Rediscovering Waddington in the post-genomic age

Operationalising Waddington's epigenetics reveals new ways to investigate the generation and modulation of phenotypic variation

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Introduction

Conrad Hal Waddington was a revolutionary interdisciplinary thinker well ahead of his time. Many of his ideas have been subsumed into our current understanding of developmental biology [1]. His pioneering theories, first published in the mid-20th century, continue to find validation 50 years later in the molecular era of developmental genetics [2]. Among his many contributions, Waddington [3] introduced the term *epigenetics* to describe the full variety of emergent[1] developmental phenomena above the level of the genome, and elegantly expressed these ideas in the form of his widely recognised and explicitly evolutionary epigenetic landscape metaphor [3]. These emergent phenomena bridge the gap between genotype and phenotype, and comprise the epigenotype [5]. Because of this close relationship between development and evolution, it is important to grasp how such epigenetic mechanisms function.

The diverse use of the term *epigenetics* in the subsequent literature has led to substantial disagreement about what exactly is being discussed, and at which level(s) of inquiry, despite several attempts to achieve consensus [6, 7]. In most contemporary biological contexts, epigenetics refers to chromatin modification [8]. Not only does Waddington's more inclusive definition appear to have been largely abandoned, also the different uses of his term have coincided with the near disappearance of the original concept of epigenetics from models of evolutionary change [9]. We see this as a potentially significant problem for evolutionary biologists.

In this essay our focus is on the theoretical concepts originally specified by Waddington's epigenetics. We argue that, in this age of powerful post-genomic laboratory and bioinformatics tools, epigenetics sensu Waddington is more informative and instructive than it has been for decades. Waddington's epigenetics has the potential to shed new light on the means by which both selectable variation and innovation, two key features of evolutionary theory, are

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[1] By *emergent*, we intend a holistic perspective in which entities with different properties are modified, re-shaped or transformed by their participation in the whole [4]. The result of interactions between lower-level entities in a hierarchy is a higher-level outcome that is not directly predictable on the basis of a reductionist understanding of the lower-level constituents. On this account, for example, a thought might be reducible to its biochemical basis, but the understanding of this basis would not provide an explanation of the meaning and intention behind the thought. Similarly, a forest might be construed as an emergent phenomenon resulting from the interaction of many individual trees.

generated, and bears careful consideration in light of new systems approaches to developmental biology [2, 10]. Indeed, Waddington's epigenetics is fundamental to the evolving theoretical framework of evolutionary developmental biology. We describe a research programme that is uniquely equipped to explore the generation, through development, of selectable variation at the organismal level that makes use of Waddington's epigenetics framework.

Waddington's epigenetics, the epigenotype and the epigenetic landscape

Waddington [3] proposed the term *epigenetics* to refer to the study of the complex set of emergent phenomena, produced by mechanisms operating between entities that result from developmental processes which help to connect genotype to phenotype. He described this research programme as the 'causal study of embryological development' [3]. He was specifically interested in the mechanisms by which a particular genotype results in a particular phenotype. He was acutely aware of the role of the products of developmental processes, as phenomena separate from the genome, in establishing and modulating that phenotype [5]. This level of analysis, above the level of the genome, is a key component of modern evolutionary developmental research.

Waddington [3] described a system in which, as development proceeds, individual cells of an organism follow particular paths at certain junctures. The decisions taken at these junctures determine the phenotypic outcome of a particular cell. The results of a classic series of experiments using fruit flies [11–13] led Waddington to propose that cells have a tendency to follow favoured paths to particular steady states, and that these paths are heavily buffered against external perturbation. Waddington [11] introduced the term *canalisation* to describe this genetically controlled ability to hold the mean phenotype unchanged in the face of environmental disturbances or genetic changes [14]. He went on to suggest that canalisation helps to produce an integrated phenotype, where the various parts of an organism exhibit correlated variation and are organised to form a functional whole at the end of ontogeny. Here, Waddington is specifically discussing the properties of developmental architecture that exist above the level of the genotype in order to modulate and control phenotypic outcomes. Developmental stability, the tendency for development to follow the same trajectory under identical genetic and environmental conditions and therefore minimise the generation of phenotypic variation, is a closely related concept. Canalisation refers to the suppression of phenotypic variation among individuals, while developmental stability refers to the suppression of phenotypic variation within individuals [15, 16].

