. Nor is the circularity a simple feedback loop that can be described as a linear sequence of causation: A leads to B which leads to C and so on. This way of thinking leads to the need to specify the *direction* of the causation, in turn leading to the idea of emergence, usually interpreted to mean that the higher-level organization emerges *from* the lower level activity. But how can that be? At the lower level we can't even *see* the organization. Low-levels do not possess such organization. The constraints of higher-level organization will be represented by a seemingly disorganized set of initial and boundary conditions. We don't for example "see" the organization of bird haemoglobins as they vary according to different altitudes by sequencing their genomes. At that level, the different species have used different molecular level solutions to evolve haemoglobins for high and low altitudes. At

the functional level, the haemoglobins can be characterized as functional for the altitude at which they live so that all high-altitude birds show higher affinity for oxygen even though the DNA sequences are different (Natarajan et al., 2016). Only at the higher level of organization is the function of the genome changes evident.

We have elaborated on this problem in a previous article (Noble and Noble, 2017). From the molecular level of DNA, RNA, proteins, metabolites, ions etc., we will not be able to see the organization. As we noted earlier, it was not the athlete's calcium ions that caused his decision to be an athlete.

Emergence – a-mergence?

For these reasons, we have argued elsewhere for replacing the term e-mergence (suggesting privileging upward causation) with the neutral term a-mergence (Noble and Noble, 2019). In terms of causation, this requires replacing the linear sequence A causes B which causes C etc., with the existence of the state X, the occurrence of which means that A, B, and C etc., will also occur. This is the characteristic of high-level attractors. Once they occur, they take over the organization of the system. This fact becomes hidden when we insist on a linear causation viewpoint. Yet it is implicit when we solve model differential equations numerically since all factors are taken into account at each integration step. In a cell model we don't, for example, first calculate the influence of all the global cell parameters (such as potentials and concentrations) and then calculate the influence of the microscopic elements (such as transporter and enzyme states) separately.

This issue of simultaneity of action is fundamental. Another way of expressing it is to ask whether circular causality can be said to have a direction. Diagrams often strongly imply that they do, by giving the impression that, if one could be a nano-level observer, one would see one stream of causation running upward and another flowing downward. That picture is far from the reality. This is where the mathematical interpretation of circular causality is so useful in providing a totally different picture of the situation, since the integration procedures must proceed simultaneously (Noble, 2012). A nano-level observer would surely see something more like a cloud of happenings, which would not be resolvable into separate streams of happenings[2].

In this respect, the Biological Relativity interpretation of multi-level causality resembles wave theories of quantum mechanics. Electrons circling a nucleus, for example, are referred to as a cloud because the wave interpretation does not, and cannot, identify where any particular electron may be. The cloud exists as a quantum mechanical state that is precisely and quantitatively described by quantum mechanical wave equations. What matters is the existence of that state, not where any particular electron may be.

Similarly, it is the *state* of a multi-level biological system that matters, not just its breakdown into any particular separate sequences of causation. In any case everything else depends on the existence of the combined state of the system, which is unresolvable into two streams of causation. Not only would there not be two separate streams of causation, what is happening would not be evident in a single slice in time. The attractor or any other organizational property would only be apparent in a phase space representation within which the organizational pattern can be appreciated in an extended time period.

Purely reductionist thinking tends to avoid such language, which is usually criticized as being somehow fuzzy. But it is no more so than quantum mechanics. The analogy is quite close, since the breakdown of an attractor state can be viewed in much the same way as the collapse of a QM wave function. The same criterion for success is also applicable: is the resulting theory empirically predictive? Multi-scale physiological modeling is increasingly successful by this criterion. Vecchi et al. (2018) have introduced the term Entangled Causation to represent their conclusion that "there is no biological rationale for assuming that every switch point should be regulated by a single causal factor and that development generally involves interactive causation in the form of multiple simultaneously contributing difference-making causes to the regulation of the threshold mechanism at every switch point." The resemblance of their conclusions to ours is clear.

Representing organisms as high-level attractors and similarly organized states therefore corresponds much better to what we know experimentally. Most changes at the level of DNA are buffered by the high-level attractors. As Baverstock and Rönkko have shown, the phenotype can best be "represented by high dimensional attractors, evolutionarily conditioned for stability and robustness" (Annila and Baverstock, 2014; Baverstock and Rönkkö, 2014).

