Studies in History and Philosophy of Biol & Biomed Sci xxx (xxxx) xxxx

Contents lists available at ScienceDirect

Studies in History and Philosophy of Biol & Biomed Sci

journal homepage: www.elsevier.com/locate/shpsc

DNA is not an ontologically distinctive developmental cause

Davide Vecchi

Centro de Filosofia das Ciências, Departamento de História e Filosofia das Ciências, Faculdade de Ciências, Universidade de Lisboa, 1749-016, Lisboa, Portugal

ARTICLE INFO

Keywords:
Development
Causation
Determination
Switch-point
Causal specificity
Causal parity
Robustness

ABSTRACT

In this article I critically evaluate the thesis that DNA is an ontologically distinctive developmental cause. I shall critically analyse different versions of the latter thesis by taking into consideration concrete developmental cases. I shall argue that DNA is neither a developmental determinant nor an ontologically distinctive developmental cause. Instead, I shall argue that mechanistic analysis shows that DNA's causal role in development depends on the higher robustness of the developmental processes in which it exerts its causal capacities. The focus on process and developmental system implies a metaphysical shift: rather than attributing to DNA molecules biochemically unique properties, I suggest that it might be better to think about DNA's causal role in development in terms of the causal capacities that DNA molecules manifest in a rich developmental milieu. I shall also suggest that my position is distinct both from the view advocating the instrumental primacy of DNA-centric biology and developmental constructionism. It is different from the former because it provides a substantial answer to the question of what makes DNA causally central in developmental processes. Finally, I argue that evolutionary considerations pose an important challenge to developmental constructionism.

1. Explaining DNA-centrism

The focus of my analysis shall be the thesis that DNA is an ontologically distinctive developmental cause. I shall briefly illustrate the genesis of this idea and then critically analyse its different versions by taking into consideration a variety of concrete developmental cases. I shall argue that DNA is not an ontologically distinctive developmental cause. The position I defend is distinct from both the view advocating the instrumental primacy of DNA-centric biology and developmental constructionism. The former view states that DNA-centrism is a uniquely powerful investigative strategy for understanding and modelling biological processes. The epistemological and heuristic primacy of DNA-centrism is underpinned by a variety of technologies for the manipulation of and intervention on DNA sequences. This view has been defended in several publications by authors such as Schaffner (1969), Waters (2006) and most recently Esposito (2017). Even though I largely agree with this position, I find it unsatisfactory because it avoids giving an answer to the question of what makes DNA causally central in development. Alternatively, developmental constructionism has strongly highlighted errors in traditional DNA-centric narratives such as genetic determinism. One limit of this approach is that it is not easy to articulate the causal parity thesis on which it is based.[1] If causal parity is the claim that all causes contributing to an organism's ontogeny are on a par, developmental constructionists complain, rightly or wrongly, that a straw man misrepresentation is created. Thus:

> "The real developmentalist position is that the empirical differences between the role of DNA and that of cytoplasmic gradients or host-imprinting events do not justify the metaphysical distinctions currently built upon them." (Griffiths & Knight, 1998, p. 254, p. 254)

Interpreted in this way, developmental constructionism is the thesis that DNA is not ontologically distinctive. As I shall show in the article, several authors have defended exactly the opposite thesis. Interestingly, and in contradiction with the instrumentalist stance defended in other publications, Waters (2007) has defended a similar view tailored to reject generalised causal parity claims, arguing that in specific developmental contexts DNA sequences are ontologically distinctive. Given that my analysis rejects various claims about ontological distinctiveness, and given that the developmental constructionist position denies the ontological distinctiveness of DNA, it seems that I automatically support some form of causal parity. Nevertheless, in section 5 I shall argue that evolutionary considerations pose a particularly important challenge to developmental constructionism. I shall thus suggest that my position is alternative to both the thesis of the instrumental primacy of DNA-centric biology and developmental constructionism.

The general analytic approach endorsed in this article is neo-mechanistic (Bechtel & Richardson, 2010). In particular, the switch-point model of development illustrated in section 2 and adopted throughout

E-mail address: davide.s.vecchi@gmail.com.

[1] I thank an anonymous reviewer for stressing the importance of distinguishing naïve and biologically feasible interpretations of causal parity.

https://doi.org/10.1016/j.shpsc.2019.101245
Received 20 July 2018; Received in revised form 14 December 2019; Accepted 27 December 2019

the analysis is an attempt to provide a biochemically-informed mechanistic interpretation of developmental processes. The ethos of the analysis is that an "injection of molecular detail" (Griffiths & Knight, 1998, p. 253) is instrumental in order to critically evaluate the various ontological distinctiveness claims analysed. This investigative strategy is akin to mechanistic decomposition and I will make ample use of it in this article. However, "injection of molecular detail" is not sufficient for mechanistic analysis. What is also needed are recomposition and situating analyses (Bechtel & Abrahamsen, 2009). Recomposition analysis tries to conceptually assemble how parts and their activities are orchestrated in order to realise the phenomenon under study. Recomposition is challenging because the behaviour of highly integrated dynamical systems often involves transformation of parts, their multiple activities, causal cycles and feed-back.[2] Complementing decomposition and recomposition analyses, situating analysis aims to conceptualise how mechanisms interact between themselves and within the larger cellular, organismal and environmental context. The upshot is that, while mechanistic analysis is partially reductionist because couched in terms of decomposition, it is not merely reductionist as far as it involves recomposing and situating (Bechtel, 2009, p. 559).

The structure of the article is as follows. In section 2 I shall argue that developmental determinants are a biological myth. In section 3 I shall show that DNA cannot be considered an ontologically distinctive developmental cause. In section 4 I show in what sense DNA plays a crucial causal role in development. In section 5 I propose that evolutionary considerations are in tension with causal parity claims.

2. DNA as developmental determinant

Even though August Weismann probably introduced the notion of developmental determinant in the biological literature (Weismann, 1893, p. 134), a central figure in substantiating the same notion was Conrad Hal Waddington (Sarkar, 2005). Already in 1939, Waddington proposed an interpretation of gene action that was deterministic despite the well-known complexity of the genotype-environment relationship. In that period, the central concept in developmental biology was that of organiser, introduced by Hans Spemann in order to account for developmental processes such as cellular differentiation or morphogenesis. The material basis of the organiser was debated as well as the nature of its causal influence. Spemann, for instance, considered organisers irreducible to their biochemical bases and thought that their causal influence was irreducible to that of their constituents (Hamburger, 1999). Conversely, Waddington gave a reductionist as well as a deterministic interpretation of the concept:

> "…the factor which, in the development of vertebrates, decides which of the alternative modes of development shall be followed is the organiser, or, more specifically, the active chemical substance of the organiser which has been called the evocator." (Waddington, 1939, p. S37)

Most importantly for my analysis, the evocator "decides" which developmental path is taken. The idea of epigenetic landscape was already implicit in Waddington's conceptualisation of development at the time: development can be represented as a system of branching paths, that is, as a series of discrete steps or bifurcations with no intermediates between them; evocators "decide" which path is taken by the developing organism at every bifurcation. The last inference in Waddington's argument was that (at least some) genes, also known to be discrete factors, are evocators:

> "The characteristics of each path will depend upon the developmental potencies of the tissue, that is to say, they will be under the control of the genes. We may also expect to find genes which act in a way formally like that of evocators, in that they control the choice of alternatives." (Waddington, 1939, p. S42)

The upshot was that genes, as evocators, "control" developmental stages and are therefore developmental determinants. When, in the 40s and 50s, the material basis of genes was discovered, the idea that DNA is a developmental determinant had somehow already found its conceptual underpinning.

