Abstract

This paper aims to clarify the consequences of new scientific and philosophical approaches for the practical-theoretical framework of modern developmental biology. I highlight normal development, and the instructive-permissive distinction, as key parts of this framework which shape how variation is conceptualised and managed. Furthermore, I establish the different dimensions of biological variation: the units, temporality and mode of variation. Using the analytical frame established by this, I interpret a selection of examples as challenges to the instructive-permissive distinction. These examples include the phenomena of developmental plasticity and transdifferentiation, the role of the microbiome in development, and new methodological approaches to standardisation and the assessment of causes. Furthermore, I argue that investigations into organismal development should investigate the effects of a wider range of kinds of variation including variation in the units, modes and temporalities of development. I close by examining various possible opportunities for producing and using normal development free of the assumptions of the instructive-permissive distinction. These opportunities are afforded by recent developments, which include new ways of producing standards incorporating more natural variation and being based on function rather than structure, and the ability to produce, store, and process large quantities of data.

1. Introduction

Extensive and successful efforts have been made to analyse and conceptualise the philosophical and scientific consequences of changes and new movements in developmental biology (e.g. Amundson 2005, Burian 2005, Robert 2004). The idea that development is the unfolding of a programme contained in the genome is challenged in many of these accounts. Instead, development is conceptualised as the interpretation by the organism, or its parts, of the genome and other (possibly environmental) factors considered as developmental resources (Gilbert 2003a; Griffiths and Stotz 2013). In addition, disciplines such as evolutionary developmental biology (evo-devo) have led to a renewed focus on the origins, causes and significance of phenotypic variation. Yet, criticism has been levelled at evo-devo that, as a result of using highly standardised model organism strains and normal stages, it generally fails to take account of organismal and ontogenetic variation (Love 2010; Bolker 2012; Bolker 2014; Minelli 2015).[1] Richard Lewontin, for example, has criticised the way in which experiments in developmental genetics deal with variation by focusing on the (difference-making) effects of changes to DNA, to the exclusion of other possible difference-making variables (2000).

This critique is at the heart of the programme known as developmental systems theory (DST; see, in addition to Lewontin 2000, Oyama 2000; Oyama, Griffiths & Gray [eds.] 2001; Gilbert 2002; Griffiths and Gray 2005; Griffiths and Stotz 2013; for criticisms of DST see Waters 2007; Weber 2006). DST emphasises context-sensitivity, an epigenetic model of development, the interaction of many "developmental resources" in the production of any one trait, and causal parity (see previous references, and also Oyama, Griffiths & Gray 2001). The latter means simply that no one cause, type of cause, interactant or developmental resource is special, privileged, or in some way ontologically, methodologically, or causally primary. This part of the critique is normally aimed at biologists and philosophers (such as Waters 2007) who argue that DNA is a cause that is an "actual difference

[1] Exceptions that do deal with variation include: Hall & Hallgrímsson 2005; Hallgrímsson et al. 2012; Klingenberg 2010; Minelli 2015.

maker" in development, in a way that other factors are not. In this way, it is variation in DNA sequence that is deemed to be the cause that makes a difference to changes in an organism. The most comprehensive recent criticism of this position can be found in Griffiths and Stotz (2013).

In this paper I do not only want to replay or develop such arguments, but build on them to make two more contributions. Firstly, I want to identify what and how different kinds of variation other than genomic variation matter in understanding key processes and events in organismal development. In part, this task will involve reconceiving precisely what those key processes are. Secondly, I want to assess how variation, thus more broadly conceived, might be properly investigated. In identifying ways in which the critiques of DST makes concerning the methodological and theoretical treatment of variation in developmental biology, I aim to provide insights which might aid DST in constructing new research programmes, for instance by designing and adopting new methods of standardisation.[2]

This paper thus analyses the different ways in which variation matters in experimental work in developmental biology to assess the way in which variation is managed – practically and conceptually – in the prevailing, or orthodox, framework of developmental biology. To do this, three interrelated concepts, at once ontological and informing practice, will be assessed: the distinction of instructive and permissive causes, the distinction between organism and environment, and normal development. In this paper, I use the term 'ontological' to mean what people believe to be the true nature of the entities and processes they are working with.

The distinction of instructive and permissive causes has been introduced to the literature to distinguish causes of phenomena of interest from the mere conditions that allow the causes to

[2] Though some scientists have adopted a DST approach, it is reasonable to say that DST itself is marginal within developmental biology, partly because of perceptions of its "inutility" and that "it motivates relatively little research" (Shea 2011). I find much of what DST has to say about development compelling, but acknowledge this criticism.

operate (as examined in Gilbert 2003a).[3] In practice, this distinction very often relies on the distinction between organism and environment. The instructive cause is located in some part or parts of the organism, such as the genome, and the permissive conditions are located outside of the organism, in the environment. A consequence of the distinction is that difference-making causes become assigned to internal factors that vary, and not to external factors that can of course vary as well, but in experiments are usually held constant to study the effects of internal factors.

Such strategies meet the demand that, to make an experiment tractable, the experimenter must control variation. In biology, variation "refers to the actually present differences among the individuals in a population, a sample, or between the species in a clade" (Wagner, Booth & Bagheri-Chaichan 1997, 330). There is variation within individuals as well, such as between the left and right sides of organisms (Carlson Jones and German 2005, 74). There are, conceivably, unlimited ways in which comparisons between two (or more) parts of organisms, individual organisms or groups of organisms can reveal, or attempt to measure, variation. Controlling variation in experimental embryology often involves the establishment of a 'normal development', as "a strategy for managing complexity and variation via a practice of categorization that proceeds, not by applying definitional criteria, but rather by comparison to some reference standard" (DiTeresi 2010, 29).