In addition to his theories on the types of developmental constraints that help to ensure viable phenotypes, Waddington [3] elaborated on the concept of developmental noise. This was characterised as variation about the mean phenotype that is not directly attributable to genetic or environmental factors. Developmental noise might also be construed as imprecision, or perturbation within the developmental trajectory of an individual, resulting from processes that oppose those that buffer and stabilise development [15]. Essentially, this noise is a manifestation of the complexity of developmental processes.

Waddington was always aware of the multidimensional nature of development and the multifactorial nature of induced variation. *Epigenetics* was not intended to specify a particular mechanism, or even a group of such mechanisms, but rather a collection of emergent phenomena that bridge the gap between genotype and phenotype. These phenomena relate to each other through a set of hierarchical and interconnected mechanisms, and no one particular process has claim to the actual word *epigenetic*. Collectively, these emergent phenomena constitute Waddington's [5] hypothesised *epigenotype*, the understanding of which is critical to elucidating the relationship between genotype and phenotype. A focus on epigenetics as the causal analysis of development allows access to an important and largely neglected level of analysis in our understanding of the developmental trajectory of an individual. This level of analysis encompasses key aspects of the research programme of evolutionary developmental biology (Fig. 1).

We characterise epigenetics sensu Waddington as a collection of physical interaction(s) between the products of developmental processes that alters their final form. The products of developmental processes may be thought of as physical entities that interact with each other, resulting in outcomes that are not directly predictable based upon the developmental genetic

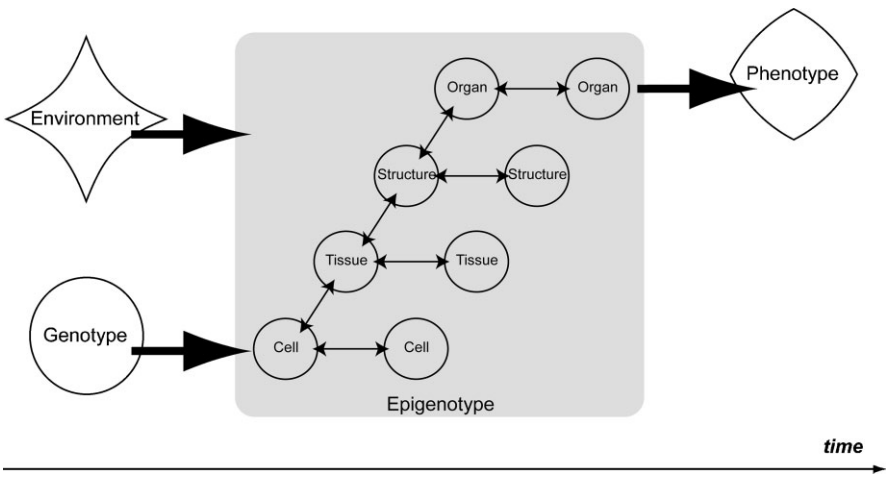

Figure 1. Epigenetics sensu Waddington [3] and as construed in the present essay. Small arrows represent epigenetic interactions among parts of a developing organism. The complete assemblage of these interactions is grouped as the epigenotype.

underpinnings of these entities. This notion is very similar to Newman and Müller's [17] definition of epigenetics as the interactions of cells and tissues with each other and with their environments. While not referring specifically to epigenetics, Oster and Alberch [18] likewise proposed that physical interactions of tissues with their extracellular matrices are critical determinants of the outcome of developmental processes, a notion that has recently been empirically demonstrated [19]. As Müller and Newman [20] have argued, the study of the physics of development is a vast and barely tapped area that has great potential for understanding the developmental basis for phenotypic variation and evolutionary change. We suggest that this gap in knowledge results in part from the absence of the epigenetic perspective articulated by Waddington.

Waddington proposed a metaphor [3], the *epigenetic landscape*, based on Wright's [21] adaptive landscape, to describe how developmental processes span genotype and phenotype. The epigenetic landscape is a sculpted surface offering a choice of several developmental pathways, represented as valleys, to an individual cell, represented by a rolling ball. The surface of the landscape is underpinned by a complex system of interactions resulting from the actions of gene products. As ontogeny proceeds, the number of available pathways for the rolling ball increases, representing differentiation possibilities for the cell. The curvature of the valley floor represents the intensity of canalisation along a particular pathway. Waddington proposed that natural selection would result in the construction of a landscape that filters, or selects among, the phenotypic effects of available mutations. In Waddington's time it was not possible to identify the processes responsible for modulating epigenetic landscapes, largely because of technological constraints. Now many of these methodological barriers have been overcome by modern molecular and bioinformatic techniques [2], and it is time for evolutionary developmental biologists to revisit, test and expand on Waddington's theoretical work and hypotheses, and to use epigenetics sensu Waddington as a stimulus to develop new post-genomic theories.