Given this brief historical background, two questions can be now addressed: are developmental determinants just genetic? And do they exist al all in the first place? In order to answer these two questions, let me first provide a representation of the nature of the developmental process based on a refinement – proposed by West-Eberhard (2003) - of Waddington's epigenetic landscape, a pictorial device representing the developmental trajectory of a ball (i.e., the phenotype) that starts at the top (i.e., the pre-developmental condition) and rolls down the slopes of a corrugated terrain, encountering many bifurcation points (i.e., canalising events), eventually reaching the bottom of the slope (i.e., the "adult" phenotype). The first element of this interpretation is that development is a process in which a responsive developmental system (e.g., zygote, blastocyst, gastrula, adult organism) acts as a transducer (i.e., a converter, modulator or "interpreter") of impinging stimuli. This process of transduction can be assimilated to the action of a complex regulatory mechanism. The second element refers to the fact that the process of developmental regulation is structured in a multi-step fashion; namely, it is paved with bifurcations or switch points:

> "Switch-mediated developmental pathways occur at all levels of phenotype organization and in all forms of life, including viruses… Switch points are the organizing points of development." (West-Eberhard, 2003, p. 67, p. 67)

A switch point refers to a point in time when some element of a phenotype changes from a default state or pathway to an alternative one. The third element is that this change is of a particular sort. Phenotypic expression depends on a condition-sensitive and quantitatively variable regulatory mechanism that flip-flops when a threshold is reached. Hence the name switch point: "A switch implies some change in state, for example, between on and off, under certain conditions" (West-Eberhard, 2003, p. 68). The change in state or pathway is therefore abrupt upon reaching the threshold because threshold effects are all-or-none. In this sense, switch points are equivalent to the discrete steps with no intermediates postulated by Waddington. A developmental determinant would be a developmental factor exerting a predominant causal influence at specific switch points by tipping the threshold, causing the change of default state or pathway.[3]

Answering our first question, the switch point model is clearly compatible with the hypothesis that both genomic and environmental inputs can be developmental determinants. The model thus makes sense of the extended use of the language of determination to include environmental determination. Various agents of environmental induction can "determine" phenotypic outcomes, e.g., temperature in the case of sex "determination" in reptiles, concentration of kairomones in *Daphnia*'s helmet formation, physical contact with peers in grasshopper's morphology (Gilbert & Epel, 2009). Consider the mechanisms regulating sex morphogenesis. Sex "determination" is triggered in some cases by genomic and in some cases by environmental inputs. In mammals, genomic "determination" is the norm because otherwise the hormonal environment of the uterus would produce a vast majority of

[2] A clear example of parts transformation is represented by the complex series of structural modifications that RNA polymerase, sigma factors and DNA sequences undergo during transcription initiation (Glyde et al., 2017).

[3] Development can be characterised in many ways, some restrictive and some less so. In this article I favour the latter avenue and characterise development, following West-Eberhard (2003, pp. 89), as the series of phenotypic and qualitative changes a responsive biological system undergoes due to environmental and genomic inputs during its life history. In this sense, DNA replication and transcription are developmental processes.

females. However, in many species of insects and reptiles environmental sex "determination" is common. In the case, for instance, of the leopard gecko, a temperature of 26 °C produces 100% females (Crews, 2003).

The switch point model also helps to dispel a couple of conceptual errors. The first is that, given that it is unlikely that there exist phenogenetic processes that are entirely either genomically or environmentally determined, the use of the language of determination in the latter sense – i.e., extended to the entire developmental trajectory – is misleading (West-Eberhard, 2003, p. 99–100). The switch point model clarifies that the hypothesis according to which gene G determines phenotype P is an indiscriminate claim about the causal role of a DNA sequence on the entire developmental trajectory. As such, it is false; however, a more refined version of the determination hypothesis might be that DNA causes a particular switch point, or a series of switch points. Consider Halder, Callaerts and Gehring (1995) genetic experiments with *Drosophila* in which the expression of gene *Ey* is argued to be "necessary and sufficient to induce ectopic eyes" (Halder et al., 1995, p. 1791) even in wings and antennae. Halder et al.'s claim that *Ey* "determines" eye morphogenesis should be qualified: admittedly, *Ey* is not the only gene regulating this morphogenetic process because "…we estimate that more than 2500 genes are involved in eye morphogenesis" (Halder et al., 1995, p. 1791). Nonetheless, some determination claims might be compatible with the evidence, namely that either *Ey* causes one or a series of switch points, or even that the entire developmental trajectory is entirely regulated by genomic resources (i.e., that all its switch-points are caused by genomic inputs, e.g., *Ey* and thousands of other genes). This latter hypothesis is a form of genetic determinism because an adult phenotype would be fully determined by genomic inputs.

The second conceptual error is that predominant influence on the tipping of the threshold is not enough for determination. West-Eberhard seems to argue that, given that the switch point model is based on the postulation of the action of a condition-sensitive threshold regulatory mechanism at every bifurcation, a causal factor might be considered determinative in two cases: either when it causes single-handedly the tipping or when it is the most causally relevant factor in tipping the threshold of the regulatory mechanism. However, the latter case is problematic because cases of multiple developmental factors simultaneously contributing to the regulation of the threshold mechanism cannot be instances of determination. Thus, the genuine question concerning determination is more complex: are there any genetic or environmental inputs exerting dictatorial causal influence at specific switch points by tipping the threshold and hence individually causing the change of state or pathway?

I strongly doubt it. In order to show this, consider the first switch point in prokaryotic transcription, i.e., the generation of a DNA coding strand from a starting DNA sequence. Even in this case, there is no way to make sense of the idea that DNA is a determinant. In fact, without the holoenzyme (i.e., the molecular complex constituted by an RNA polymerase and a sigma factor protein), DNA cannot unwind itself and cause the generation of the coding strand. The relevant upshot is that neither DNA nor the holoenzyme can be seen as the determinative input tipping the threshold of the regulatory mechanism and causing the appropriate decision, hence canalising the developmental process in one specific direction down a specific slope of the epigenetic landscape, i.e., by producing a DNA coding strand ready for transcription. So, even if the first step of phenogenesis is taken into account – that is, the first step of arguably the simplest developmental process - neither DNA nor the holoenzyme can be necessary and sufficient causes for the occurrence of a specific phenotypic outcome. Both causes are necessary for unwinding the DNA template strand, but neither is sufficient. By extrapolation, every switch point will be causally influenced by a multiplicity of developmental factors and characterised by interactive causation. This general point applies to genetic and environmental determination cases alike. On the one hand, gene expression processes do not happen in a vacuum but in a developmental context that is rich in extra-genomic developmental resources such as molecular factors and environmental inputs. Returning to Halder et al.'s research, to emphasise *Ey*'s and the 2.500 other genes' causal contribution to the process of eye morphogenesis in *Drosophila* is to dismiss as causally irrelevant these extra-genomic resources of the developmental context. Analogously, temperature in environmental sex "determination" is not a necessary and sufficient cause of any switch point because the processing of this environmental input is necessarily modulated by the developing organism, i.e., by a multitude of cells and an even greater number of subcellular molecular factors. Thus, even though the notion of determination can be explicated via the switch point model of development, necessary and sufficient condition for the occurrence of developmental outcomes are most probably a myth. Given the fallacy of genetic (and environmental) determinism, it is difficult to make sense of the ontological distinctiveness of DNA's causal role in development via the notion of determination (see Vecchi, Miquel, & Hernandez, 2019 for further analysis on the issue of determination). But the impossibility of characterising ontological distinctiveness in terms of the notion of determination does not mean that there are no other possible characterisations.

3. DNA as ontologically distinctive developmental cause

Another way of characterising the ontologically distinctiveness of DNA's causal role in development emerged with the informational interpretation of protein synthesis. Crick (1958) made a particularly important contribution in this sense by proposing the sequence hypothesis:

> "In its simplest form it assumes that the specificity of a piece of nucleic acid is expressed solely by the sequence of its bases, and that this sequence is a (simple) code for the amino acid sequence of a particular protein." Crick, 1958, p. 142

The sequence hypothesis expresses the concept of co-linearity between genes and gene products, that is, the idea that a sequence of DNA bears a part-by-part correspondence to transcriptional and translational phenotypic outcomes, that DNA molecules determine the order in which the components of transcripts and polypeptide chains are orchestrated. The sequence hypothesis transformed the concept of biological specificity, historically stereo-chemical, generating its informational interpretation:

> "… stereochemical specificity results from the unique, complex 3-dimensional structure of a molecule that allows some molecules but not other to bind to it and interact. In contrast, informational specificity is produced by exploiting combinatorial complexity within a linear sequence …" (Griffiths et al., 2015, p. 6, p. 6)[4]

Sarkar has argued that Crick's informational specificity was reified as a peculiar kind of relationship between DNA sequence and developmental outcome:

> "The specificity of the gene-gene product (nucleic acid or protein) relationship was informational and thus different from specificity at every other level of biological organization, which remained physical (or stereospecific)." (Sarkar, 2005, p. 367, p. 367)

To ground the ontological distinctiveness of DNA in terms of "informational specificity" is, however, problematic.[5] Crick proposed to

[4] The frequent use in the literature of the spurious distinction between the "three-dimensional" structure of proteins and the "bi-dimensionality" of nucleic acids or, even, amino acids (Hüttemann & Love, 2011, p. 539), highlights another element of the informational interpretation.