I begin my discussion of variation in developmental biology by providing an account of the history of normal development, and explaining its role in experimental systems, particularly in the conceptualisation and management of variation. Following this, I explore the instructive-permissive distinction and its role in underpinning experimental practices concerning the relegation of certain types of variables to background conditions to be held constant. I take the instructive-permissive distinction to underpin what I take to be the prevailing framework of developmental biology. After

[3] I am informed by an anonymous referee that the origin of the term "permissive" in this sense dates back to Holtzer (1968). While I have not been able to obtain that source, consulting a review of the book in which Holtzer's work was published I am satisfied that this is indeed the case (DeHaan 1968). Scott Gilbert has also cited Holtzer's coinage of the term (e.g. Gilbert 2003b, 349).

detailing the other key elements of that prevailing framework, including the central organising principle of differential gene expression, I move on to explore the examples. Before I do that, I begin the section by identifying different aspects or dimensions of variation in development, variation in unit, temporality and mode. I demonstrate that how these aspects of variation are managed is linked to how experiments are conceived, designed and conducted, for example through how a particular experimental system is spatially or temporally partitioned, which affects what unit or temporal variation can be identified, measured, and either kept constant or deliberately varied.

To make sense of new insights arising from biology itself, as well as from the philosophy of biology, I then examine some examples of the different ways in which variation can be characterised in experimental and conceptual practice in biology. These examples include the role of the microbiome in development, the phenomena of developmental plasticity and transdifferentiation, and different ways of conceptualising causation and organising and conducting experimental work. These examples call into question how variation is managed in the prevailing framework, which takes differential gene expression to be the central organising principle of developmental biology.

Finally, I revisit the instructive-permissive distinction. As well as demonstrating that if one aims to investigate certain problems, the practical-theoretical framework of modern developmental biology has problems, I aim to show that considering the different ways in which variation can be managed can guide the formulation of alternative frameworks and practices. The project of ascertaining the significance of different kinds of variation will entail abandoning the once-useful conceptual crutch of the instructive-permissive distinction.

By focusing on the role of variation, and how it relates to normal development, I am able to direct focus on what conceptual and practical changes might be needed to cohere the disparate critiques of the existing framework, and to signal more clearly and constructively what can be done to forge a

new framework. These changes will entail finding ways to incorporate dimensions of variation in development other than that of differential gene expression into conceptual and experimental practice in developmental biology. I conclude this paper by suggesting some concrete ways in which this might be done, and detail some of the many challenges faced by such a project.

2. Normal development and the instructive-permissive distinction

Aristotle was the first to conduct systematic studies into embryology, and his work led him to adopt what has been characterised by Elliott Sober as the Natural State Model (Sober 1980). This views apparent variation as masking an underlying reality. In the case of a process such as organismal development, there is a "natural tendency" which is an essential characteristic of that process towards the attainment of the "natural state" of a particular object which does not itself require explanation (Sober 1980, 360). Deviation from that course, however, is explained with an appeal to "interfering forces" (ibid). For adherents of this Aristotelian point of view, "within the possible variations that a species is capable of, there is a privileged state – a state which has a special causal and explanatory role" (Sober 1980, 365). Sober observes that "our notion of normality as something other than a statistical average enshrine[s] Aristotle's model. ...[W]e preserve the form of model he propounded, but criticize the applications he made of it" (Sober 1980, 363).

Normal development was formalised by comparative embryologists at the end of the nineteenth-century in tables of normal stages (Hopwood 2007). In experimental embryology, the development of model organism systems drove the production of standards such as normal development, against which the effects of experimental manipulations could be compared. Standardised organisms, produced by the breeding of particular strains, with variation within the strains reduced to a minimum, needed to be accompanied by standard, or normal, series, tables, or stages (DiTeresi 2010). The normal stages have much of the apparent variation of embryonic development

abstracted away, and the stages themselves are an abstraction from the continuous process of development. These stages then, and now, are a fundamental part of the material, intellectual and educational infrastructure of modern developmental biology. They differ from previous representations of development in their identification of criteria for determining whether an organism was normal and, if so, in which stage of development it was. Karl von Baer's mid nineteenth-century account of the normal development of the chick, produced before the introduction of normal stages to embryology, is therefore quite different to the Hamburger-Hamilton version of normal development (Hamburger and Hamilton 1951; Love 2010). So scientists need to be trained and develop experience working with such staging systems and organisms. In using the stages, they need to quickly process many samples, to determine whether they are 'normal' and to which stage of development they correspond.

Few texts question normal development and its role in biological practice, but the following excerpts attempt to make the position of normal development, in the methodological and ontological framework of developmental biology, more explicit:

> Various definitions of normality [...] depend heavily on an intuitive sense rather than on a systematic, empirical investigation of normal development. [...] One implicit assumption is that the course and outcome of development are universal and immutable. [...]This view leads to research that is focused on observed regularities and that neglects contextual differences. Thus, normal is defined as the typical rate of progression through common universal patterns that results in a typical outcome. Factors that alter the rate or end point of progression result in deviations from the typical and are considered the causes of abnormal development. This is at variance with the understanding of development achieved from a natural history orientation, which incorporates contextual factors into the explanatory system. [...] The assumption that

> normal development consists of a universal, relatively immutable, developmental sequence is challenged by the discovery of both *equifinality,* that is, the same psychological ability may arise from quite different developmental pathways and processes, and *multifinality,* a specific developmental pathway or process does not invariably result in the manifestation of one specific ability.
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> Michel and Moore 1995, 411-412

This challenge to normal development originated in developmental psychobiology, which is primarily concerned with the study of behaviour. It contains many pertinent points, however, as developmental psychobiology takes behaviour to be "a function of whole, living organisms," and therefore investigating the ontogeny of those individual whole organisms which manifest the behaviour is a central part of the enterprise (Michel and Moore 1995, 3). The three points made by the authors I wish to highlight are the arbitrariness of normal development (that it is not based on "systematic, empirical investigation"), that it is subject to a naturalistic critique (it "is at variance with the understanding of development achieved from a natural history orientation"), and that it fails to take account of the variation in the process of development which manifests itself in equifinality and multifinality. Their critique also highlights another concern with normal development. In accounts of development, the normal is not merely descriptive, it is normative. Georges Canguilhem noted these linked meanings of normal, observing that "[s]ometimes it designates a fact that can be described through statistical sampling [...]. And yet it also sometimes designates an ideal, a positive principle of evaluation, in the sense of a prototype or a perfect form (Canguilhem 2008, 122).

