C H Waddington, Canalisation and Genetic Assimilation

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Conrad Hal Waddington was an English biologist who was among the earliest to emphasise that the proper understanding of multicellular development required an evolutionary approach. More than 50 years ago he carried out a series of experiments with intriguing implications for the evolution–development link. They appeared to demonstrate that it was possible for evolutionary change to take place and a qualitatively different developmental outcome to result without the action of selection on spontaneous mutations – that is, without the conventional neo-Darwinian route coming into play. The findings suggested that Lamarckian mechanisms might be at work, namely that acquired traits might be heritable. When Waddington is remembered today, it is for this set of experiments and for the explanation that he gave, which he named *genetic assimilation*. The explanation postulated that as a consequence of evolution, the course of normal development was *canalised* or buffered against perturbations. What follows is an informal and non-technical account of his work on genetic assimilation and his less successful effort to build a theoretical biology.

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Introduction

Among biologists generally, the current upsurge of interest in the evolution of multicellular development (often abbreviated as Evo Devo) has made Waddington's name more familiar now, over 30 years after his death, than it was during his lifetime. Even those who are not acquainted with his work acknowledge his contributions indirectly whenever they use the words *canalisation* or *epigenetics*. For Waddington 'epigenetics' was simply the backdrop to multicellular development. He used it to refer to the coordinated working of genes in different cells at different times that resulted in the same outcome – the characteristic adult form

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of a species – time after time. This is quite different from the sense in which the word is used today, which is in the context of a transfer of heritable information other than via the primary DNA sequence. Waddington tried to come to grips with general phenomena rather than focussing on detailed results. This made him adopt a theoretical stance in his numerous writings, and the special terms that he coined (there were many more) are pointers.

Career

Soon after his birth in 1905, Waddington was taken to live with his Quaker parents, first to Wynaad and then to Coimbatore, both in South India, where his father was a tea planter. He was about four when he returned to the UK to be brought up by an aunt. Almost two decades were to pass before his parents moved back; by then he was already married. After high school he joined Sidney Sussex College in Cambridge University and went on to study a wide range of subjects including geology, palaeontology and philosophy. Climbing and folk dancing were serious hobbies. A diversity of tastes and the desire to bring together concepts from different areas remained characteristic of him all his life. The titles of some of the books he wrote show this: *The Scientific Attitude, The Ethical Animal,* and *Behind Appearance; A study of the relations between painting and the natural sciences in this century*. He wrote many books dealing with the themes of evolution and development. Even here he was among the first to propagate the daringly radical explanation for biological patterns that had been put forward in 1952 by the mathematician Alan Turing. Later he tried to persuade biologists that René Thom's catastrophe theory[1] could provide useful insights into morphogenesis (the development of biological form), but the attempt did not get very far.

A friendship with Gregory Bateson, son of William Bateson (among the more vigorous proponents of the laws of Mendel after they were rediscovered in 1900), sparked an interest in genetics. In 1931, together with J B S Haldane he published a somewhat involved algebraic analysis on a difficult problem concerning the

[1] 'Bifurcation theory' is the name of a mathematical framework to study dynamical systems that undergo large and sudden changes following small perturbations in the parameters defining the system. 'Catastrophe theory' is a branch of bifurcation theory that classifies the changes when certain conditions are specified and shows that they can be described by a limited number of characteristic shapes (topologies).

effects of inbreeding. How did the correlated transmission of genes, known as linkage, affect the expected consequence of inbreeding, which is to make the offspring genetically more and more alike? The experience would seem to have left him dubious regarding the possibility of making quantitative statements regarding evolution by using the mathematics-based approach known as population genetics (see the article by J T Bonner in this issue). He expressed his reservations in a symposium held in 1952, but Haldane rebutted him forcefully when he wrote the Foreword to the volume containing the symposium proceedings.

A serious interest in embryology began at about the same time. Like many others, he had become fascinated by the 'induction' experiments of Hans Spemann and Hilde Mangold in Germany. Spemann and Mangold discovered something astonishing when they transplanted the dorsal lip of the blastopore, a small peiece of amphibian embryonic tissue, to a different location. The transplant seemed to redirect the fate of its neighbours to such an extent that it made them organise themselves into a second embryo.