[5] Co-linearity is a form of informational specificity. Most generally, informational specificity might be characterised as the causal capacity of an

conceptualise the relationship between DNA and its proximate phenotypic outcomes by abstracting away from the complexity of unknown biochemical interactions. Crick (1958, p. 144) argued that the crucial problem of protein synthesis resides in solving the puzzle of "sequentialization", i.e., the fact that "for any particular protein *the amino acids must be joined up in the right order"* (italics in the original). Crick also reasoned that, given that protein synthesis involves the flow of matter, energy and information, the problem of sequentialization should be conceptualised in terms of information flow.[6] However, even though an informational interpretation of co-linearity might play a heuristic role in developmental biology, it cannot be reified as ontologically distinctive. Crick's conceptualisation of protein synthesis was merely an alternative way to tackle a complex problem rather than an ontological gamble. Indeed, Crick (1958, p. 144) recognised that "… it is obviously important to discover the exact chemical steps which lead up to and permit the crucial act of sequentialization". In brief, the reification of informational specificity as an ontologically distinctive property of DNA sequences was never proposed by Crick and surely remains unwarranted: informational specificity is a form of biochemical specificity after all.[7]

The notion of informational specificity cannot thus ground the ontological distinctiveness of DNA sequences. Waters (2007) has proposed an alternative: in the context of certain developmental processes, DNA is distinctive for being the actual difference making cause.

3.1. DNA as the actual difference maker

In order to show that DNA is the actual difference making cause, Waters focuses on the process of prokaryotic transcription. Waters (2007, p. 22–23) shows that "… only the activated DNA segments (the genes) are actual difference makers of RNA sequences in a bacterium." He grants that, apart from DNA, also RNA polymerases and other molecular factors are potential difference makers. The actuality and potentiality of causes is relative to the production of the relevant differences in a population of interest (in this case variation in RNA molecules in a bacterial cell): an actual cause would explain the occurrence of different phenotypic outcomes through its variation; while a potential cause is one that does not vary and that, as a consequence, cannot explain the occurrence of different phenotypic outcomes. Hence, in bacterial transcription RNA polymerases and other molecular factors are merely potential (rather than actual) difference makers not in the sense that they have no causal role in the process, but in the sense that, not varying, reference to their causal role has no explanatory relevance in order to account for the differences in RNA molecules in a bacterial cell. Thus, the actual variation in DNA sequences completely accounts for the actual variation in the sequences of the population of RNA molecules. This implies that individual RNA molecules synthesised from the same DNA sequence have the same RNA nucleotide sequence. In the case of bacterial transcription, Waters argues that DNA is the actual difference making cause of the differences in the linear sequences of the population of RNA molecules in a cell. For this reason, DNA is an ontologically distinctive cause. This type of causal influence might be called *determination of structure*, distinct from the *determination of occurrence* at switch points seen in section 2.

Let us now critically evaluate the supporting premises of Waters' argument. The first premise – which is, as a matter of fact, a simplifying assumption - is that RNA polymerases and other aspects of the transcription milieu do not vary. The second, crucial, premise is that the variation of the transcription milieu does not cause the differences in the linear sequences of the population of RNA molecules produced during transcription. I shall now argue that both premises in Waters' argument are false.

To show that the first premise is false is easy: the transcription milieu varies in an indefinite number of ways. Waters considers only RNA polymerases as potentially varying aspects of the transcription milieu in his analysis of prokaryotic transcription. He argues that RNA polymerases as a matter of fact do not vary because prokaryotes possess only one type. This claim is, however, misleading. RNA polymerases, as complex enzymes with hundreds of amino acids and several subunits, come of course in a variety of biochemical tokens, making the transcription milieu variable; for instance, amino acid differences localised in non-binding sites do not necessarily render the enzyme defective, even though, by supposition, differences in amino acid composition affect the degree of accuracy of the polymerase during transcription (e.g., its capacity to produce an accurate transcript or its capacity to correct the misincorporations produced, see below). As related in section 2, RNA polymerases also constitute holoenzymes with sigma factors in the initial phase of transcription. Sigma factors come in different natural conformations:

> "… several distinct sigma factors have been identified, and each of these oversees transcription of a unique set of genes. Sigma factors are thus discriminatory, as each binds a distinct set of promoter sequences." (Clancy, 2008).

This means that also holoenzymes vary, thus making the transcription milieu variable in a different sense. The concentration and localisation of nucleoside triphosphates precursors also constantly varies, making the transcription milieu variable in an additional sense. More generally, given that transcription involves, as I shall show below, a variety of enzymes with different biochemical structures (e.g., RNA polymerases, sigma factors, Gre proteins) and other biochemical entities varying in concentration and localisation within the cellular environment (e.g., nucleoside triphosphates, pyrophosphates), it is clear that the transcription milieu can vary in an indefinite number of ways. Given this knowledge, Waters effectively black-boxes transcription, a process whose complexity is mind-boggling. Further analytic decomposition and attention to mechanistic detail is instrumental to show why considering DNA the actual difference maker is an artefact of black-boxing. The level of mechanistic detail that I shall take into account is minimal but sufficient to show why the second premise at the basis of Waters' analysis is false.

The simplest way to show how the variation of the transcription milieu causes differences in the linear sequences of the population of RNA molecules produced is to focus on so-called "errors" in protein synthesis (Drummond & Wilke, 2009). Indeed, to conceptualise protein synthesis as a straightforward process with no glitches is a relic of a bygone age. Focusing on transcription "errors" - a significant biological

(*footnote continued*)
entity (e.g., a DNA molecule) to determine the order in which the components of another entity (e.g., an RNA transcript) are orchestrated. This causal capacity is putatively possessed by DNA, which is said to determine the order in which the nucleotides are orchestrated within the RNA transcripts. If there are other entities apart from DNA (and RNA) sequences that possess this causal capacity, then DNA (and RNA) are not ontologically distinctive developmental causes. Griffiths and Stotz (2013) answer the latter question positively by endorsing the hypothesis of distributed specificity, arguing that what they call "Crick information" (nothing else than co-linearity) is not "… located solely in coding sequences of DNA, but is distributed between the coding sequences, regulatory sequences and their RNA and protein products, and the environmental signals that act via that regulatory machinery" (Griffiths & Stotz, 2013, p. 5).

[6] The contentious postulation that information is an additional dimension of reality beyond energy and matter is a further aspect of the informational interpretation of biological processes.

[7] The notion of Crick information is used in order to defend an informational interpretation of developmental dynamics that has yielded important theoretical contributions (Griffiths et al., 2015). It might thus be interpreted as a different instance of reification of informational specificity, encompassing other entities apart from DNA molecules (see note 5). However, Griffiths and Stotz (2013, p. 5) seem to acknowledge that, ultimately, the analysis in terms of Crick information is complementary to the mechanistic: "The way in which genes in combination with other actors determine the activity of cells is mechanistic, but it is not reductionistic."

phenomenon occurring quite frequently (at a 10^{-5} rate per nucleotide, Evans et al., 2018) -, the crucial question concerns their causes.[8] It turns out that RNA polymerases are often involved in several ways in the production of transcription errors. This might happen for several reasons: it might be that the specific biochemical token of the RNA polymerase is somehow error-prone, having a higher tendency to produce misincorporations (i.e., inserting the erroneous nucleotide) than other RNA polymerases[9] or that the set of nucleoside triphosphates precursors localised in its vicinity biases its incorporation performance (e.g., if the concentration of ATP precursors surrounding the RNA polymerase is much higher than that of GTP, CTP and UTP precursors, then the erroneous incorporation of adenine in the transcript is more probable, as in the example in note 8). Independently of how the error happens mechanistically, these are clearly cases in which the variation of the transcription milieu causes the differences in the linear sequences of the population of RNA molecules.[10] In order to show this, suppose that the same DNA sequence is transcribed twice, once correctly by a "good" RNA polymerase and once erroneously by a "bad" polymerase; suppose also that the only aspect of the transcription milieu varying concerns the two token RNA polymerases; in such case, DNA clearly fails to be the actual difference maker of the linear sequence of the transcripts because it does not vary (by assumption, there is no actual variation in DNA sequences); thus, unless causal responsibility is attributed to the RNA polymerases, the variation in the population of transcripts is unaccounted for. Thus, contrary to what Waters' black-boxing analysis shows, in such cases the RNA polymerase is an actual difference maker with respect to the linear sequence of the population of RNA transcripts.