Further Physiological examples

We have already used a specific example, that of muscular exercise, to illustrate some of the main points of this article. We will now give further physiological examples. These will illustrate the variety of the forms of boundaries in physiology. It will be through understanding this variety that we will be able to summarize some general principles in See Sections "Delayed differential equations" and "Boundaries between levels: how do they differ?"

Anatomical and functional boundaries in the heart. The heart as an organ has many anatomical boundaries within it since the cells from the sinus node, the atrium, the AV node, the ventricular conducting system, and the ventricle all have different electrophysiological properties, which reflect different protein expression patterns. These in turn are susceptible to different dynamic states within the regulatory networks. The anatomical boundaries between these parts of the heart will therefore experience different magnitudes and direction of ion current flow between them.

These differences also occur within each area. Ventricular cells, for example, differ between epicardial cells and endocardial cells and between the base and apex of the ventricle. These

[2]In any programming of the integration procedure the precise algorithm used depends on the integration formula used. Usually this consists in successive iterations until a preset level of accuracy is achieved. It would not make sense to divide the integration step up into parts. The step itself is just an approximation to an infinitesimally small step. From the viewpoint of this article everything computed in each step can be regarded as an approximation to true simultaneity.

differences are very important in the interpretation of the electrocardiogram. Cells within the sinus node also differ in a graded way. Cells from the periphery have a higher natural frequency than cells near the center.

These differences led to a surprising result when multicellular models of the sinus node became possible, as a result of the increase in computer power offered by the first parallel computers in the 1990s. Using a 64,000 parallel array with each computer processor representing a single cell model, it was found that the origin of the heartbeat, defined as the first cells to depolarize, occurred at the periphery of the model node, creating a wave that spreads inward toward the center (Winslow et al., 1993). This is surprising since in a real heart the beat originates near the center and spreads outward toward the periphery.

The solution to this puzzle was given by the experimental work of Boyett et al. (2003). When the sinus node is carefully separated from the atrium by surgical dissection, the node does indeed behave like the computer model. The sinus-node/atrium boundary is therefore functionally important in creating the conditions in which the beat begins toward the center of the SA node. The high negative resting potentials of the atrial cells together with their high membrane conductance due to high expression of inwardly rectifying potassium channels create the functionality of the complete structure.

Furthermore, the shape of the boundary involved here is not a simple circle or ellipse. The regions of atrial and sinus cells interdigitate in a pattern that enables the weak sinus cells to succeed in depolarizing the stronger atrial cells by almost entirely surrounding cells at the tips of the interdigitations. The impedance-matching process at this boundary is critical in enabling the SA node signal to succeed in spreading through every part and so exciting the whole heart in a functionally important sequence. This functionality is clearly constrained by the high-level geometric structures (Boyett et al., 2003).

Intercellular potassium waves generate oscillatory growth patterns in bacterial films and in vertebrate circulations. Not all bacteria are free swimming single cell organisms. Many form multicellular colonies in the form of films, strings and various matted structures. In their patterns of growth these colonies can behave as intercommunicating networks resembling those of multicellular organisms. Thus, a bacterial film may not grow at a constant speed. It may instead display oscillations in growth rate. These oscillations have been shown to be produced by communications between the cells involving intercellular potassium waves. In effect the cells at the center of the colony are informing those at the periphery when to divide since the release of potassium ions is linked to metabolic activity which in turn enables division to occur (Prindle et al., 2015). Prindle et al. (2015) conclude: "The ensuing "bucket brigade" of potassium release allows cells to rapidly communicate their metabolic state, taking advantage of a link between membrane potential and metabolic activity. This form of electrical communication can thus enhance the previously described long-range metabolic co-dependence in biofilms" (Liu et al., 2015).

Intercellular communication is widespread even in nominally single cell organisms. Potassium wave communication occurs in many organisms, particularly in the circulation in vertebrates, where it is responsible for functionally important phenomena like retrograde vasodilation (Longden et al., 2017). The evolutionary origin of such communication between cells and tissues is clearly very ancient.