After spending some time in the laboratory of Honor Fell, a pioneer in the technique of organ culture, Waddington managed to demonstrate embryonic induction in the chick. The hope of discovering the inducer, a chemical entity that was expected to possess the property of inducing naïve tissue to form a whole new embryo, fuelled much activity. The early excitement in embryonic induction gradually petered out as it became apparent that inducer-like properties were present in the strangest substances: in sterol-like compounds, the dye methylene blue and even in silicaeous earth. (See *Box* 1)

Waddington spent the Second World War years in an Operations Research establishment that was headed by the physicist P M S Blackett. After the war ended, he was offered a Chair in animal genetics at the University of Edinburgh, a position that was combined with having to oversee work on plant and animal breeding. It was at Edinburgh that he carried out the work on genetic assimilation that made him well-known. He had a number

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Box 1.

Embryonic Induction

Research on embryonic induction was revived more recently after it was realised that the phenomenon of induction had as much to do with the responding tissue as with the inducer, and that the various non-specific artificial inducers seemed to be affecting the same set of genes in one way or another. The inducers themselves seem to be members of the family of molecules known as growth factors. They are active at very low concentrations and the non-specific effects seen earlier were very likely due to their being released from the tissue.

of distinguished coworkers at Edinburgh and the laboratory attracted many famous visitors. J T Bonner, another pioneer of the modern approach to looking at multicellular development from an evolutionary viewpoint, was one of them. Tokindo S Okada, who played a major role in post-war biology research in Japan, was another. Waddington published many papers on egg ultra-structure with Eiko Okada. Sivatosh Mookerjee and Leela Mulherkar were two Indians who were exposed to the techniques of studying embryonic induction under Waddington. Both came back to set up laboratories in India, but it would not be unfair to say that in neither case did a tradition of carrying out modern developmental biology take root.

Genetic Assimilation

[2] The wild-type is the experimentalist's and field-biologist's ideal prototype. For a given age and sex, all wild-type flies look essentially the same and behave similarly. They are healthy and vigorous, what one usually thinks of as 'normal'.

The experiment involved wild-type[2] fruit flies. A stock of flies was briefly exposed to an environmental shock, for example to high temperature or ether vapour. This was done when they were at an early developmental stage, that is, when they were eggs or pupae. Many died as a result of the shock. Among those that did not, some died after developing a bit further. A few managed to complete metamorphosis and emerged as adults. Many of the adults were aberrant in appearance and sub-normal in vitality.

Next Waddington did something that resembles what August Weismann did when he bred from generation after generation of rats whose tails had been cut off. As is well known, Weismann made an unsuccessful attempt to get a line in which the tailless condition appeared at birth. (This experiment is often quoted – with poor justification – as an argument against Lamarck's theory of evolution based on the inheritance of acquired characters.)

Waddington looked for those adults that resembled – even if very slightly – known mutant flies. The important point was that the environmental shock (increased temperature or ether vapour) was not mutagenic. It would not have been expected to cause genetic alterations, certainly not in the way that ultraviolet light or X-rays would have. Therefore the aberrant-looking flies, while not mutated themselves, copied the appearance of mutants. Such

individuals are known as *phenocopies*. Phenocopies are believed to develop when a critical developmental step is perturbed by an external agency in a similar way and at the same time that it is affected in a mutant animal. Some of the phenocopies that he worked with resembled mutants named *bithorax* (more correctly, *Ultrabithorax*; flies have four wings instead of the usual two) and *crossveinless* (some cross-veins in the wings are missing). He picked out male and female flies of the sort that he wanted, let us say the ones that showed a tendency to develop four wings. Then he allowed them to mate, waited until the eggs developed into pupae, treated these pupae as before, and so on, generation after generation. In each generation the flies that were used to breed the next generation were the ones that showed the most extreme transformation in the desired direction – that is, those in which the two extra wings looked most like normal wings (*Figure* 1).