It could be argued that transcription errors should be discounted as irrelevant because they produce, by supposition, dysfunctional transcripts that will be eventually degraded rather than used in protein synthesis. Mechanistic analysis shows why this dismissal misses the mark: for instance, transcription errors are often successfully edited by quality control mechanisms. Indeed, transcription fidelity is a complex process involving a variety of proofreading mechanisms that molecular biology is starting to unravel in detail (see for instance Wang et al., 2015). Quality control mechanisms do not just remove errors; rather, they recognise misincorporations, remove them and insert correct nucleotides. This complex process of recognition + removal + insertion is realised by a variety of transcription fidelity mechanisms controlled by the cell. As far as I understand, three mechanisms have been identified so far: pyrophosphorolytic editing, hydrolytic editing and Trigger Loop (Gamba & Zenkin, 2018). Again, it is useless, in the context of the present analysis, to understand the astonishingly complex mechanistic details of these processes. It is sufficient to stress that the RNA polymerase is actively involved in all of them. However, often it is not the RNA polymerase that performs the removal task, which is left to other molecules. For instance, in pyrophosphorolytic editing, a pyrophosphate removes the wrong nucleotide, finally leaving the field to the RNA polymerase that will eventually insert the correct one. In the case of hydrolytic editing, proteins of the Gre family induce the stop of the RNA polymerase and remove a few nucleotides (among them the incorrect ones) that will be then inserted by the RNA polymerase itself after it backtracks. By opening the black-box of transcription, other ways in which variation in the molecular milieu can be the actual difference making cause of the variation in the population of transcripts can be identified. In case quality control mechanisms successfully correct the error however produced (e.g., spontaneously, by RNA polymerases etc.), even though they are causally responsible for the functionality of the final RNA transcript, they are not actually difference makers of the variation in the population of transcripts; the reason is that they just remove such variation. Suppose instead that a correct transcript is subjected to hydrolytic proofreading and that the RNA polymerase is error-prone; suppose also that the only aspect of the transcription milieu varying concerns the presence of two tokens of the Gre protein, one of which error-prone. At least a two-fold scenario can be imagined at this juncture: in the first, the functional Gre protein keeps the correct transcript intact and the RNA polymerase is not required to act; in the second, the error-prone Gre protein transforms a correct transcript into an erroneous one and, given the error-proneness of the RNA polymerase, the newly produced erroneous transcript is not corrected; in the latter case, quality control mechanisms are actual difference makers with respect to the linear sequence of a population of RNA transcripts. In fact, unless causal responsibility is attributed to the Gre proteins, the variation in the population of transcripts is unaccounted for. Given that all three proofreading sub-processes can go wrong in many ways (e.g., failed recognition, failed substitution and failed insertion might produce errors) and, more generally, that the transcription milieu - as shown so far - can vary in an indefinite number of additional ways, the thesis that DNA is the actual difference making cause in prokaryotic transcription can be properly understood as an artefact of black-boxing. In all such cases, recomposition and situating analyses show that a proper conceptualisation of the transcription process requires reference to a much more encompassing orchestration of parts and activities inhabited by the richer set of molecular factors involved in the regulation of transcription fidelity.

I thus conclude that characterising the ontological distinctiveness of DNA in terms of actual difference making is incorrect even in the most promising case of prokaryotic transcription. Indeed, there is a general agreement that the concept of causal specificity is more fruitful in this respect.

3.2. DNA as causally specific difference maker

Waters argues that when eukaryotic transcription is concerned, DNA is not the only actual difference making cause. For instance, given that eukaryotes possess three types of RNA polymerases, they too could be actual difference makers.[11] Therefore, in order to ground DNA's ontological distinctiveness, the concept of causal specificity is needed. Framed in the terms of the manipulationist account of causation

[8] The notion of error is defined in comparison to the DNA sequence used as a template in transcription: when the linear sequence of nucleotides in the RNA transcript is not exactly complementary to the linear sequence of the reference DNA, an error occurs. For instance, if GGA is the DNA reference sequence and the RNA transcript has linear sequence CUU, an error at the second location occurs because the uracil of the RNA molecule is not complementary to the guanine in the DNA molecule; this error, if not corrected, might be used in translation to form a polypeptide with leucine. As we shall see, quality control mechanisms often correct the sequence, generating the correct transcript CCU, with cytosine as the correct complementary of guanine; this codon will then be translated to form a polypeptide with proline. These C→U errors - the most common in transcription - are caused by "spontaneous" cytosine deamination. Suppose, however, that deamination is caused by deaminase enzymes, which vary as tokens; then my general argument showing how variation of the transcription milieu might be actual difference makers can be applied to deaminases too. In any case, I shall focus on the causal role of the RNA polymerase and quality control mechanisms instead of spontaneous conversions due to the chemical instability of nucleotides.

[9] Indeed, mutations affecting the catalytic site of RNA polymerases might produce substantial increases in transcription errors (Kireeva et al., 2008).

[10] Unless we assume that other chemical properties of the DNA molecule make a difference, for instance the isotope variation in the nitrogen atoms of the nucleotides. For the sake of argument, I shall hereby assume that sameness of DNA sequence implies identity in all compositional and structural respects.

[11] I have already shown in section 3.1 that actual difference making ascriptions in developmental processes are token rather than type-dependent: even in prokaryotic transcription, an error-prone RNA polymerase might in fact produce errors. Note also that error-proneness might be a property of the polymerase itself (e.g., possessing the incorrect amino acids and, henceforth, a defective binding site) or one that is manifested within a particular relational context (e.g., dependent on the availability of a biased pool of NTP precursors).

articulated by Woodward (2003, 2010) and adopted by Waters, causal specificity refers to a pattern of causal influence that captures the "fine-grained kind of control" (Woodward, 2010, p. 305) exerted by causes on their effects that, at the extreme, could be represented as a bijective function (Woodward, 2010, p. 305). For instance, given a number of possible states of the cause variable C (c_1 …. c_n) and a number of possible states of the effect variable E (e_1 … e_n), maximal fine-grained influence of C over E occurs when the possible states of C exclusively cause the possible states of E (i.e., c_1 causes e_1, c_2 causes e_2 …. c_n causes e_n).[12] DNA seems to exert maximal fine-grained influence over transcripts and is therefore an extremely causally specific difference maker: any nucleotide change in any location of any DNA template might be matched by a nucleotide change in the corresponding location of any RNA transcript. Suppose C is the DNA sequence variable (for simplicity reduced to two nucleotides) while E is the complementary RNA transcript. There are 16 possible states of C (AA, AG, AC, AT, GA, GG, GC, GT, CA, CG, CC, CT, TA, TG, TC, TT) each of which is exclusively associated to the 16 possible states of E (UU, UC, UG, UA, CU, CC, CG, CA, GU, GC, GG, GA, AU, AC, AG, AA). In this sense, DNA is a developmental cause of unparalleled fine-grained influence in transcription. The upshot of Waters' analysis is that - relative to certain developmental processes (e.g., prokaryotic transcription as well as eukaryotic transcription before splicing) - there is a significant asymmetry between the causal specificity of DNA sequences and that of extra-genomic developmental causes that grounds the former's ontological distinctiveness. This is in my opinion by far the most promising way to ground the ontological distinctiveness of DNA. But there are a number of caveats that make this representation of the process incorrect and that allow me to highlight a number of limitations of Waters' argument.