Many biologists, however, do not recognise the key role of normal development in the ontological framework of their discipline. For example, normal development is defined by biologist Jonathan Slack as "the course of development which a typical embryo follows when it is free from

experimental disturbance." With normal development understood in this way as a control based only on description, rather than appealing to any pre-conceived ideas of normality, Slack demands that "It must not be confused with pathways of development which give a normal outcome" (Slack 1983, 11). Contrary to Slack's definition, however, to establish normal development is not actually as simple as allowing development to unfold unmolested. Normal development is not simply a control, a non-manipulated arm of an experiment. It is, even in its simplest form at the outset of experimental work in embryology, a product of observation, interpretation, abstraction, a work of standardisation. It also has come to involve the shaping, production and maintenance of actual organisms acting as instantiations of normal development. Consequently, normal development takes on greater significance than a mere control. The conditions to allow this to happen must be constructed, and observed results abstracted to produce a standard. The conceptual distinction between the course of development followed when free from external intervention, and pathways resulting in a normal outcome, is not so easily made in practice. Assumptions regarding normal outcome help to establish what is normal in the first place. What is methodologically possible thus becomes what is theoretically possible. Pragmatic scientists will not explore what they cannot empirically investigate. Theoretical precepts that rule out the possibility that such non-investigable areas are biologically relevant provide a way to legitimate the narrowing of investigative focus (Hacking 1992, 55). What *cannot* be investigated, perhaps because of a belief that it is too difficult to do so, can be ignored (e.g. Love 2010).

One example of this, which I take to underpin the whole ontological, methodological and epistemic framework of modern developmental biology, is the instructive-permissive distinction touched on in the introduction. In this distinction, external, environmental factors cannot produce new *functional* forms of an organism (Gilbert 2003a). They can merely permit the instructive causes – internal to an organism – to act properly or not, by providing more or less clement conditions. If the proper permissive conditions are not in place, the result is not a new functional form, but an abnormal or

dead organism. Here and in the rest of this paper, I take functional to mean the level of functional performance exhibited by the organism as a whole (Amundson 2000), not modes of function or the performance levels of sub-organismal functions. In this sense, provided the overall functional performance is at least adequate, it does not matter whether certain parts of an organism are not functioning at a high level, or if the mode of functioning is atypical.

If the instructive-permissive distinction is accepted, it becomes epistemically unproblematic to ignore the environment by controlling and standardising it (rendering environmental factors invariant), and concentrating on manipulating certain variables internal to the organism, as there are presumed to be no significant causal relationships between organism and environment that are being missed. This has its roots in early experimental embryology, and accepting it allowed experimental work to be conducted. Hans Driesch's complex dynamic interactive picture of ontogeny precluded experimental investigation faithful to that theoretical background (Churchill 1969). The distinction and dichotomy between organism and environment, in turn, has its roots in the work of Auguste Comte and Herbert Spencer (Pearce 2010). Once the organism and its environment are distinguished, it becomes possible to attribute causes to one realm and mere permissible conditions to the other, thus enabling the development of the instructive-permissive distinction. The instructive-permissive distinction is a concept that is indissolubly ontological, epistemic, and methodological. It is ontological, because it makes a claim about what exists; primarily the organism, relative to which the environment is determined. Furthermore, it makes a claim about the nature of organismal development: that there exist two kinds of causes, those originating within the organism, and those originating outside the organism, and that the former are more important than the latter, and even determine which of the latter matter. It is epistemic because it structures beliefs concerning the knowledge of development that biologists deem it possible to attain, namely knowledge of the interaction over time of what can be demonstrated to be instructive causes with other instructive causes, and with permissive conditions. It is

methodological because it guides researchers by highlighting what possible variables might be relevant to a causal understanding of the developmental processes or features they are interested in, allowing them to identify some as permissive causes that simply need to be controlled, and others as candidate instructive causes that they may wish to manipulate and vary. On its own terms, normal development is, "in a greater or less degree the response of the developing organism to the normal conditions" (Wilson 1896, 326). We don't have to deal with the complexity and multi-dimensionality of the ongoing relations between organisms and their environments, and can unproblematically assume a distinction between the two.

What are the other elements of the existing orthodox framework of modern developmental biology? As I have noted, it is underpinned by a mutually-supporting framework of theoretical, conceptual, epistemic and methodological elements such as the instructive-permissive distinction, the distinction between organism and environment, and normal development. It delineates what processes, entities and potentially causally-relevant factors exist, which ones are significant, and what concepts make sense of them. It centres on the identification of differential gene expression as the central organising principle for understanding the production of a complex, heterogeneous adult organism from a simpler, relatively homogeneous organism at a prior stage of development (see Pradeu 2014 for a discussion and criticism of this central organising principle). This principle provides a way of resolving the central problem of experimental embryology first raised in the nineteenth-century of how a complex, differentiated organism is produced from a relatively simple undifferentiated single-cell, despite all cells containing exactly the same hereditary material.[4] In different cells, different parts of the genome are switched on or off, upregulated, downregulated, epigenetically marked, in a particular temporal order.

[4] This problem was perhaps most cogently stated, alongside an early version of the principle of differential gene expression, by Thomas Hunt Morgan in his 1934 book *Embryology and Genetics*.

Since the early twentieth-century, the orthodox framework has included concepts like normal development, differentiation, potency, fate, induction, and specification. All of these concepts are used and reproduced in practice by developmental biologists. Recent debates have questioned whether developmental biology is an enterprise in which it is possible to develop theories (Pradeu 2014), or whether it is organised differently, perhaps in terms of a problem or question-centred framework (Love 2014). By focusing on the challenges to, and the practices associated with, one of the elements of the previously sketched outline of a theoretical framework – normal development – I sidestep this debate. Whether these challenges lead to the formulation of a theory of development, or a reformulation of the problems guiding developmental biologists, is not important here. What is important here is whether key aspects of the existing framework, namely the central organising principle of differential gene expression, the instructive-permissive distinction, and the methodological role of normal development, inhibit the investigation of the role of variation in organismal development, and if so, what alternative approaches might be appropriate.

3. Problems with the instructive-permissive distinction

In this section I detail objections (explicit and implicit) to the instructive-permissive distinction. They imply that the instructive causes of developmental form may lie outside, as well as inside, the traditionally defined boundaries of the organism. Further, they indicate that there are problems with the way the existing framework of developmental biology conceptualises or treats variation. To identify what aspect of variation is relevant in a particular example, I have identified the following dimensions of variation within development:

1) Unit(s) – what is it that is recognised to vary? To what extent is it observed and measured to vary?