There were three clear outcomes of the experiment. First, the proportion of adult flies that were close to the desired type kept rising from one generation to the next. Second, they began more and more to resemble four-winged flies of the *bithorax* mutant type. Finally, and perhaps most interestingly, the intensity of the environmental shock required to get the desired effect kept falling from one generation to the next. This last trend led eventually to the strangest outcome of all. After about 15 generations, Waddington discovered that there was no need to provide the environmental shock at all, because from then on the four-winged 'phenocopies' began to breed true (as a noteworthy aside, simultaneously they had lost the ability to fly).

Even though they had not been mutagenised at any stage, the flies appeared as if they were *bithorax* mutants. However, their ancestors of a few generations earlier had belonged to the wild type. It appeared that what had first looked like as an environmentally induced character had gradually worked its way, so to speak, into the genome. At the time when Waddington carried out these experiments, the Lamarckian notion that traits acquired during an individual's lifetime could be inherited had long been discredited – certainly in the case of animals. It was noted that the change
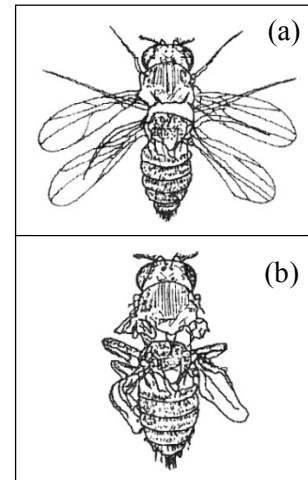
Figure 1. Schematic drawings based on originals.
(a) A multiply-mutant fly with four wings. The pair of wings in front is as usual; the second (posterior) pair is not found in normal wild-type flies. It develops because tiny dorsal metathoracic appendages, the halteres, have been transformed into almost normal-looking wings.
(b) An example of the outcome after genetic assimilation for the Ultrabithorax phenotype [4]. The normal wings have been removed to show more clearly the modified, wing-like halteres.
Adapted from [2].

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from two to four wings was a major transition, that it had occurred in almost a handful of generations, and that the change seemed to mimic an evolutionarily ancestral form (two-winged flies are believed to have evolved from four-winged ancestors). No wonder, then, that the results caused consternation.

In order to keep this description simple I have omitted a clever twist that Waddington introduced. It made the phenomenon appear even stranger. The twist was this. After the first episode of environmental shock, he separated the adults into distinct groups of siblings. That is, all the flies in one group had the same parents – the group consisted of brothers and sisters. For breeding the next generation of flies, those brothers and sisters that had given the best results were chosen for mating. But before being allowed to mate they were separated into two sets. The pupae resulting from one set of matings (A) were given the environmental shock but the ones from the set made up of the remaining flies (B) were not. The procedure was repeated in all subsequent generations. The progeny of those brother-sister matings that had given the best results were used to bring out the following generation. But the adults used for breeding were always chosen from set B. The implication was extraordinary. Not only had the flies that bred true for the four-winged form been derived from quite recent ancestors that had bred true for the normal two-winged form, but, besides that, *no individual in the entire lineage had actually experienced the environmental shock.*

How was one to make sense of the findings? Waddington himself provided the explanation. It invoked nothing more than standard Drwinian selection but the genes in question influenced the working of other genes. Part of the explanation involved something that plant and animal breeders had known and practised for centuries. Called sib-selection, it depends on the principle that if an individual possesses a heritable trait, it is likely that close relatives will carry the genes that are involved in the appearance of the trait. It does not matter whether they display the trait or not. For example, suppose there is a genetic basis for milk yield in cattle (which is a fact). Then, one way to improve the yield of milk

is to breed from cows that are high yielders and bulls *whose sisters* are high yielders. The second part of Waddington's explanation went to the heart of what the term 'wild-type' means. In order to appreciate it we need to remember that one belief held sway for many years following the explanation of evolution by natural selection. The belief was that a species had been so moulded by evolution that at every genetic locus, individuals had the 'best' genes appropriate to that locus. (A small minority of mutant individuals, and differences between males and females because of reproductive functions, were acknowledged as exceptions to the rule.) In other words, the members of a species were assumed to be genetically identical. The assumption was believed to be confirmed because it was noticed that by and large they looked the same in their natural habitats – they were 'wild-type' individuals.