First of all, in order to ground ontological distinctiveness, the claim about DNA's causal specificity should identify a unique pattern of causal influence that can be represented by a bijective function. But this cannot be done, a point on which clearly Waters and Woodward agree. But the causes of the failure of maximal fine-grained influence should be understood. In a sense, the conversion of DNA templates into RNA transcripts (pre-mRNAs in eukaryotes) seems code-like. However, as already seen in section 3.1, mechanistic analysis shows that DNA can exert its causal specificity capacities only within a particular developmental context. The nature of the causal influence of the transcription milieu is particularly visible when glitches in transcription occur. Whenever transcription errors occur, it becomes clear that DNA's causal influence is not maximally fine-grained whenever the RNA polymerase fails to accurately incorporate matching nucleotides in the nascent RNA strand; DNA's causal specificity capacities of generating downstream effects in transcription (i.e., by causing RNA transcripts matching DNA sequences) are thus dependent on the accuracy of the RNA polymerase. Thus, causal specificity is not a unique biochemical property that DNA molecules manifest spontaneously but, rather, one that is manifested within a particular relational context or mechanistic orchestration. More generally, it could also be argued that causal specificity, mechanistically speaking, can only be considered as a property of the developmental system: by black-boxing transcription, it looks as if the DNA sequence exerts fine-grained causal influence over the final state of the transcript. However, this conceptualisation is simplistic, as mechanistic analysis helps showing. While recomposition analysis suggests that DNA's causal role in transcription can only be properly conceptualised within the context of the larger orchestration involving the quality control mechanisms seen in section 3.1, situating analysis suggests that DNA's causal role in transcription can be properly conceptualised only within a cellular context. For instance, erroneous transcripts can be co-opted to perform other functions in the cell instead of being degraded; they can also be used in protein biosynthesis and, according to some estimates, be translated several times (up to 40, Traverse & Ochman, 2016, p. 3311); at the extreme, they could even be used to synthesise a novel functional protein (Drummond & Wilke, 2009). Thus, to ground ontological distinctiveness on a property like causal specificity that DNA exhibits only within a particular relational context or mechanistic orchestration and that, in my opinion, should be more properly ascribed to the developmental system does not seem a very promising avenue.

Secondly, Waters' claim concerning the ontological distinctiveness of DNA is extremely limited in scope. In order to be philosophically and biologically significant, the thesis of the ontological distinctiveness of DNA should ideally apply to a variety of developmental processes. As a matter of fact, it can only be applied to error-free transcription and cannot be extrapolated to any other developmental process. The more the causal relationship between DNA and phenotypic outcome is indirect, the more redundancy becomes important.[13] For instance, several codons are matched in translation to the same amino acid, making DNA's fine-grained causal influence invisible to the translation apparatus. Suppose that from two different DNA sequences TTA and TTG, two transcripts AAU and AAC are formed; given that these transcripts will be matched in translation to the same amino acid asparagine, the fine-grained causal influence of DNA will be nullified. To put it crudely, ribosomes just do not care about some of the variation generated by DNA sequences. Consider now protein synthesis: if different DNA sequences (for instance, two recently duplicated genes with a few nucleotide differences) are used to produce the same folded protein, the fine-grained causal influence of DNA is somewhere lost in the way; assuming the different DNA sequences are transcribed and translated correctly (i.e., no errors in transcription and translation occur), different polypeptide chains would ensue; hence, the bottleneck screening off DNA's fine-grained causal influence would be post-translational, i.e, during protein folding. Similar processes of screening off are of course not isolated to DNA. For instance, even though several mRNA transcripts are produced through alternative splicing, the cell often uses only one variant for protein synthesis (Tress et al., 2017); hence, the fine-grained causal influence of mRNA in protein synthesis is nullified in translation (see also section 4 on this point). All of this is not surprising in the light of the switch model of development articulated in section 2. Developmental processes can be represented in terms of the relative proximity between cause/developmental input and effect/developmental output. The longer the developmental pathway between developmental cause and phenotypic outcome (i.e., the longer the number of its switch points), the higher the probability that the constantly increasing number of molecular factors and environmental inputs involved in the pathway will dilute the causal influence of the developmental cause chosen as the variable of interest. From this perspective, the causal influence of DNA in prokaryotic transcription is less sensitive to the vagaries of the developmental context than that of any other developmental process, at least for the simple reason that prokaryotic transcription involves less biochemical interactions and less molecular factors than the other processes.[14] For instance, the

[12] Woodward (pp. 310–314) distinguishes between two senses of causal specificity, the fine-grained influence notion on which I am focusing and the *one cause-one effect* notion (i.e., that one cause has one single and exclusive effect, a causal relationship for instance realised by an hypothetical enzyme binding just to one kind of substrate and catalysing just one specific kind of biochemical reaction). As Woodward (2010, p. 313) notes, the two senses are interconnected in a sense that can be grasped by decomposing the causal influence of C over E when maximal fine-grained influence is at stake: c_1 has only one effect e_1, c_2 has only one effect e_2 …. c_n has only one effect e_n.

[13] Lack of maximal fine-grained control because of redundancy is clearly envisaged by Woodward (2010, p. 305), whose non-mechanistic analysis is illuminating in several respects.

[14] This idea of variable sensitivity of the causal dependence between developmental cause and phenotypic effect within a specific developmental context is related to Woodward's (2010) concept of stability. For instance, Woodward (2010, p. 295) argues that the causal relationship of a DNA sequence with its

developmental pathways of translation and, even more so, of morphogenetic processes like ptilopody and sex determination involving cell-to-cell and cell-to-tissue interactions (see below) are longer, more complex and, ultimately, more sensitive to the actual variation of the developmental context. By extrapolation, I surmise that, even though DNA's fine-grained causal influence in transcription is unparalleled for reasons that I shall make clear in section 4, mechanistic analysis shows that the more the causal relationship between DNA and phenotypic outcome is indirect, involving additional processes and mechanisms, the more the probability its fine-grained causal influence will be partially screened off.[15]

Thirdly, Waters acknowledges that causal specificity comes in degrees (as initially stressed by Weber, 2006) and that other developmental causes might be somehow causally specific. Let me consider the implication concerning the gradual nature of causal specificity in more detail. Let us first ask what kind of developmental factors can be causally specific. Waters suggests that the causal influence of molecular factors such as RNA polymerase is switch-like (i.e., they can merely slow down or stop RNA biosynthesis). Woodward (2010, p. 307) tries to make causal sense of the claim that RNA polymerase and other aspects of the cellular machinery involved in protein synthesis are switch-like, even though he also adds that these are open empirical questions. Their suggestion (Woodward, 2010, p. 306) might not be surprising if it is thought that the only possible kind of intervention on molecular factors is on their concentration, where the only causal effect that altering such concentration would have is to slow down or stop synthesis of RNA molecules rather than to change their structural properties. But the manipulation of concentration level is not the only kind of intervention that can be envisaged. An interesting "anomaly" (somehow incoherent with the above suggestion) concerns the molecular factors involved in splicing. Waters acknowledges that splicing factors are causally specific difference makers in eukaryotic protein biosynthesis. Note that if causal specificity is not a unique property of DNA, then grounding its ontological distinctiveness on this basis seems even more unpromising. In any case, take for instance the *Drosophila* Dscam gene that can be potentially alternatively spliced, according to some estimates (Schmucker et al., 2000), into more than 38.000 possible different mRNAs. In order to understand how splicing factors can be highly causally specific developmental factors, what should be shown is that different states of the splicing factor variable exert fine-grained influence over different states of the mRNA variable. One way to conceptualise this pattern of causal influence is the following. Let us suppose that the relevant splicing factor is the spliceosome (S), a very complex enzymatic molecular machine constructed by the cell and consisting of many different amino acids, subunits and active sites. Let us suppose its possible states are s_1, s_2 …. s_n. The population of mRNAs alternatively spliced (R) has possible states r_1, r_2 …. r_n. Suppose also that the other aspects of the molecular milieu are invariable. Given same pre-mRNA molecule, the spliceosome exerts maximally fine-grained influence over mRNAs when the possible states of S exclusively cause the possible states of R (i.e., s_1 causes r_1, s_2 causes r_2 …. s_n causes r_n). The question then is: what are the possible states of S? I admit my ignorance of the biochemical details at this juncture and propose two possible mechanistic interpretations. The first is that the same spliceosome splices the pre-mRNA at different times. In this case, the possible states of S are the possible conformational states of the spliceosome, particularly the different ways in which the subunits and active sites of the molecular machine are orchestrated at a particular time. According to this interpretation, the possible conformational states are the actual difference making causes of mRNAs. That is, being in conformational state s_i accounts for the specific intron-excision and exon-packaging splicing behaviour of the spliceosome, i.e., producing a r_i with that specific arrangement of exons. The second mechanistic scenario is that different spliceosomes alternatively splice the same pre-mRNA molecule. In this case, the possible states of S are the different token spliceosomes, which act as the actual difference making causes of mRNAs. That is, spliceosome s_i has a specific intron-excision and exon-packaging splicing behaviour that produces a r_i with that specific arrangement of exons. If this mechanistic reconstruction is somehow reasonable, I see no reason why it should not also be applied to the behaviour of other complex molecules such as RNA polymerases. After all, why should the spliceosome be such an anomaly? Waters and Woodward suggest that the only causal influence RNA polymerases can have is switch-like. In such case, they have arguably no effect on the *structure* of the transcripts but only on their rate of production. I have already stressed in section 3.1 that RNA polymerases do not merely affect the rate of biochemical reactions but also the *structures* of the transcripts whenever they generate a transcription error. Using the above scenarios, it follows that RNA polymerases, analogously to spliceosomes, might have fine-grained causal influence on RNA transcripts: given same DNA sequence, different RNA polymerase tokens c_i might cause the structural differences of corresponding RNA transcripts r_i. It is of course unlikely that this causal dependency can be represented as a bijective function. However, this causal influence would be very different from the switch-like behaviour suggested in the literature. Thus, spliceosomes, RNA polymerases and other complex enzymes that can assume different conformational states and that come in different biochemical tokens, can be causally specific difference makers.