Such boundaries can be maladaptive. In the brain, the phenomenon known as spreading depression is due to the generation of a wave of potassium efflux arising principally from glial cells that leads to the depolarization of neurons, resulting in their refractoriness to the nerve impulse with consequent loss of neural activity.

In such forms of communication, the boundaries are fuzzy and distributed. What is a component from some levels may be a boundary at others. Functional boundaries can come and go according to the state of the whole system. Boundaries are themselves therefore interactive. Thus, in the life history of Amoeba *Dictostylium* (?), intercellular boundaries exist at some phases of the cycle and not at others since the organism can function either as an integrated well-ordered colony or as single cells or spores.

Cancer formation and suppression. The standard theory of cancer formation is the somatic mutation theory according to which the accumulation of mutations cause some cells to proliferate abnormally to develop the cancerous tissue. A competing theory is the tissue organization field theory which attributes the cause of cancerous development to properties at a tissue rather than cell or genetic level (Soto and Sonnenschein, 2011). This theory locates the main action at the boundaries between individual cells and the state of the surrounding tissue. A key prediction of this explanation of cancer is that cancers may be "normalized" by changing the boundary, i.e., by transplanting the cancerous or precancerous tissue into normal tissue. This has been shown to happen (Mintz and Ilmensee, 1975; McCullough et al., 1997; Maffini et al., 2005; Kasemeier-Kulesa et al., 2008).

Sponges. All multicellular organisms and colonies of unicellular organisms face the problem of the open boundary requiring exchange with the environment. If the cells are packed too close together some will not be able to exchange nutrients and waste rapidly enough. In See Section ("Intercellular Potassium Waves Generate Oscillatory Growth Patterns in Bacterial Films and in Vertebrate Circulations") above we saw that bacterial colonies solve this problem by signaling when parts of the colony experience metabolic stress. Sponges solve this problem in a different way: the organism is structured using collagen forming open networks of spaces through which freshwater or seawater can flow. Water is wafted through the channels by flagella on the lining of cells, so enabling all cells to exchange freely with the environment. This movement of fluid is the sponge's equivalent of a circulation. There is experimental evidence that this slow-moving aqueous boundary enabled the earliest animal sponges to survive in very low oxygen levels and therefore to evolve before the general oxygenation of the environment around 580 million years ago (Mills et al., 2014).

Delayed differential equations

Equations of this form are sometimes used to represent situations in which there is a significant delay in the action of a part or level of the system on its components (Bocharov and Rihan, 2000). These are important because they also show that chaotic behavior can arise from deterministic equations (Ikeda and Matsumoto, 1987). This form of mathematical representation may seem to contradict our earlier claim of simultaneity of upward and downward causation. That this is not so can be understood by noting that such equations represent an *ordinary* differential equation simplification of any real system, where a full representation would require *partial* differential equations in which the delay would be modeled as a diffusion process in space. This more complete representation would then satisfy the simultaneity condition, with the delay being properly computed in time at each point in space. At each point in space there would be no delay.

Boundaries between levels: how do they differ?

Figure 3 shows the original diagram of multi-level causation (Noble, 2006). The downward arrows were drawn as large and as separate arrows to emphasize the importance of downward causation [see also (Tasaki, 2013)]. These are the forms of causation that constrain the lower levels and which are necessary for an organism to be alive.

However, there are two aspects of this diagram that could be misleading.

First, both the upward and downward forms of causation differ in their details as we move between the levels. We have discussed examples of these differences in the present paper. An important difference that we will highlight here is the difference between the downward forms of causation onto the genome. The arrow between *Protein and DNA Networks* and *Genes* (the smaller left downward arrow) will consist of molecular details concerning the set of transcription factors, regulatory RNAs and methylation by which molecular events at the network level control gene expression. The higher level causation of the same process (right downward arrow) will include properties at the highest levels of the organism that would enable these controls of the genome to be understood functionally, for example why some cells are constrained to produce the patterns of expression for bone cells while others are constrained to become heart cells, albeit from the same genome. Comparable differences occur between the upward arrows. The arrow from *Genes* to *Proteins and RNAs* consists in the transcription and translation machinery of cells. That between *Cells* and *Tissues* consists in the processes that bind cells together to form tissues. The causation at the different levels depends on all the other forms of causation between lower and higher levels. There is a form of nesting of causation, both upward and downward.