2) Temporality – over what timescales do the units vary, and what variation is there in timescales for a given mode?
3) Mode:
    a) Outcome – variation in outcome at defined point of the developmental process, usually an adult stage but not necessarily. Variation in the outcome mode is multifinality.
    b) Pathway – variation in the route taken by the developmental process, either to the same outcome (equifinality) or to different outcomes (multifinality).

The different ways these dimensions are conceptualised, measured, investigated, manipulated, standardised, and made background conditions constitute the management of variation. This involves the identification in biological practice of the boundaries of a system, and its partitioning, into organism and environment, or variables and background conditions. It also entails making judgements as to what is causally, and experimentally, relevant. Practices of establishing types also fall under this. For any given unit, a given temporality and mode is instantiated in normal development, and the management of variation – in terms of identification of variables to be manipulated, and background conditions to be controlled – is conditioned by assumptions about normal development as well. In the following examples, I identify what significance these assumptions have for the various ways in which variation can be dealt with. My aim in this is to show that a concept of normal development that relies upon the assumption that there is a single unit, temporality and mode of, and hence way to manage, variation is no longer sustainable, and needs to be replaced with concepts and practices which take account of the possibility that all of these parameters may also vary.

Units of variation - the microbiome's role in development

The need to take account of naturally occurring variation is central to the programme of research into the developmental and physiological effects of the dynamic interactions between metazoans (also known as macrobes) and associated microbial communities. Investigations into those interactions, and the role of microbiota in providing signals or cues as inputs into developmental processes, have inspired much recent philosophical work assessing its significance, some of which I touch upon shortly.

Organisms acquire communities of microbes by direct transmission from the environment, or by transmission into a (female-derived) gamete, or into the developing embryo (McFall-Ngai 2002, 2-5). The presence of certain microbes or communities of microbes has been linked to the maintenance of physiological function and avoidance of dysfunction (for example, concerning the gut, see Hooper et al. 2001; Blumberg and Powrie 2012), and even animal behaviour (Ezenwa et al. 2012). Furthermore, microbes are increasingly thought to play an instructive role in the development of the individual organism, and in differentiation and other countervailing processes (e.g. Blaser et al. 2013; Cho et al. 2012; Hooper 2004; Ito and Ohta 2015).

A simple, but classic, example is the association between the Hawaiian bobtail squid *Euprymna scolopes*, and the luminescent bacterium *Vibrio fischeri*. Early after hatching, *V. fischeri* colonise the juvenile squid in an organ, the light organ, at the centre of its body cavity. Once there, the bacteria interact with host epithelial cells, and colonisation by other species of bacteria is prevented by host immune cells. The host provides the bacteria with the sources of carbon, nitrogen and minerals it requires for survival and growth, and once it has established itself the community of bacteria bioluminesces. This light, on the underside of the squid as it feeds nocturnally, prevents the squid from casting a shadow from the moonlight, and therefore increases the squid's chance of avoiding detection by predators (Visick and McFall-Ngai 2000). The bacteria are provided with a nutrient-rich environment free from competition by other species, and the squid benefits from the

bioluminescence, which it could not produce itself. Importantly, the bacteria also play a role in the morphogenesis of the light organ (McFall-Ngai 2002, 8).

How does the microbiota associated with a host organism affect development? Firstly, there is the possibility that the host organism acquires genes from one or more microbes, a process known as Horizontal Gene Transfer (HGT). Genomic evidence indicates that as well as historically shaping genomes in at least some metazoans, this continues to take place today, and that the transferred genes are expressed (Crisp et al. 2015). Secondly, and undoubtedly more commonly, there are the epigenetic effects of microbiota on host gene expression, for instance by DNA methylation (Kellermayer et al. 2011).

John Dupré has argued that the role of associated microbiota in an organism's development and health means that the conceptualisation of organisms as monogenomic differentiated cell-lineages is limited and partial (Dupré 2012). The microbial communities that are associated in a generally stable (but not unchanging) way with a given macrobe have been conceived of as commensals (e.g. Hooper et al. 2001), symbionts (e.g. Ezenwa et al. 2012) or as part of a "holobiont," an "integrated organism comprised of both host elements and persistent populations of symbionts" (Gilbert et al. 2012). The latter view rejects the conceptualisation of organisms as "individuals." A similar view is advanced by Thomas Pradeu, who argues that the mechanisms underpinning the immunotolerance of symbiotic bacteria exist because the developing organism requires the presence of certain communities of microorganism at certain periods of development, important aspects of which continue even into maturity. As a consequence of this need for the host organism to tolerate and even acquire and incorporate microbes for their "crucial" and "indispensable" roles in development, the distinction between organism and environment is preserved, but only if the organism is considered to be the "unity" of a "plurality" (Pradeu 2011). The organism maintains its distinction from the environment,

by selectively incorporating parts of the environment into its own self, or to join the holobiont of which it is part.

If one does not accept this (and Pradeu's argument is dependent on accepting a particular hypothesis concerning the developmental function of certain immune system mechanisms), provided one does not make the assumption that the source of instructive causes must be internal to the organism, then microbiota surely contribute instructive causes. If, however, we do accept Pradeu's argument for the concept of the holobiont, the microbiota that manifest instructive causes in the developmental processes of a host are now considered to be internal to the holobiont or Pradeuian organism. If the composition of the microbial communities is specified by the holobiont as a whole, any instructive causes manifested by the microbial part of this could ultimately be attributed back to the holobiont, which selects the microbes manifesting instructive causes of some use to it. Even in non-pathological circumstances however, the host would not be in a position to completely specify the composition of the microbial communities with which it associated. Microbes that did not provide important instructive signals may be tolerated. Additionally, microbes that might have provided helpful signals may not be present. The availability of particular communities of microbes depends on environmental factors. Thus, the instructive causes exhibited by the microbes integrated into an organism would therefore rely, in part, on the environment outside of the original host organism before it is colonised. In many circumstances, therefore, the microbiota can be regarded as playing an instructive causal role, regardless of one's conception of the relationship between host and microbiota.