Epigenetics, evolvability and evolution

A complete theory of evolution must account for both variation and innovation [22] as materials for selection to act upon. Variability [14, 23, 24] is defined as the tendency to vary, and the variability of a system describes its ability to generate selectable variation. Thus evolvability depends directly on variability. Evolvability is the capacity of a system to evolve in response to selection [24]. While some determinants of evolvability, such as the presence and architecture of genetic variation, fall within the domain of quantitative genetics, evolutionary developmental biology deals with the generation of selectable variation through development. Elucidating the developmental determinants of evolvability is, therefore, a core question at the centre of evolutionary developmental biology [25]. A similarly important question, and one that is gaining increasing traction in evolutionary developmental biology, refers to the mechanisms behind innovation, which result in novel phenotypes [22]. A focus on the emergent phenomena of development illuminates both avenues of inquiry, because it is the interaction among the mechanisms acting at multiple levels to generate these phenomena that allows, or inhibits, the generation of both variation [26–28] and novelty. For the remainder of this essay, we focus on the variability aspect of evolutionary theory. We direct interested readers to the work of Müller and Newman [22] for a thoughtful treatment of the role of epigenetics in the study of innovation and novelty.

The variability of biological systems is the foundation upon which much evolutionary change occurs. Canalisation, developmental stability and morphological integration are the three major tendencies that result from developmental architecture that the study of variability attempts to explain [28, 29]. The mechanisms which are responsible for these tendencies can cause shifts in the direction and magnitude of variation generated during organismal development. In turn, such shifts in direction and magnitude of generated variation help to determine a population's evolvability [23, 24]. Waddington [3] characterised the mechanisms producing these tendencies as 'intangible internal sources of variation'. While the exact nature of these mechanisms remains elusive [16, 30], modern developmental-genetic, morphometric and bioinformatic tools, among other resources, are now beginning to be able to recognise and compare the morphological signatures, such as phenotypic covariance, of these mechanisms.

Put simply, epigenetics is the study of emergent mechanisms in developmental systems. Most such mechanisms are also likely to be developmental determinants of evolvability. It is essential to consider the hierarchical and mutually informative relationships between these mechanisms and their resulting emergent phenomena, at the level of analysis specific to the question under consideration, as we seek to understand both the process of development and the *evolution* of development. Changes in these patterns of relationship are the key elements that allow development itself to evolve. By extending Waddington's epigenetic landscape metaphor to include these higher-level interactions (Fig. 2), we can appreciate that an epigenetic landscape underlies each level of organismal organization. These landscapes are arranged in a hierarchical manner such that mechanisms acting at a particular level influence levels both above and below. Within a population, variability, and by extension evolvability, result from the superposition of all epigenetic interactions, overlain upon genomic instructions, and influenced by the external environment.

Waddington's concept of epigenetics as a field of inquiry focused on the complexity of developmental mechanisms that operate between the genotype and phenotype. Thus, his concept fits squarely into the theoretical framework of contemporary evolutionary developmental biology. A broad construal of those processes that do not directly impact the genome, but instead generate the epigenotype, as originally proposed by Waddington, is the proper focus of epigenetic studies. Such studies occupy a unique place within biological inquiry, providing the means with which to frame novel hypotheses at the appropriate