Second, as we have already shown, it would be a mistake to think of the upward and downward causations between any levels as sequential, with one occurring before the other. The lesson we learn from representing these forms of causation in mathematical models is that they are necessarily simultaneous.

Figure 4 gives a different representation in which double-headed arrows are used on the left to indicate the simultaneity of action between the different levels. Yet it is still formally correct to say that each of these consists of different kinds of causation. Some will be stochastic, others are ordered constraints. We can therefore imagine these as formally separate lines, as illustrated on the right hand diagram.

The brown colored arrow between DNA and the level of proteins and RNAs is special. The upward influence is a kind of template: genes as DNA sequences act as a template for amino acid sequences in proteins. The downward influences are twofold:

Normal. Influence on expression levels of proteins and RNAs with no change in DNA sequence.

Special. Creation of new DNA by, e.g., the immune system, and other forms of targeted mutations and natural genetic engineering.

Boundaries beyond the organism

Figure 4 also illustrates the fact that, since organisms are open systems, there are necessarily levels above that of the whole organism, extending into the various forms of social interactions and, in the case of humans, the constraints of laws and ethics. Here we simply note that they also introduce different forms of causation, including constraints on behavior exerted by reasons and habits. The blue arrow at the top therefore represents the very different forms of causation that depend on reasons and contextual logic The relations and distinctions between reasons and causes are deep philosophical issues which we do not deal with here. This is part of the reason why we have represented the social and cultural factors involved all together as a single cloud. The diagram does not imply fuzziness or "ghostliness" in the actions on organisms. On the contrary, there is nothing ghostly about the fact that choice of lifestyle affected the muscles of the identical twins in **Figure 2** so differently, nor in the fact that Bathgate et al. (2018) have now identified the specific RNA changes involved at the molecular level.

This is a suitable point to comment on Craver and Bechtel (2007) case against the use of "causation" in top-down influences. Their case is that "the notion of top-down causation is incoherent or that it involves spooky forces exerted by wholes upon their components." We see nothing incoherent in the expression of top-down influences in terms of boundary and initial conditions. Open systems necessarily have boundaries. The forms of causation across those boundaries differ in the two directions, as we have shown and acknowledged throughout this article, but they are nonetheless real. Both forms are mathematically rigorous. As differential equation models show, they are both also necessary. An important clue to the substantial difference between our viewpoints is their statement that "both phrases describe *mechanistically mediated effects*" (their emphasis). We agree that setting boundary conditions is not "mechanistic" in the same sense as the dynamic role of upward causation represented in the differential terms in model equations. Moreover, processes that harness stochasticity are not well represented by the term "mechanistic." It is precisely their non-mechanistic nature that is important.

We are not the first to draw attention to the fact that the causal effects of organization at higher levels are exerted through

the boundary conditions at lower levels. The physical chemist Michael Polanyi made exactly this point as long ago as 1968 (Polanyi, 1968):

> "Therefore, if the structure of living things is a set of boundary conditions, this structure is extraneous to the laws of physics and chemistry which the organism is harnessing. Thus the morphology of living things transcends the laws of physics and chemistry."

Polanyi's article is remarkably close to our use of differential equation models to illustrate the different forms of causation in multi-level interactions. The only aspect of his work that has dated is his complete acceptance of Watson and Crick's Central Dogma. He wrote "the morphogenetic process is explained in principle by the transmission of information stored in DNA." He did not know that organisms can influence DNA sequences (the downward aspect of the brown arrow in **Figure 4**) and that much more than DNA is involved in the morphogenetic process.

It is difficult to represent all of these important theoretical distinctions in a single diagram. **Figures 1**, **3**, **4** in our article should therefore each be taken as partial guides to understanding. They each have their limits in representing the conceptual distinctions we are making.

DISCUSSION

The Questions in Our Title: What, Where and When Are Boundaries?

What?