One of the key problems with the instructive-permissive distinction is that it allots the former part of the distinction to 'internal factors' and the latter part to 'external factors', or 'the environment'. It therefore limits the kinds of variation that are deemed to be important to investigate to elucidate the causes involved in the production of developmental outcomes. If the constitution of microbiotic

communities in organisms is conceived to have an environmental source (even if one does not consider the microbiome itself to be part of the environment) the role of the microbiome in development and health demonstrates that the instructive-permissive distinction as an heuristic means of identifying which variation is methodologically and theoretically important, is deeply flawed.

It is in the ongoing dynamic variation in and between microbial communities in different developing individual organisms, and their relationship to that developing individual organism, that scientists make sense of the reciprocal, iterative relationships between the macrobe and its succeeding microbiotic communities in the development of both. This requires not only a serious focus on the investigation and importance of microbiome variation, but also on the dissolution of the framework of developmental biology which is centred on the instructive-permissive distinction that inhibits biologists' ability to explore the variation in a wider range of ontogenetically-related units. The microbiome's role in development is one example of the way in which the formulation of new *explananda* require the forging of new *explanantia* and ways of constructing them. Two more examples are the phenomena of developmental plasticity and transdifferentiation.

Modes of variation - developmental plasticity and transdifferentiation

Plasticity is defined as "the ability of an organism to react to an external or internal environmental input with a change in form, state, movement, or rate of activity," and we might add, developmental fate (West-Eberhard 2003, 33). It therefore responds to, and creates, variation. The processes involved in developmental or phenotypic plasticity and the process of differentiation are opposing, the former widening developmental cell fate, the latter supposedly narrowing it (Bateson and

Gluckman 2011; Sánchez Alvarado and Yamanaka 2014).[5] Developmental or phenotypic plasticity has been demonstrated to be an evolutionarily primitive, rather than derived, state (Nijhout 2003). In evolutionary history there was therefore no default phenotype for ancestral forms of extant organisms, and in organisms that have retained plasticity this may remain the case.

Contingent events (which include stochastic events within the organism) are not 'accidents', and the response of the organism to them should be an important target of biological research, instead of merely studying the response to reliable, controlled, and predictable conditions. After all, developmental or phenotypic plasticity is the response of an organism to changing and varying environments and environmental factors. The phenomena of developmental plasticity suggest that organisms exhibit variation in the outcomes and pathways towards outcomes, such as the different phenotypes exhibited by the planktonic crustacean *Daphnia* depending on early exposure to chemicals indicated the proximity of predators (Gilbert and Epel 2009) or the formation of the different castes of social insects (Rajakumar et al. 2012). These are two examples in which environmental signals are causally efficacious in selecting between limited numbers of possible phenotypes. What is important about them is that there is not a default phenotype. An example which exhibits a greater range of possible phenotypes (and, within one individual organism, phenotypic heterogeneity) is angiogenesis and the formation of an integrated vascular network, in which the endothelium is in constant interaction with the tissue environments it encounters (Aird 2012). The variation in pathways and outcomes of development also includes heterochrony, a change in the timing of developmental events, which is defined as "a series of morphological states through which a given embryonic structure passes" that "reflect distinct changes in the embryo"

[5] Though, it must be stressed that 'plasticity' encompasses a large number of different processes and mechanisms that operate in different ways and in relation to environmental factors in different ways, so any inferences deriving from one kind of plasticity may not be applicable to others (Forsman 2014). I have tried to make my own general statements on plasticity to be ones applicable to most types of plasticity, and have stated otherwise when my comments concern particular types.

(Bininda-Emonds et al. 2002, 299).[6] An altered order of developmental stages or events may even be exhibited, a sequence heterochrony (see Blomquist 2009; Harrison and Larsson 2008; Jeffery et al. 2005; Smith 2002; Velhagen 1997, for methodological discussions concerning the construction, analysis and comparison of sequences).

Developmental plasticity depends on the capacity of the organism not merely to react to the environment, but to use cues from the environment as signals to be interpreted by developmental processes. These cues can therefore constitute instructive causes of the products of those developmental processes (Gilbert 2003a). The organism can therefore evolve developmental processes that allow them to detect cues and respond to environmental conditions in such a way as to produce a functional outcome (Whitman and Agrawal 2009, 12).[7] In this way, the phenotypic plasticity of the organism can be exploited to enhance fitness (Nijhout 2003). Given plasticity, the range of relevant variation (be it internally or externally induced) that can produce functional organisms at a given end-point is therefore widened – a wider range of variation becomes relevant for understanding the variation between forms of a given type.

As questions of the origin of phenotypic variation are central to evo-devo investigations, Love has criticised the ways that certain practices in evo-devo excessively abstract from variation. If too much variation is abstracted away, phenotypic plasticity would present itself to a far lesser extent. His proposed solution is to use a greater range of model organisms and create alternative periodizations or stagings, based on using different characters to those currently used in normal stages. This would ensure that periodizations are not collapsed into a single periodization only, and that, as a consequence, variations would be revealed rather than abstracted away (Love 2010).

[6] This concept of heterochrony is inclusive of more kinds of variation than the changes in size and shape dealt with by Stephen J. Gould and Pere Alberch (see Smith 2002).

[7] The type of plasticity in which the result of the environmental input is mediated by the developmental processes of the organism is known as active plasticity, and can be contrasted with passive plasticity, in which the environmental input (such as temperature) is directly proportional to the extent of the change effected (for example by increased temperature speeding up certain metabolic processes) (Forsman 2014, 3).

Another problem is that even though the production and distribution of particular strains of model organisms aim at standardisation and a reduction in variation between individual organisms, evidence indicates that considerable (biologically relevant) variation remains (Carlson Jones and German 2005). If it does – and it would take investigation of what variation exists and is biologically relevant to demonstrate this in particular cases – then to fail to take account of it in experimental design and the interpretation of results is to allow an unwarranted assumption and source of error to remain. If, however, the variation were controlled, measured and noted, or made into an extra experimental variable, not only would this error be avoided, but it might provide useful information that would have otherwise been missed.