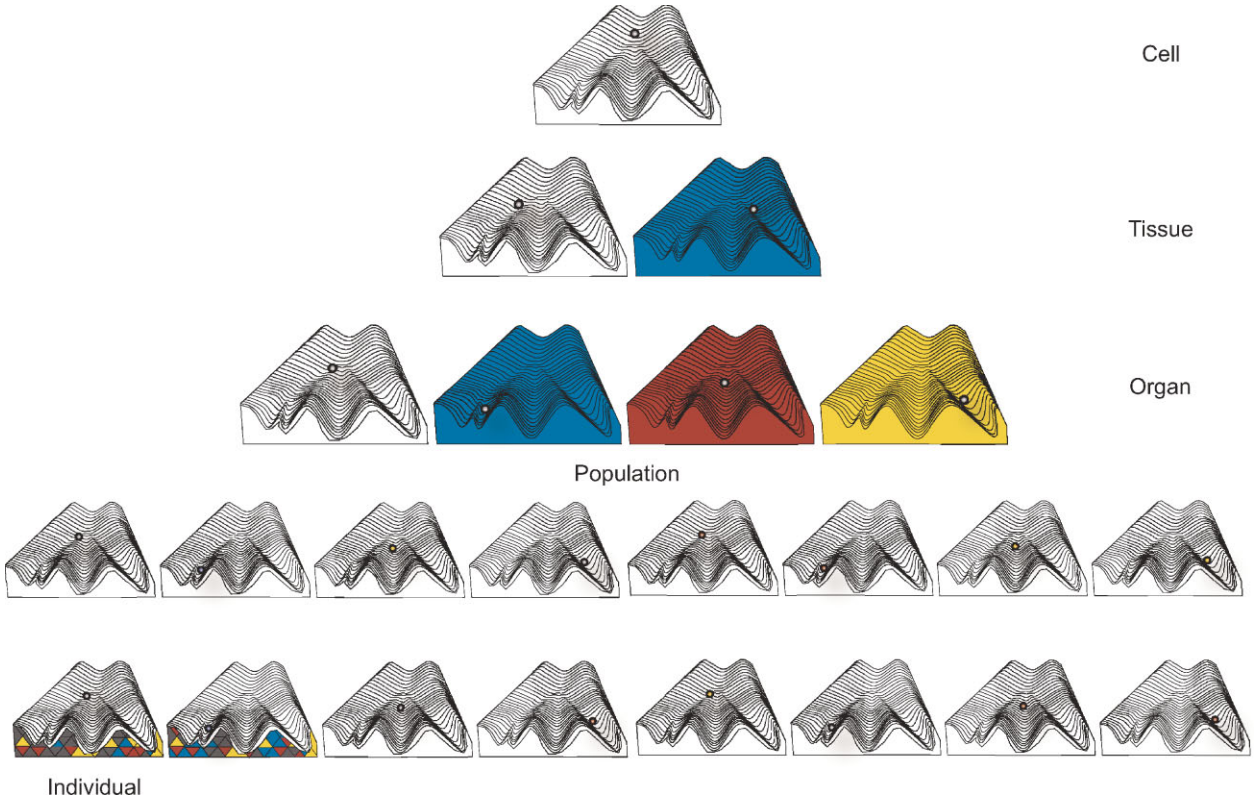

Figure 2. Waddington's epigenetic landscape extended to an evolutionary framework. Epigenetic mechanisms operating at multiple hierarchical levels form the underpinning of the organismal epigenetic landscape. Each organism has a different underpinning, represented by the combination of coloured landscapes under the final landscape, resulting in selectable variation within populations.

level of analysis and elucidate answers to previously unapproachable questions about the evolution of development. Waddington's epigenetics, effectively a systems biology approach to the study of development, was thus the forerunner of an area of increasingly intense research interest in evolutionary developmental biology that is unconstrained by a gene-centric worldview.

An epigenetic research programme

Following the interpretation presented here, Waddington's [3] original epigenetic landscape provides a practical way to frame evolutionary and developmental questions. This approach has already proven powerful in answering complex evolutionary developmental biological questions, some of which are long-standing problems that have previously eluded study. For example, Lieberman *et al.* [31] were able to test and validate the hypothesis that spatial packing in the cranium influences craniofacial shape by examining mouse models with mutations affecting craniofacial growth. They determined that the cranial base angle is variable and that it changes in order to accommodate shape changes in other parts of the head, specifically the brain and the face, regardless of the underlying genetic cause of those changes. This work showed that physical epigenetic interactions between the developing cranial base and other parts of the developing head are at least as important in determining organismal form as are the developmental processes that underlie the generation of the interacting parts [31, 32] (Fig. 3A and B).

Mammalian molars constitute one of the most thoroughly studied anatomical systems using an evolutionary developmental approach, and provide a second powerful example of how emergent developmental phenomena help explain evolvability. Cusp development is controlled by signalling centres formed by densely packed cells, the primary and secondary enamel knots, which interact with surrounding tissues by secreting molecular signals [33]. Every molar cusp expresses the same family of genes, which means that there are no specific genes that identify individual cusps. Instead, cusp size and pattern results from activation time and position of enamel knots; small changes in the spacing of earlier cusps can increase or decrease the number of cusps and the size of later cusps [34]. Such epigenetic interactions among intermediate products of development (enamel knots and surrounding tissues) thus participate directly in the generation of variation, and are able to influence the evolvability of tooth cusps. The distolingual cusp in the upper molar (the hypocone), for example, has evolved many times in unrelated mammalian groups [34].