A further question is whether there any good biological reasons to deny that the manipulation of the concentration level of a certain molecule in the cell cannot be a specific cause. Zuckerkandl and Villet (1988) proposed that a phenotypic outcome could be either produced through a structural modification caused by mutation or through a change in the concentration of a molecule. They called their hypothesis the "principle of concentration-affinity equivalence in phenotypic expression": there exists equivalence "… between the effect of a change in component concentration (activity) under a constant structural state and the effect of a structural modification under constant component concentration (activity)" (Zuckerkandl & Villet, 1988, p. 4784). This means that, apart from DNA sequences and particular token molecular conformations of molecular factors, also precise concentrations of certain chemical substances might exert fine-grained causal influence on phenotypic outcomes. For instance, ptilopody (i.e., feathering of the foot) in chickens can be equally caused by a mutation and induced by administration of 125 μg of retinoic acid to 10-day old chick embryos (Dhouailly et al., 1980). Framed in Woodward's terms, this causal influence would be fine-grained rather than switch-like if, by modulating amount of retinoic acid injected (e.g., 75 125, 175 μg etc.) or developmental stage (e.g., administering the acid to embryos at 5, 10, 15, 20 etc. days), different patterns of feathering result. It is difficult to evaluate such claims because ptilopody as a morphological phenotype can be represented as a set of continuous states that are not as precise and discrete as the nucleotide differences in RNA transcripts or polypeptide chains. This limitation also suggests that, when non-molecular factors are concerned, it is difficult to evaluate the thesis of distributed specificity (see note 5), that is, the hypothesis that causally specific difference makers are distributed in the developmental context. The principle of concentration-affinity equivalence in phenotypic expression deals, strictly speaking, with the equivalence between the developmental

(*footnote continued*)
associated RNA transcript is more stable than that of DNA with the eye-colour phenotype.

[15] One interesting suggestion is that sensitivity will depend on the level of "generative entrenchment" of the developmental resource within the developmental system. In this sense, Schank and Wimsatt (1986, p. 39) argue that earlier expression of a developmental resource implies that, on average, it will have a larger number of downstream features dependent on them: "*Given that earlier features have a higher probability of being significantly generatively entrenched, mutations which are expressed earlier in development are more likely to have larger, more pervasive, and more deleterious effects*". Given this larger dependency, it makes thus sense for the developmental system to "armor" the developmental pathway. I shall return to this point in section 5.

changes produced by different conformations of molecular factors and by their different concentration. However, the big idea behind Zuckerkandl and Villet's principle is that it can be broadened as to encompass the equivalence of genomic and environmental developmental causes:

> "The interplay between equilibrium constants and concentrations and, as a result, between mutations and effects of the environment (external and internal) turns out, upon examination, to seem destined to be of general importance in biology." (Zuckerkandl & Villet, 1988, p. 4785).

Similarly, West-Eberhard (2003, p. 99) has proposed the hypothesis of inter-changeability of genetic and environmental factors: environmental effects on development can be as specific as genomic ones. Part of the rationale of West-Eberhard's hypothesis, as already seen in section 2, is that phenotypic effects are mediated by a responsive developmental system acting as a transducer of impinging stimuli, where transduction depends on a condition-sensitive regulatory mechanism that flip-flops when a threshold is reached. If development is thought in these terms, the origin of the stimuli is irrelevant:

> "Neither genes nor environmental conditions have any developmental significance without a phenotype already organized to respond… The nature-nurture dichotomy disappears with the realization that the developing phenotype responds to both internal and external stimuli in much the same way. As a result, genetic and environmental influences are equivalent and interchangeable …" . (West-Eberhard, 2003, p. 99, p. 99)

In the light of this hypothesis, it can be asked whether there are any good reasons to believe that causally specific developmental factors must be confined necessarily to some part of the biochemical machinery of the cell (including, of course, its genome). Weber (2006, p. 607, note 5) argues that "…environmental factors are no candidates for causal specificity because they are usually not *discrete* variables." While Weber might be right in claiming that environmental factors are often continuous variables from the observer's perspective, their developmental effect depends on the nature of the responsive developmental system (e.g., zygote, blastocyst, gastrula, adult organism) under consideration. Importantly, if developmental switch points are the result of a threshold mechanism of regulation, then a specific-enough agent of environmental induction might trigger the switch. If this were the case, environmental factors might have specific effects on developmental processes and there might be no good reason to deny them the status of being causally specific difference makers. In this sense, genomic and environmental stimuli can be seen as interchangeable. But it is difficult to make sense of the interchangeability thesis in terms of Woodward's account of causal specificity. For instance, in the American alligator species *Alligator mississippiensis*, temperature is somehow a specific difference maker in the sense that definite morphological effects are caused by a temperature of 30 ° Celsius (i.e., production of 100% females) and a temperature of 32.5 ° Celsius (i.e., production of 100% males; Gilbert, 2000, Fig. 17.20). Framed in Woodward's terms, the causal specificity of temperature in *Alligator mississippiensis*'s sex morphogenesis would be fine-grained if, by modulating temperature, specific morphological effects ensue. Note that in the ptilopody case the effect variable was continuous (i.e., the feathering of the chicken foot is similar to baldness in humans). In the sex morphogenesis case, the effect variable has just two possible states: male and female (excluding intersexes). This mismatch between the possible states of the temperature cause-variable (which, despite being continuous, might be somehow representable as discrete) and the two-states of the sex-effect variable suggests another limitation in the evaluation of causal specificity claims concerning environmental factors. Even though it is true that some states of the cause variable (any temperature up to 30, temperatures between 32.5 and 34 and any temperature above 35) are correlated to specific effects, and even though it is true that they are actual difference makers of the structural features of the sexual morphology of *Alligator mississippiensis* organisms, it is difficult to make sense of the claim that temperature exerts fine-grained causal influence in sex morphogenesis. This might be a consequence of the fact that Woodward's account of causal specificity is tailored to protein synthesis and sets the bar too high in order to account for distributed specificity. Alternatively, it might be that the causal effects of molecular factors, precise concentrations of certain chemical substances and environmental variables are incommensurable (I shall come back to this issue in section 5).[16]

In this section I have argued that the thesis of the ontological distinctiveness of DNA does not identify a unique pattern of causal influence and that it has an extremely limited scope as it only applies to error-free transcription. I have shown that the causal influence of DNA can be considered specific only within a specific developmental context and, as a consequence, causal specificity is not a unique biochemical property that DNA molecules manifest spontaneously. I have additionally shown that many other developmental causes can be relatively specific, always within a particular developmental context of course. I thus doubt that a claim concerning ontological distinctiveness can be grounded on a property that is neither unique nor manifested independently of the particular relational context or mechanistic orchestration provided by the developmental system.

4. Making sense of DNA's causal role in development

In this section I shall argue that developmental processes involving DNA are more robust than other developmental processes and that this robustness accounts for the pivotal role played by DNA in development. In this way, I provide a way to complement the thesis of the instrumental primacy of DNA-centric biology by showing why DNA is causally central to understand developmental processes. In the context of the present discussion, robustness refers to a property of a developmental process, that is, the ability to produce a functional phenotypic outcome despite the existence of what are called, somehow elusively, "disruptions" or "perturbations".[17] A developmental pathway is robust when the "normal" phenotypic effect is caused despite such disruptions. In developmental processes, robustness is achieved through the exploitation of the resources of the developmental system that manages to resist perturbations by cancelling out the causal effects of perturbations. The way in which this resistance is realised depends on the mechanistic details of the developmental pathway considered.