Conventionally, the process of development is envisaged as a hierarchical process of differentiation. Germ-layers are specified, then tissues, then cell-types. Cells become ever more differentiated. With the exception of stem-cells, this is conceived as a one-way process towards terminal differentiation. However, growing empirical evidence suggests that this may not be the case, and that rather than being terminally differentiated, it might be more appropriate to say that mature cells exhibit stable differentiation (Sánchez Alvarado and Yamanaka 2014). There is growing interest in the phenomena of transdifferentiation, defined as the "transformation of one *differentiated* cell type into another" [italics in original] (Slack 2009). The discovery of transdifferentiation in plants and animals, aside from the promise it holds for cell reprogramming in the laboratory (Graf and Enver 2009), calls into question the view that differentiation is necessarily a hierarchical and one-way process, or even that a cell can be ever be described as fully differentiated (Sánchez Alvarado and Yamanaka 2014).

As with developmental plasticity, transdifferentiation leads one to consider the equifinality and multifinality of developmental processes. Given that equifinality and multifinality can be manifested in development, the variation in processes and outcomes of development, as well as the variation in

the (internal and external) factors and processes that affect these, is an important part of understanding organismal development. In addition to the variation in factors, there may also be variation in the reliability or stability of their presence in the life cycles of organisms. The variation in that reliability, and also in the temporality, may also be worthy of investigation.

Rather than transdifferentiation being merely an exception to the 'normal' model of hierarchical and irreversible differentiation, the causes underpinning it may in fact constitute one developmental process or tendency that interact or counteract with other tendencies as part of the developmental process as a whole. Other processes that may act in this way besides transdifferentiation may include stochastic processes in cells (Kupiec 2014). Due to the presence of potentially antagonistic or opposing processes and tendencies, the maintenance of a (stable) differentiated state is itself an active process – there is for example the need to faithfully transmit epigenetic marks through DNA replication and cell-division (Bateson and Gluckman 2011, 58).

The phenomena of transdifferentiation therefore directly challenge the central organising principle of differential gene expression. The product of differentiation is the variation in cell types at a particular point in time. As the product of the maintenance of these varied differentiated states, the variation in cell types can be attributed to the ongoing active stabilisation of that variation (Minelli 2014). Therefore, the existence of that variation can be attributed to a wider set of causes beyond the genealogy of a particular cell, and its history of differential gene expression. To understand the existence, persistence and potential fate of differentiated cell types therefore requires investigation of a greater range of causal factors than at present. Furthermore, in challenging the conception of development as a one-way process with a default way of unfolding, the phenomenon of transdifferentiation lends support to critiques of "adultocentric" conceptions of development in which development is conceived as the finalistic process of producing an adult from an egg (Minelli

2003 and 2014). Such critiques highlight the need to consider the variation that may be apparent and significant over different temporalities.

Management of variation - experimental conditions and assessing causal factors

Standard laboratory methodology dictates that factors which are not being tested must be held constant, as invariant background conditions, while one is conducting a scientific experiment. However, while this reduction in the variation of factors may help to improve test sensitivity, there are indications that it may hinder the reproducibility of results. For example, Richter et al. (2009) argue, based on data analysis they conducted on behavioural research conducted in multiple different laboratories, that despite the considerable efforts to standardize conditions between laboratories, this is not in fact possible, and that the effect of this is that they "standardize to different local environments." Consequently, the generalizability and reproducibility of findings produced in such circumstances must be called into question.

An alternative to strict standardisation they propose is the employment of "systematic environmental variation," or "heterogenization" (ibid.).[8] Here, changing the practices of managing variation in the experimental set-up is crucial. While scientists are assiduous in ensuring that every conceivable variable in the 'background conditions' are controlled, it may not always be possible to either control known variables or even apprehend certain variables. The craft of working in a particular laboratory ameliorates this somewhat, but the strategy of heterogenization, of incorporating a wider array of explicit variation in the experimental set-up, provides a way to ameliorate it still further. The history of agricultural science demonstrates that when scientists have needed to deal with multiple environmental variables, which they may not be able to sufficiently control but need to take account of, they have been able to develop methods of managing this

[8] The proposers of heterogenization believe that it is just as valuable a strategy within a single laboratory as between multiple laboratories.

variation. As an example, in pursuit of the "epistemic goal" of managing "variable environmental conditions," agricultural researchers in the first half of the twentieth-century in the UK developed (sometimes competing) techniques of statistical analysis and experimental design, such as the analysis of variance (ANOVA) tests and factorial experiments testing different combinations of factors (Berry 2015, 247; Parolini 2015, 266-270 & 274-275). For heterogenization in the laboratory, other tools may need to be produced, including tools to identify exactly what variables might be appropriate, and what can still be confined to controlled 'background conditions'.

One of the strengths of James Woodward's manipulationist account of causation is that it allows one to distinguish between causal factors by various criteria, rather than merely distinguishing between causes and non-causes (Woodward 2003, 2010; the framework was used to analyse the role of causal factors in development by Waters 2007, and in response to Waters by Griffiths and Stotz 2013). The criteria are specificity, stability and proportionality. Rather than be content with qualitative and intuitive comparisons of candidate causes in these terms, some researchers are developing formal methods to quantify specificities (Griffiths et al. 2015). This modelling involves identifying the relationship between the range of variables of a particular cause, and the range of variables of a particular effect. The measure of specificity depends on what is included as part of the experimental variables, and what is excluded, and therefore part of the invariant background conditions. Such a method, if developed, could provide us with insights into the relative importance of particular causal factors for particular developmental events or processes. It makes explicit what is being backgrounded and made invariant, and what the consequences are of this. To identify what should be backgrounded, measures of specificity (incorporating ranges of variation for particular variables) will be required for a multitude of factors. The importance of variation is therefore foregrounded in this approach.

This has two important implications for our main theme, the ways of managing variation and how that relates to the prevailing framework of developmental biology. Firstly, rather than the world being divided into causes and conditions, taking the Woodwardian approach entails seriously considering a world in which putative causal factors for any given phenomenon are assigned greater or lesser weight or strength. In the light of this, the instructive-permissive distinction seems obsolete and an obfuscation, providing an inappropriate and misleading model of causation for the analysis of complex, multidimensional and context-sensitive biological systems. Secondly, an alternative criterion to the instructive-permissive and organism-environment distinctions is presented and made available. For pragmatic reasons, in an experimental set-up, many variables need to be made part of the background conditions and therefore controlled and if possible kept constant. Empirical identification of how the constellation of variables might be partitioned in a given experimental context into experimental variables and background conditions means that experimenters need not rely on the ontological assumptions that alternative partitioning strategies or assumptions like the instructive-permissive distinction bring with them. In the absence of any set of given universal background conditions, normal development would therefore be relativised to the particular experimental context, and the background conditions empirically identified by a prior assessment of the relative strength of putative causal factors.