Other body systems, including both hard and soft parts, have also been shown to be influenced by emergent developmental phenomena. Vertebrate limbs, for example, exhibit self-organising behaviour that results in the generation of particular anatomical patterns [35]. This self-organisation is followed

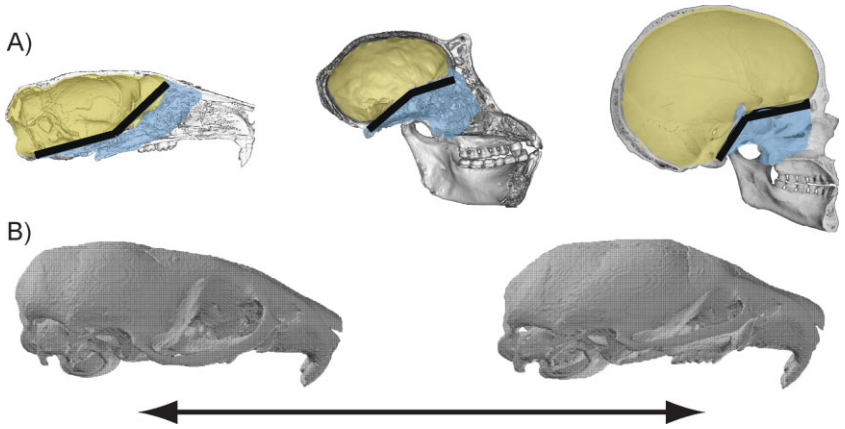

Figure 3. An example of an epigenetic interaction as construed herein. **A**,**B**: The cranial base angle is modified in response to brain growth, which in turn affects facial shape. Testing this hypothesis across a variety of mouse strains reveals that almost all variation (~87%) in the cranial base angle can be explained by three-dimensional neural and facial packing [31]. Physical interactions between brain, face and cranium thus influence the final shape of the skull in ways that are unpredictable by genetics.

by the establishment of biomechanical regulatory processes that maintain and expand upon the basic pattern. Another example can be drawn from the study of the developing heart [36]. Biophysical processes related to hemodynamics have a direct impact on the timing of differentiation of developing cardiac tissue, and thus on the developmental trajectory of the conduction system of the heart. All of these examples illustrate the pervasiveness of emergent phenomena in shaping the outcome of development within individual body systems and at the level of the whole organism. Research programmes that emphasise an epigenetic approach are thus uniquely positioned to provide novel insights into the generation of variation in development.

Conclusion

The concept of epigenetics as articulated by Waddington is the study of emergent phenomena that drive the generation of selectable variation. New phenotypes necessitate new developmental patterns; development must evolve for adult morphology to evolve. The process and evolution of development are therefore important to understand because propensity for change in developmental architecture allows development itself to evolve. Waddington's epigenetics is a central focus of evolutionary developmental research. It has the potential to shed new light on the means by which both selectable variation and innovation, two key features of evolutionary theory, are generated.

This middle level of activity, bridging genotype and phenotype, has both intrinsic and genetically determined sources of variation. In other words, the study of variability in the epigenotype will facilitate the study of evolvability. The genomic approach provides only an incomplete picture of the relationship between genotype and phenotype. Similarly, examining the phenotype in isolation does not afford sufficient insight into the means by which it is established and how variants are generated. A return to Waddington's original epigenetics and a focus on the epigenotype results in a 'middle-out' approach, which combines elements of genomics with quantitative phenotypic study while focusing directly on the developmental processes that connect the two at the most appropriate level of analysis. A 'middle-out approach' allows evolutionary developmental biologists to establish novel hypotheses and approaches that better bridge genotypic and phenotypic variation. Current work in developmental biology is rapidly deciphering some of the genetic mechanisms behind developmental processes, but few researchers focus on the generation of selectable phenotypic variation. This is a significant gap in our knowledge. Focusing on the epigenotype will allow us to identify sources of internal variation, and understand how these sources ultimately contribute to observable phenotypic variation.

The examples presented above illustrate that there are crucial levels of analysis above the genome, and below the final phenotypic outcome, focused on emergent developmental phenomena, which require thorough study for us to be able to fully account for the ontogeny of selectable phenotypic variation. It is at these levels that Waddington focused his work, and it is at these levels where we must focus evolutionary developmental biology research in order to extend our understanding. At these levels of analysis we can begin to answer questions that are central to evolutionary developmental biology and to evolutionary theory, namely what are the mechanisms driving variability and evolvability, and how the interplay of developmental and evolutionary processes has shaped the history of life. Using the powerful tools of bioinformatic and genomic approaches, in combination with a systems-oriented focus on intermediate levels of analysis, evolutionary developmental biology is poised to make a unique and valuable contribution to an understudied aspect of evolutionary theory.

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