Our paper shows that there are many kinds of boundaries in and around living organisms. Furthermore they are not usually, or ever, passive. They are an essential ingredient of functionality. The reason is that organisms are open systems, operating far from equilibrium. Boundaries are where many of those non-equilibrium processes take place. We cannot therefore understand the behavior of organisms or their parts from their composition alone, and certainly not from the genome alone. The consequences for physiological research are profound. Isolated components of organisms, whether molecules, cells, tissues or organs, do not necessarily behave in the same was as those components *in situ*. This fact is evident even at the molecular level. Proteins, for example, assume different forms in different environments (Balchin et al., 2016) and so do the processes in which they take part (Garcia-Contreras et al., 2012).

Where?

In answering this question we need to remember that it is we who decide what to study in physiological research, whether whole organisms or their components. The way in which we divide nature up determines where the boundaries lie in modeling systems. Where a boundary exists therefore depends on our choice (see the example of the DiFrancesco-Noble equations cited in the Introduction). These choices are not arbitrary, they depend on what has already been discovered. As an example, before the discovery of the variety of epigenetic controls of the genome, the idea of a boundary between the genome and its control by cellular and higher level processes would not have been conceivable. The discovery of these processes and the relevant boundary has far-reaching consequences for physiological research, including interpretations of the Central Dogma of Molecular Biology and of the Weismann Barrier (Noble, 2018).

Choice of boundary also plays a major role in the way in which multi-scale physiology discovers the relative importance of different molecular components. Examples in this article include how the extensions of heart muscle modeling in the 1980s led to the discovery of the quantitative importance of the sodium-calcium exchanger, and how the importance of this exchanger and its regulation has now been discovered using a similar shift from cell to tissue level modeling in skeletal muscle.

When?

Organisms develop, so many boundaries do not exist in the same way at the earliest, single cell, stages. Furthermore, they may differ in their ingredients from system to system even though achieving similar objectives. Boundaries between levels can obviously only arise when those levels develop.

Clarifications of the Principle of Biological Relativity

Our article clarifies several aspects of the Principle of Biological Relativity.

(1) The forms of causation involved in downward and upward causation are fundamentally different. Downward causation consists in constraints exerted by higher levels on the initial and boundary conditions within which the dynamics of lower level elements operate. By contrast, upward causation is the way in which those dynamics influence higher level states.
(2) These two forms of causation do not form a temporal sequence. They occur simultaneously.
(3) It is the state of organization of a higher level that can constrain lower levels. Causation by a state means that it does not make sense to separate out causation by any one element of the state.
(4) Conditioned causation exists in attractors since any perturbation of the state will be resisted. The strength of an attractor can be measured by the speed with which it re-establishes itself (Kaneko, 1998). The strength of downward causation in organisms is generally high since organisms are very effective at resisting changes in phenotype in response to changes at the molecular level, including changes in DNA sequences. Some authors describe conditioned causation as entangled causation (Vecchi et al., 2018). This is a term borrowed from quantum mechanical theory. The analogy is correct to the extent that the causal states involved should not be separated and the entanglement involved resembles that in quantum mechanical states. But there is also an important difference, which is that entangled states in quantum mechanics are very fragile, collapsing in a fraction of a second, whereas the attractor states in biology are often very robust.