A chief example of robustness is related to organismal responses to so-called "errors" in protein synthesis or phenotypic mutations, e.g., the nucleotide and amino acid misincorporations produced by RNA polymerases and tRNAs respectively. Significantly, errors in protein synthesis are orders of magnitude higher than DNA replication errors:

> "The *E. coli* genome is 4.6×10^6 base-pairs long, such that at the typical mutation rate of approximately 10^{-9} per base pair, one bacterium in 200 will bear a mutation in its genome. By contrast, the average *E. coli* coding sequence is 335 codons long, and at a canonical per-codon missense error rate of 5×10^{-4}, 15% of protein molecules will contain at least one error. At the bacterial scale,

[16] How to compare the nucleotide variations in RNA molecules (including those due to errors in transcription) to the morphological variations in ptilopody or to the morphological and physiological variations involved in sex development? Different chicks will develop the feathering of the foot in slightly different ways and different alligators will exhibit sex organs with slight physiomorphological variations. Seemingly, the three cases above are different patterns of variation.

[17] The concept of robustness is somehow related to what Woodward (2010, note 11, p. 296) calls "modularity": causal chains are modular when disruption to certain causal interactions does not affect other causal interactions in the chain.

perfectly replicated genomes are commonplace, but perfectly synthesized proteomes never occur." Drummond and Wilke 2009[18]

One obvious reason for this is that "phenotypic mutations" are not generally inherited by daughter cells, so that the organism can tolerate them more than genomic mutations. Another is that often errors do not impair proteins' functionality (e.g., an amino acid change in a non-active site will have less probability of impairing proteins' functionality and might henceforth be tolerated by the organism), that some erroneous products of biosynthesis (e.g., transcripts) decay rapidly and are henceforth unused in protein synthesis (Wilusz, Wormington, & Peltz, 2001) while others (e.g., spliced variants, see below) are probably used for other metabolic reasons but not to produce proteins (Tress et al., 2017). Thus, organisms have evolved ways to reduce the costs associated with transcription, translation and post-translation errors by increasing tolerance to phenotypic mutations. Indeed, organisms do not simply tolerate errors but (as anticipated in section 3.1) have also evolved ways to reduce error frequency and ways to increase the accuracy of protein synthesis - for instance by increasing translational accuracy of conserved or functionally crucial sequences or by enhancing ribosome accuracy. Significantly, DNA replication is more accurate than transcription, which in turn is more accurate than translation etc. (Drummond & Wilke, 2009, Table 1).

Thus, focusing solely on replication and transcription (the only developmental processes in which DNA is directly involved), I suggest that replication and transcription are more robust processes than all other developmental processes. This hypothesis applies to all developmental processes taken into consideration in this article: translation, ptilopody and sex morphogenesis are less robust processes than replication and transcription. The most important point is that such higher robustness is not due to the putative biochemically unique properties of DNA molecules, but to the mechanistic nature of the developmental processes in which DNA plays its causal role. In order to substantiate this hypothesis, let us focus on the comparison between prokaryotic transcription and translation. RNA biosynthesis is basically a process in which a complementary RNA molecule matching a DNA template is generated by an RNA polymerase. Translation requires more than complementarity between the RNA molecule and the tRNA. It also requires the association of the tRNA with a specific amino acid (carried out by the aminoacyl-tRNA synthetases), the discharge of the amino acid when attached to the ribosome etc. In brief, translation involves more switch points than transcription (as seen in section 3.2). From this perspective, it is easy to grasp that the causal influence of DNA in prokaryotic transcription is less sensitive to the vagaries of the developmental context than that of translation given that transcription involves less biochemical interactions and less molecular factors than translation. It is therefore not surprising that "simpler" processes are generally more accurate and that the causal dependence of transcripts on DNA is so strong.[19] Additionally, the higher relative insensitivity of transcription to the vagaries of the developmental context is mechanistically achieved: as seen before, the quality control mechanisms of transcription are more efficient than those involved in translation (Rosenberger & Hilton, 1983; Shaw, Bonawitz, & Reines, 2002) etc. Of course, the higher level of accuracy is dependent on the simpler nature of the developmental process. Using a mechanistic analogy, a machine with less parts can be defective in less ways. Thus, there must be a correlation between the fewer switch points and the possibility of evolving efficient quality control mechanisms. But this dependency does not impinge on the crucial point that transcription accuracy, as a pivotal dimension of robustness, should not be interpreted in terms of the biochemically unique properties of DNA molecules, but rather in terms of the causal capacities that DNA molecules manifest in a rich developmental milieu. In this respect, an additional and crucial dimension regarding the relatively higher robustness of DNA-involving processes should be highlighted. As I have shown above (section 3.2), some of the unedited errors due to the inaccuracy of RNA polymerases are not only "tolerated" by the developmental system under consideration, but can be rather re-deployed by it. This crucial aspect of DNA-involving processes can be conceptualised from the developmental-system-centric perspective endorsed by West-Eberhard and from that of mechanistic analyses alike. For instance, situating analysis shows that it is the causal capacities of the cell (or, in the case of multicellular organisms, of a larger developmental system) that accounts for the higher robustness of DNA-involving processes: the capacities of the developmental system to re-deploy developmental resources for other metabolic roles and co-opt for new functions developmental resources aimed for different functions has nothing to do with the putative ontologically distinctive properties of DNA molecules. In a nutshell, ascribing peculiar causal capacities to DNA in order to explain this dimension of robustness of DNA-involving processes is an easy shortcut. Let me explain this crucial point with two additional significant examples.

Causal parity advocates aim to find ways to show that DNA molecules do not possess an unrivalled degree of specificity compared to other macromolecules like splicing factors (Griffiths et al., 2015). But if the context of analysis is the entire process of protein synthesis rather than on isolated sub-process (e.g., transcription, translation, folding), experimental data suggest that splicing agents have not such pervasive causal influence on protein synthesis. For instance, the extrapolation that transcriptomics data map proteome complexity seems unwarranted:

> "The gap between the numbers of alternative variants detected in large-scale transcriptomics experiments and proteomics analyses is real and is difficult to explain away as a purely technical phenomenon. While alternative splicing clearly does contribute to the cellular proteome, the proteomics evidence indicates that it is not as widespread a phenomenon as suggested by transcript data. In particular, the popular view that alternative splicing can somehow compensate for the perceived lack of complexity in the human proteome is manifestly wrong." Tress et al., 2017 p. 108

This research suggests that organisms (the relevant developmental system) have evolved ways to either edit out spliced variants or to use them for other cellular roles rather than for protein synthesis. Indeed, as Tress et al. (2017, p. 98) argue, proteomics evidence shows "… that most human genes have a single main protein isoform", meaning that there is little evidence of proteins produced from alternatively spliced variants. While the robustness of protein synthesis processes vindicates the central causal role of DNA in development, it does so not by ascribing unique causal capacities to DNA molecules. Eukaryotic protein synthesis is often a convoluted process that from one DNA sequence generates a variety of splicing variants (analogous to perturbations) and then, through a bottleneck (e.g., the degradation or alternative use of splicing variants), often leads to just one functional protein. DNA remains central in this process, but not because of its unique causal capacities: it is obviously neither the only actual difference maker in

[18] As anticipated in note 8, the concept of error makes reference to the expected outcome of a process. In DNA replication, the expected outcome is a daughter DNA sequence perfectly matching the nucleotide structure of the mother sequence. In the case of transcription, it would be an RNA transcript perfectly matching the structure of the DNA coding strand etc. Thus, error does not imply damage. Indeed, it has been suggested that the fact that "there is not enough evolutionary pressure to increase the accuracy of the transcription and translation apparatus to DNA replication standards" (Bürger & et al, 2006, p. 197) is due to the fact that such errors can be beneficial (Drummond & Wilke, 2009; Halfmann et al., 2012; Koonin, 2012). The general point is that both genomic and phenotypic mutations are a source of potential beneficial changes for the cell both in protein synthesis and in the regulation of other cellular processes.