The preceding examples have shown that it is no longer valid to strictly partition the world into a dichotomy of internal and external. The way that variation is managed, and the causal relevance of certain types of variation for given outcomes of interest, should depend instead on a more explicitly articulated justification for dividing the system up into potentially relevant causes and background conditions. More varying factors would be incorporated into the realm of potentially relevant causes than in the analogous category of internal, and there would need to be more explicit reasons within the context of the research situation for making certain potentially varying factors invariant. The

ways of measuring causal specificity and the strategy of heterogenization offer ways to do this that can be explored and developed.

4. Opportunities and possibilities

All of the preceding objections are rooted in a rejection of the instructive-permissive distinction. They are bolstered by empirical data that suggests an instructive role for 'signals' from the environment in development (Gilbert 2012; Gilbert and Epel 2009), and there have been philosophical critiques of the distinction, such as the causal parity thesis (Griffiths and Gray 1994; Oyama 2000). The distinction locates the cause of developmental form within the organism, and underpins a normative sense of normal development based on a restricted range of variation, and allied concepts. The instructive-permissive distinction is one way of conceptualising variation, and of identifying what variation is relevant and not relevant for the investigation and explanation of particular developmental phenomena. It therefore plays a key role in structuring how variation is dealt with in the practices of experimentation in developmental biology.

The examples given in the preceding section indicate that the way in which the distinction conceptualises and manages variation is questionable. It precludes us, at least some cases, from apprehending or investigating the origin and significance of the range of variation present in nature, not merely in and between genomes, but also other manifestations of variation. The role of the microbiome and the phenomena of developmental plasticity and transdifferentiation call into question the idea that there is a single unit, mode and temporality to developmental processes in any given type (such as, but not restricted to, a given species or variety) of organisms. These, and the new methods proposed in two of the other examples (heterogenization and assessing the causal relevance of potential variables), undermine the justification for unequivocally identifying the unit of

variation by partitioning based on a firm distinctions between organism and environment, internal and external, or even intrinsic and extrinsic factors. If context is important (in the sense of being instructive), and it varies, this variation must be investigated. The distinction therefore prevents us from developing the tools and knowledge to investigate that variation and its role in health, disease, conservation, and other fields where a more naturalistic understanding might bear fruit in the laboratory, and from the laboratory to arenas of human activity. Consequently, it will be productive to identify ways in which variation can be conceptualised and managed in experimental set-ups (aiming to explore particular questions) that are congruent with the way that variation has been demonstrated to be relevant across the full range of its dimensions.

I am not new in desiring a biology which incorporates a wide range of interacting varying factors. In fact, at the very dawn of biology as a science, G. R. Treviranus outlined such a programme (Grene and Depew 2004). This failed to happen because the statistical and computational tools were not present, and it was not possible to produce the amount of data needed for findings to be statistically significant. Furthermore, the experimentalisation of embryology which might have newly instantiated such a desire, needed to introduce the instructive-permissive distinction and its methodological and epistemic consequences in order to proceed. For early experimental embryology, the instructive-permissive distinction was a way of managing variation. At that point the technical means to investigate the full complexity of ontogenetic processes that were becoming apparent were either absent or underdeveloped. This, I contend, is no longer the case, and the instructive-permissive distinction, which has become entrenched in developmental biology, is no longer required. We have the computing power and the statistical tools to deal with multivariate complexity. We are now aware of the shortcomings of the instructive-permissive distinction, and have the means to produce, analyse and interpret large amounts of data concerning embryonic development (and increasingly, in an automated way). The latter point refers to the linked developments of computational methods in biology (Stevens 2013), and the rise of ‘Big Data’. Some

accounts of ‘Big Data’ argue that it will revolutionise practices in a wide range of areas, including science (Mayer-Schönberger and Cukier 2013). When considering biological research, however, continuities between modern (e.g. ‘Big Data’) and historical data practices are apparent (Charmantier and Müller-Wille 2014; Leonelli 2014a). ‘Big Data’ infrastructure and methods, however, can and will “enable scientists to spot patterns and trends in new ways,” which will at least provide a guide towards further research, if it cannot be considered an end in itself (Leonelli 2014a, 8).

One historical example of the generation of large number of samples and data is T. H. Morgan’s fly lab, which operated in the first half of the twentieth-century. In Robert Kohler’s (1994) account of the lab and its work, he emphasised the importance of what he termed the “breeder reactor” in generating a large number of flies for inspection. Given the sheer number of the flies, even rare mutations became apparent. Once investigators were aware of a particular mutation, they were primed to observe it when it appeared again. Large numbers of samples may reduce the variance exhibited in a particular population of samples, but they can increase the number of variants observed. Methods of dealing with high quantities of samples have been slow to enter large areas of developmental biology, but they are being developed and used (e.g. see Tills et al. 2013) and they have the potential to transform our views on the extent and significance of variation in organismal development.

Emerging model organisms offer the possibility of producing models and standards better equipped to deal with scientific approaches which investigate the role of context and variation in development (Bolker 2014). New methods to identify standard events (such as the Standard Event System of Ingmar Werneburg 2009, which features standardised checklists for the presence or absence of particular structures or processes at particular times in development) or developmental steps (proposed by Gerhard Scholtz 2012) would allow developmental variation to be identified and

assessed, rather than missed as a consequence of dividing the developmental process into highly abstracted stages. Automation of processing of embryos could allow a greater number of embryos to be assessed in this way. Automation produces an incentive towards standardisation and the reduction of variation to ensure that samples are uniform enough to be processed in a standard and efficient way. Consequently only part of the process (for example, actual videoing of samples, and computerised three-dimensional morphometric analysis) could be completely automated. The rest of the processing of samples would necessarily involve human labour and judgement. Even this level of automation would get past some of the problems associated with the human staging of embryos. Such problems include focusing on one feature as a primary criterion and assuming that all other features associated with it at a particular stage are also present or absent. This is a custom borne of practical need but it inhibits the apprehension of variation, such as variation in the timing of the appearance of particular structures.