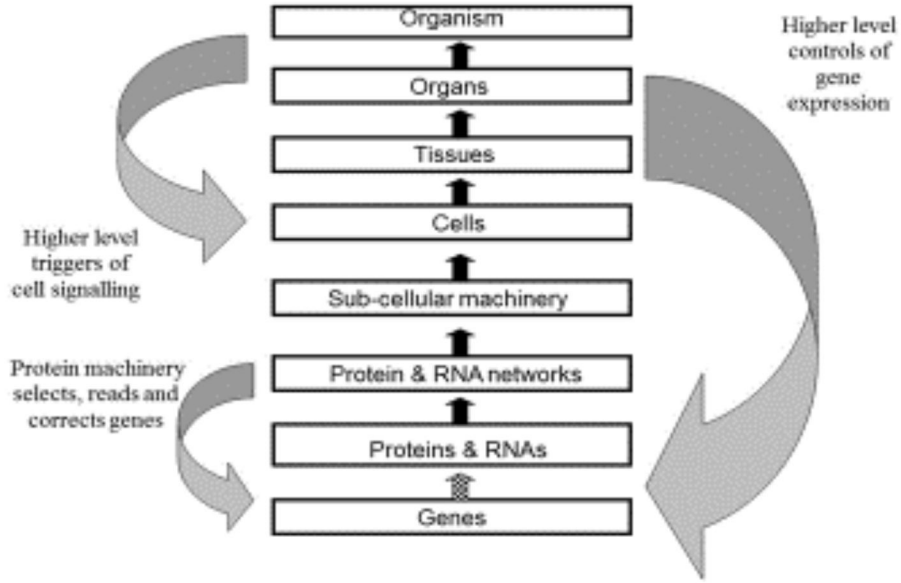

FIGURE 3 | Original multi-level causation diagram illustrating some of the forms of downward causation. Redrawn from Noble (2006), **Figure 2**.

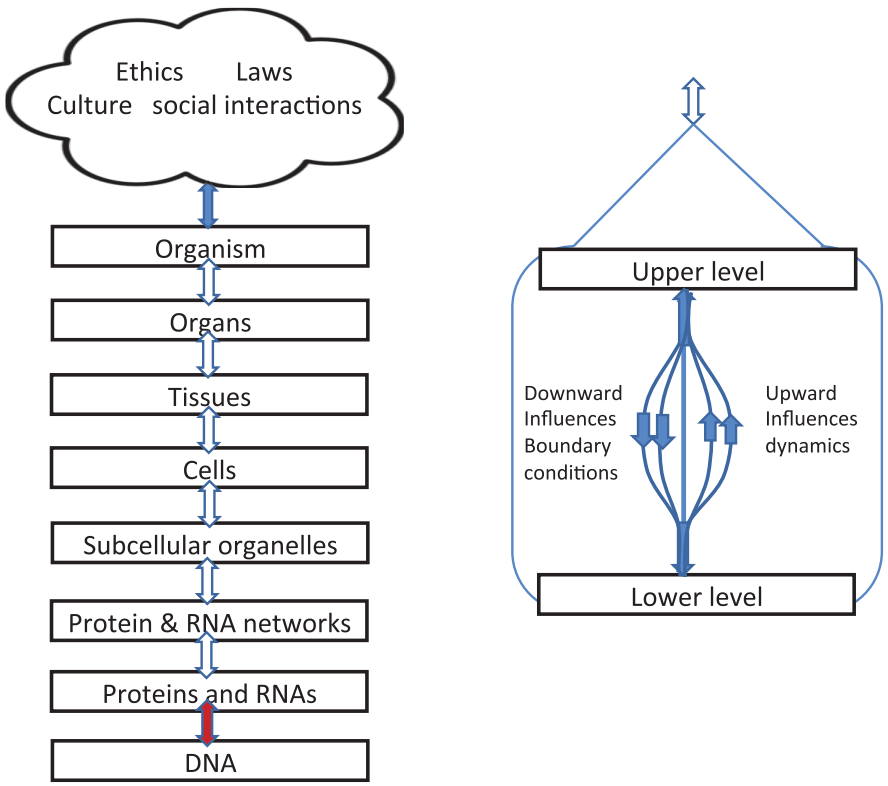

FIGURE 4 | Left: Representation of levels of interaction emphasizing that upward and downward causation operate simultaneously and are shown as double arrows. Right: diagram showing that, within each bidirectional causal arrow, there are different forms of causation, up and down.

Consequences for the Foundations of Physiology

(5) By clarifying the principle of biological relativity, and the nature of the boundaries, multi-level physiology gains rigor. We have not used specific mathematics in this article, nor are many of the points we have discussed primarily mathematical. They are points about the fundamentals of *physiology*. Expressing those fundamentals in terms of arguments drawn from mathematics simply shows that they can, in principle, be as rigorous as any form of science.

(6) What have we not explained? We believe our article opens up many further questions concerning the nature of multi-level physiology. In See Section "Boundaries Beyond the Organism" we have drawn attention to the fact that the causal relations between different levels differ in important ways. One of the most important of these is the increasing role of logic and reasons as we move up to and beyond the level of the whole organism. This is one of the most intractable problems in philosophy and clearly requires more research.

AUTHOR CONTRIBUTIONS

All authors listed have made a substantial, direct and intellectual contribution to the work, and approved it for publication.

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