[19] This point is also highlighted by Woodward (2010, p. 294): "… as a general rule, more distal causal relationships with many intermediate links will be less stable than the individual links themselves".

protein synthesis nor the only causally specific developmental factor involved; indeed, the degradation of splicing variants is achieved through mechanisms that dilute and screen off DNA's fine-grained causal influence (see section 3.2), while their alternative use is achieved through mechanisms controlled by the developmental system. DNA's centrality has rather to do with the robustness of the developmental processes in which it performs its causal role and with the causal capacities of developmental systems: organisms not only tolerate perturbations in the form of the spliced transcripts generated (for instance by degrading them) but also manage to harness and deploy this variation to accomplish in alternative ways self-maintenance tasks (for instance by using the spliced variants in other metabolic roles).

Developmental systems can also resist perturbations directly affecting DNA resources. Aminoacyl-tRNA synthetases (AARSs) attach the correct amino acid to tRNAs and are henceforth crucial molecular factors involved in translation. Given their causal importance, it could be assumed that they are highly conserved across phylogeny and that all organisms possess the 20 AARSs for the 20 different amino acids of the genetic code. However, surprisingly, the genomes of certain prokaryotic species lack certain AAARs (Chaliotis et al., 2017). The reason is that the loss of AARS-encoding genes (a perturbation) does not necessarily affect prokaryotic biochemical function. For instance, when the glutaminyl-tRNA synthetase is absent because the gene has been lost through genome reduction, some prokaryotes have the ability to co-opt the non-specific molecule ND-GluRS, modify it and finally produce a functional glutaminyl-tRNA synthetase. The ensuing causal chain or biochemical series of interactions is longer but nonetheless robust. This example illustrates the point that the developmental system does not need to rely solely on DNA's causal specificity capacities in order to perform translation functionally. DNA still exerts its causal capacities by contributing to the biosynthesis of the non-specific molecule ND-GluRS, but it is the process of transformation of ND-GluRS into a functional glutaminyl-tRNA synthetase that compensates for gene loss. Thus, it is not because of its biochemically unique properties that DNA can be said to have a central role in development. The developmental system manages to tolerate perturbations such as gene loss and has the capacity to transform alternative enzymes to finally deploy them in translation. It is rather the robustness of the process of translation within a developmental system that is causally relevant and that accounts for DNA's central role in developmental processes.

To sum up, I propose that the most promising way to make sense of DNA's causal role in development might be captured mechanistically by stressing that DNA-involving processes are more robust than other developmental processes, in part because developmental systems have the capacity to tolerate perturbations and harness them to accomplish in alternative ways self-maintenance tasks. The focus on process and developmental system implies a metaphysical shift: rather than attributing to DNA molecules biochemically unique properties at all costs, I suggest that it might be better to think about DNA's causal role in development in terms of the causal capacities that DNA manifests and potentially acquires in a rich developmental milieu. The explanation here provided of why DNA is causally central in developmental processes is aimed to complement the thesis of the instrumental primacy of DNA-centric biology.

5. Evolutionary considerations pose a challenge to developmental constructionism

In the introduction I also showed that developmental constructionism might be interpreted as the thesis that the causal differences between DNA and extra-DNA factors "do not justify the metaphysical distinctions currently built upon them." (Griffiths & Knight, 1998, p. 254). My analysis is largely in tune with this position. Given all this, should causal parity be endorsed? There are still some good reasons to endorse a more nuanced position. First of all, as I anticipated in section 3, the thesis of distributed specificity is also difficult to evaluate. Consider West-Eberhard's (2003, p. 99) interchangeability thesis:

> "Note that I am not arguing here merely that the environment affects development. Everyone admits that. I am arguing that environmental effects on development can be as specific and essential as genetic effects…".

In section 3.2 I tried to evaluate the implications of this position by considering in what sense the causal specificity of DNA, molecular factors, precise concentrations of certain chemical substances and environmental variables can be compared. But this evaluation is problematic for at lest two reasons. First, as anticipated at the end of section 3.2, analytic frameworks such as Woodward's are insufficient to evaluate distributed specificity claims. Secondly, mechanistic analysis is generally impaired. With respect to this second point, compare for instance mutation-generated and acid-induced ptilopody. The genomic change and the administration of retinoic acid are developmental events within very long causal chains. Whenever satisfactory mechanistic decomposition of long causal chains is unachievable, distributed specificity claims are difficult to assess. This is also valid for claims - see section 2 - like Halder et al.'s (1995). What we are left with is just the observation that mutation is a developmental factor affecting the entire developmental trajectory constellated by switch points, much before acid administration (which has been performed experimentally, as long as I know, only when chicks embryos are 10 days old). One way to make sense of this observation is to propose that, despite being a more distal cause, DNA has an equivalent structural effect to that of a more proximate cause like retinoic acid administration. As a consequence, it could be argued that DNA's causal influence is not on a par with that of the environmental input. The reason for this is that the first developmental pathway is more robust and insensitive to perturbations.[20] However, generalising from this specific instance seems to me dubious.

More importantly, two kinds of evolutionary considerations pose a particularly important challenge to causal parity claims. DNA replication is orders of magnitudes more faithful than transcription, where the latter in turn is orders of magnitudes more faithful than translation. Selection is weaker against protein synthesis errors than against DNA replication errors (Lynch, 2010). The evolution of highly efficient DNA-repair and transcription quality control mechanisms could be interpreted as a form of cellular control and regulation of DNA sequences aimed at protecting them from damage. Why should cells evolve such sophisticated quality control mechanisms in the first place? The reasonable hypothesis is that the function of DNA as a template in replication and transcription is causally central for the survival of the cell. This is an evolutionary argument both against causal parity (because it explains the causal primacy of DNA in developmental processes like transcription) and against the thesis of the ontological distinctiveness of DNA (because it makes reference to cellular or organismal control on transcription). I would argue that the burden of proof is on the shoulders of the defenders of the causal parity thesis to find an alternative explanation for the lack of comparatively accurate post-transcriptional proofreading and regulatory mechanisms.

Another challenge for causal parity stems from considering the asymmetry between prokaryotic and eukaryotic phenogenesis. Again, evolutionary considerations help to explain this asymmetry. Prokaryotes' genomes are streamlined, almost completely coding and lacking structural genomic contrivances. Prokaryotic DNA sequences "coding" for genes can be successfully manipulated and transferred not only from one strain to another of the same lineage, but even from phylogenetically distant species. Lateral gene transfer works on this very principle: in order to make the lateral transfer of genomic resources possible, transcription and translation should work more or less straightforwardly. Conversely, eukaryotic phenogenesis is the result of

[20] Schank and Wimsatt (1986) make a similar point in my opinion (see note 15).

an opposite dynamic of non-adaptive genomic evolution (Lynch, 2007). There is evidence that the accuracy of protein synthesis depends on effective population size, with the expectation that multicellular eukaryotes with lower population sizes exhibit error rates higher than unicellular organisms (Gout et al., 2013, p. 18588). In the case of multicellular eukaryotes - whose bloated genomes are characterised by extensive non-coding regions, intron/exon distinction, pseudogenes, ubiquity of repeated sequences and mobile DNA elements etc. – processes such as alternative splicing and RNA editing render the relationship between sequence and phenotype more convoluted and complex. Developmental constructionism builds part of its narrative on a eukaryote-centric bias. But such strong emphasis on eukaryotes has created a somehow distorted representation of development, neglecting the much more DNA-dependent way in which prokaryotic phenogenesis is regulated and missing the blatant fact of the matter that multicellular eukaryotes evolved from prokaryotes. A sound argument in favour of causal parity would have to account for the characteristic nature of prokaryotic phenogenesis.

Acknowledgments

I thank the anonymous reviewers for particularly penetrating comments. I especially thank Gil Santos for continuous stimulating discussions. I also thank Lorenzo Baravalle, Maurizio Esposito, Gabriel Vallejos, Ken Waters and several other people who gave me important feedback when I presented these ideas at the Universidad de Santiago de Chile and at the Universidad Nacional de Educación a Distancia in Madrid, Spain. I acknowledge the financial support of the FCT - Fundação para a Ciência e a Tecnologia (DL57/2016/CP1479/CT0072; Grant N. UID/FIL/00678/2019) and of the Fondo Nacional de Desarrollo Científico y Tecnológico de Chile (Grant N. 1171017).

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.shpsc.2019.101245.

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