There remain many problems. One is that if experimenters were to measure more variation, and use this to divide up samples, they will be left with very small sub-samples, below the numbers needed for statistical significance.[9] Another is that if biologists were to produce local standards, aside from the practical considerations, it raises problems concerning how the data produced using those local standards can travel beyond the context in which they were produced. *In silico* methods offer a possible solution, but these approaches still require the input of high-quality data. Additionally, in part to satisfy community standards, *in silico* methods require *in vivo* validation after results are produced (Leonelli 2014b). Inspiration might be drawn from tools that are developed to integrate small datasets produced in different contexts for different purposes, an acute issue in ecology (but

[9] There are numerous ethical implications of the changes in practice suggested here. Firstly, the number of animals needed for particular experiments will, in the absence of alternatives being used, increase considerably, counteracting attempts to reduce the number of animals used in research, and potentially coming up against legislation which, in the UK at least, strictly calculates the numbers of animals for particular experiments based on statistical significance. Another consideration is that it may even be deemed unethical to reduce the sample size and therefore the number of animals used, because for particular questions asked this may reduce the quality of the results produced, due to not taking the variation of a number of potentially important factors into account.

also plant science, see Leonelli 2008a). Part of the solution there is to formulate and adopt standards concerning data collection, the recording of the "structure, content and appropriate usage" of data, and the development and use of software to ensure that data are able to travel outside its particular context of production (Madin et al. 2008, a discussion of the challenges of using ontologies in ecology; see also Leonelli 2008b).

The elaboration and development of some of the methods outlined above could generate new ways of producing and using normal development, ones that encompass all variation deemed to be normal. These would be normal developments that would incorporate the observed results of the exposure of embryos to multiple contextual environments. They would therefore be more ecological or naturalistic normal developments that would not instantiate or reproduce the instructive-permissive distinction. The question that should guide the abstraction process to produce new normal developments should be: does the organism show the requisite plasticity to remain functioning at an appropriate level?[10] This would invert the relationship between attributions of functionality and normality, as the functional would become the standard for the normal, rather than the normal being the standard for the functional, as at present (Wachbroit 1993). If attributions of functionality have been based on what has been considered to be normal, however, we are faced with a problem. On what do we base our attributions of functionality, if not pre-existing conceptions of what is normal? The problem is reminiscent of over 150 years of debates in biology on the criteria and basis for the determination of homologies, and this history offers rich conceptual resources for suggesting ways out of the problem of determining functionality (e.g. Griffiths 2007). Developing a standard of functionality that determines what is counted as normal would be challenging, as attributions of functionality are often dependent on context and mode, rather than overall level of functioning (Amundson 2000). Here, the insights of Michel and Moore concerning the equifinality and multifinality of development can help clarify what would not be part of a functional standard – it

[10] A question that in a different form was at the heart of Canguilhem's analysis, inspired by Kurt Goldstein, of the distinction between the normal and the pathological (Canguilhem 2008).

would not include specific modes of existence, or specific routes between two modes. Instead of being concerned with particular functions, metrics of functional level could be produced. These would identify the overall level of functioning exhibited by the organism (or part thereof), from which the range of what counts as normal is derived. Marcel Weber suggests a further constraint, arguing "that the relevant counterfactuals that guide causal selection in biology are counterfactuals that describe interventions that are *biologically normal*" (Weber, 2013: 31; emphasis in the original). Such interventions are identified as being those "that could naturally occur as part of the normal biological functioning of an organism." This would still require a judgement to be made by investigators, but rather than the implicit and intuitive judgement of normality that Michel and Moore rejected, this would be explicit, and open to critique (for one example, see Hall 2014).

5. Conclusion

Since the late nineteenth-century, the allied ontological concepts and practical precepts associated with normal development and the instructive-permissive and organism-environment distinctions have enabled experimentalists to investigate and construct robust explanations of various aspects of organismal development in a wide variety of organisms. The examples I have provided indicate that the central organising principle of developmental biology – that development is predominantly explicable through differential gene expression – cannot be sustained. Additionally, the examples I provided indicate that, insofar as the treatment of variation according to that model of practice is concerned, there are problems with this framework. Primarily, this is because evidence is accumulating of the ontogenetic significance of variation in units (for example, in the microbiome and its effects on the host or holobiont) and modes (the cases of developmental plasticity and transdifferentiation I discussed) that cannot be captured by a central organising principle of differential gene expression or the instructive-permissive distinction. Furthermore, new experimental approaches such as heterogenization and methods of causal analysis not relying on a

prior partitioning of a system into organism and environment and instructive and permissive causes present opportunities for forging new investigative approaches in developmental biology. How much the appearance of problems with the prevailing framework of developmental biology is due to different questions being asked by biologists concerning organismal development, to the availability of new methods and resources, or because of fundamental problems with the framework itself (even for the problems it was originally formed to tackle appropriately) is an open question.

I have shown that the various examples which question aspects of the orthodox methodological and theoretical frameworks in developmental biology can be interpreted in ways that bring them into coherency by focusing on the role of variation in experimental and conceptual practices. I have shown that more dimensions of variation in addition to variation in differential gene expression needs to be incorporated into what is counted as normal development. This insight allows us to suggest ways in which practices might change, foreshadowed by actual approaches already developed or in development. This would provide a way of operationalising the positive elements of the critiques of the standard framework, as well as highlighting what unites the various diverse critiques.

I have already begun the task of identifying some changes (and conceptualising them as part of a wider whole), but this requires considerable development, elaboration, and collaboration.
At the moment the formal fiction is that there is a formal boundary between normal development as a (descriptive) standard or control, and a more normative conception of normal development that is grounded in the instructive-permissive distinction and an ontological separation of organism and environment. Only by highlighting the links between a conception of normal development tightly linked to the prevailing problematic theoretical framework of developmental biology and the role of normal development as a standard in biological experiments can we begin to identify how standards of normal development can be developed that minimise those links. I have advocated basing new

standards of normal development incorporating different dimensions of variation on determinations of functional level. To further these proposals, work will be required to elaborate how these different standards can co-exist, and how functional level is to be determined for structures and processes over different time frames of ontogenesis.

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