A combination of field work and laboratory experiments, many of them by Th. Dobzhansky, showed that the assumption was incorrect. If anything, there was a substantial amount of genetic variation in the wild. Evidently the variation was not apparent in terms of form or behaviour, namely in terms of those traits of the organism with which it has to tackle the world, traits that are mostly relevant for survival and reproduction – the 'phenotype'. According to Waddington, the reason for this was that the naturally-occurring genetic variation was masked by the effects of other genes. These other genes modified the effects of the first set of genes and ensured that development led to the same 'wild-type' phenotype (these days the words *regulation* and *regulatory gene* are used rather than *modification* or *modifier gene*). Why were modifiers there at all? That, he said, was because there was a fitness advantage possessed by the wild-type phenotype over other alternatives. Therefore any genetic change that tended to make the outcome of development resemble the wild type would be favoured by natural selection. The upshot would be something like buffering; changes which might have taken place on account of one set of variables were compensated by a second set of variables. Waddington named the buffering effect *canalisation.*

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Canalisation enabled development to work reliably towards the same end in the face of genetic, and possibly also environmental, variation. That end was the production of a wild-type adult. Thanks to canalisation, flies can contain a great deal of genetic variation and yet look and behave as if they are 'normal', so that they are classified as 'wild-type'. The variation remains cryptic. It was this cryptic variation that was being exposed by the environmental shock. The development of different genotypes was indeed canalised or buffered in the natural environment (in which selection for canalisation had taken place successfully). But as with any other buffering, there were limits to what could be tolerated. A sufficiently strong environmental shock could break down canalisation and make it possible for the underlying genetic variation to be exposed in terms of the different phenotypes that resulted. Once that happened, selection could be applied on the phenotypes. That was because individuals that visibly belonged to different phenotypes would also be likely to differ *in terms of their genetic capacity to respond to the environmental shock.* Normally that capacity remained invisible. In Waddington's experiment, selection, in the form of the choice of breeding partners, favoured those genotypes which were most likely to give rise to the desired four-winged phenotype. Therefore what looked like a Lamarckian outcome could be explained in conventional Darwinian terms after all. But to do so, one had to think in terms of two sorts of genes, a 'structural' set that affected the phenotype per se and a second, 'regulatory' set that influenced the *reliability* with which the phenotype developed (*Figure* 2).

Genetic Assimilation

I What appears to happen | II What actually happens
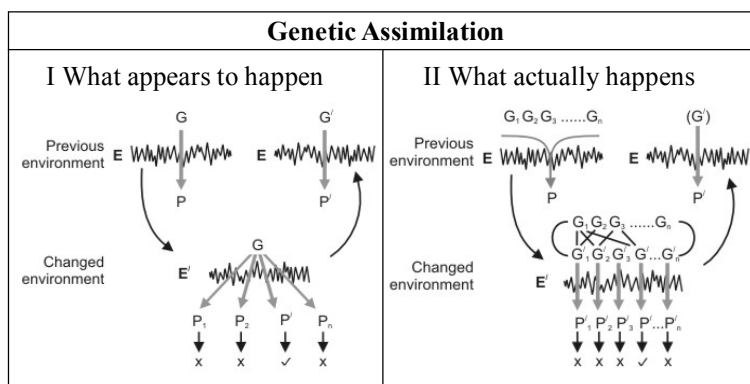
Box 2.

The gene has been said to be "the central organizing theme of twentieth century biology". Wilhelm Johannsen, a Danish botanist, coined the word in 1911. He meant it to stand for the physical and functional unit of heredity whose existence was implied by the findings of Mendel. For a long time the concept of the gene was an abstraction. And yet the abstraction enabled scientists to construct the formal, elegant, and enormously useful structure that constitutes the field of genetics. The existence of genes could be inferred in two ways. One was via mutations, heritable changes whose effects were made visible by changed traits. The other way was via recombination, which brings together and separates different genes. The understanding of genes as giant molecules of DNA (and sometimes RNA) ushered in a revolution. Its foundations appeared to be secured once it was recognised that a DNA sequence could specify implicitly ('encode') the amino acid sequence of a protein. Ever since then we have learnt more and more about what genes are and how they work. It is ironical that in parallel with these advances in knowledge a simple explanation of what 'gene' means has slipped away from us. Wisely, people have not let that come in the way of doing genetics. This theme needs a full article by itself, but in brief: genes are not indivisible, can be unstable, and need not encode proteins. Johannsen's definition was not so bad after all. See http://plato.stanford.edu/entries/gene/ for a sophisticated discussion.

Today we know that the words 'structural' and 'regulatory' are often interchangeable. A structural gene in one context can be a regulatory gene in another context, and sometimes a gene can play structural and regulatory roles at the same time. (See *Box* 2) These experiments on genetic assimilation have been repeated by others with similar results. These days genetic assimilation is used as a possible explanation for cases of rapid, and often qualitative, evolutionary transformation, for example a change in the type of symmetry of body form. (About 10 years ago there was the hint that a possible molecular mechanism behind canalisation involved a protein named Hsp90. More recent results raise a question about whether that is the full story.) Waddington's most important contribution in all this may have been to draw attention to the point that natural selection leads to two related but distinct outcomes. The first is a certain 'product' and the second is the accuracy with which the product is attained. Terms used in communication theory are useful here: both 'signal' and 'signal-to-noise ratio' can evolve by natural selection.

Theoretical Biology

The culmination of Waddington's lifelong desire to build a theory of the organism was a series of four yearly meetings in

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Bellagio in northern Italy from 1966 onwards. In an article that he wrote after the first two of them he pointed out the glaring lack of anything in biology with the status of theoretical physics within physics. The problems, he said, were three. There was the high level of complexity of biological systems in terms of both the number of variables that had to be taken into account for describing them and the number of interactions among those variables. Next, the prevailing gene-centred view failed to take into account the fact that genes were as much responders as actors. Third, evolution had to be integrated into any theory of development. One needed to understand organisms and their development by including the workings of genes and the environment in one conceptual whole. It had long been Waddington's complaint that classical population genetics, the mathematical formulation of evolutionary theory, lacked just that.

The Bellagio meetings were as notable for the range of topics that were discussed in them as for the relative absence of molecular biologists; Francis Crick and Robin Monro were the two prominent exceptions (see the article by M H Cohen). Among the themes that were taken up at the meetings, one that forms part of the currency of discourse among modellers today is the analysis of biological networks that has been fuelled largely by the explosive growth and accessibility of computational power. The conference volumes that resulted, entitled *Towards a Theoretical Biology*, attracted prestige and bafflement on the part of biologists in equal measure. Why might this be so? A look at what has happened in the fields that were Waddington's lifelong interest, genetics and embryology (or developmental biology), may be of interest.

Each field – embryology being much the older one – had its own favourite systems, experimental approaches, ways of thinking and traditions.

Each field – embryology being much the older one – had its own favourite systems, experimental approaches, ways of thinking and traditions. For a long time it was not obvious that they had anything to do with each other. Waddington was among the few who saw at the time that they provided complementary approaches to deciphering how a fertilised egg becomes an adult, and it was his hope to contribute to their unification. As it

happens, the fusion of the two fields came about in the late 1970s through an unanticipated route. At first thought this appears odd, because he was one of the earliest workers to carry out experiments that focussed on the genetic basis of embryological change – that too in the fruit fly *Drosophila*, which in the 1970s became the prototype for the genetic analysis of developmental phenomena. But the explanation stands out when one thinks about it a bit more. The crucial element in the breakthrough achieved by developmental geneticists was their focus on single gene mutations with major effects.

Waddington sought explanations involving gene networks, not genes in isolation. His early models of how genes influenced development involved many genes, each of which affected many developmental pathways. And in turn every pathway was influenced by more than one gene. Right until his death in 1975 his goal remained the understanding of living organisms in their entirety. He was what today we would call a 'systems biologist'. His attempts to further the development of a theoretical biology were in the same line. It was not easy then, and it is not easy now, to see how this approach could be integrated with the sort of thing that experimentalists do, grounded as they are most of the time in firmly reductionist concepts. Overall, we still lack a theoretical biology of the sort that Waddington was looking for.

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Suggested Reading

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