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Environment and Heredity in Development and Evolution

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Abstract

The integration of development and evolution occurs through the organisms' experience of the environment. The registering of experience and its eventual assimilation are the basis of adaptive evolution.

The material link between development and evolution is the hereditary apparatus which realistically includes both maternal/cytoplasmic effects and nuclear genes. The oocyte cytoplasm is at once a carrier of heredity independently of nuclear genes and the necessary communication channel between the environment and nuclear genes in the coordination of developmental and evolutionary processes.

Pattern formation in the *Drosophila* thorax serves as a concrete illustration of the general argument. Some experimental work bearing on canalization and genetic assimilation is reviewed and discussed in relation to (a) the developmental genetics of pattern formation, (b) the problem of the epigenetic origin of spatial organization and (c) the epigenetic mechanisms of differential gene expression and their relevance to genetic assimilation.

INTRODUCTION

The purpose of a theory of evolution is to account for the origin and transformation of organisms. Thus, to define evolution as the change in allele frequencies in populations from the outset — as neo-Darwinists do — is to defeat the purpose of the theory before we begin.

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Contrary to what is commonly supposed, the genotype–phenotype dichotomy, or Weismann's barrier, which conceptually separates evolution from the development of organisms (and hence from environmental influences), does not thereby absolve us from a theory of phenotypes. Natural selection, after all, acts on phenotypes. This leads us back to development, and to the role, not only of genes, but of the environment in the shaping of phenotypes. It is impossible to evade the problem which continues to confront us: how *organisms* evolve.

The physiological nature of development was emphasized by Goldschmidt alone among neo-Darwinists (especially in his *Physiological Genetics*, 1938). The rates of its reactions are almost unavoidably subject to influence from both environmental and genetic factors. Therefore, even a theory of evolution based solely on natural selection must reckon with developmental physiology, and with the interactions between genetic and environmental factors in morphogenesis.

Physiological considerations of development however, fit at best uneasily within the narrow confines of neo-Darwinism. This has prompted us to outline an alternative approach (Ho and Saunders, 1979; 1982a; 1982b). Our project is a logical extension of physiological genetics, and is continued in the present chapter. Here, I shall elaborate on an epigenetic framework which integrates development and evolution through the organisms' experience of the environment. The registering of experience and its eventual assimilation are the basis of adaptive evolution. An important feature which distinguishes the framework from neo-Darwinism is our concept of heredity. It includes, besides nuclear genes, maternal and cytoplasmic effects which provide the material link between development and evolution. The egg cytoplasm is both the carrier of heredity independently of the nuclear genes and the necessary interface between nuclear genes and the environment in the coordination of developmental and evolutionary processes.

In order to give focus to the discussion, much of it (especially in the second half of the Chapter) will be centred around pattern formation in the *Drosophila* thorax, which serves as a concrete illustration of the general argument. In part, this reflects our own research interests. But the main justification is that pattern formation is recognized as the major problem in development, and the *Drosophila* thorax has been subject to the most intensive investigations within the classical genetics framework. Both the successes and limitations of the latter will therefore stand in the sharpest relief.

DEVELOPMENTAL PHYSIOLOGY, THE 'NORM OF REACTION' AND THE 'EPIGENETIC LANDSCAPE'

The concept of the 'norm of reaction' was first introduced by Woltereck (1919) from his studies on cyclomorphosis in cladocerans. These freshwater animals such as *Daphnia* undergo a cycle of transformations involving a number of morphologically different generations throughout the year. Woltereck showed that the same range of variations could be produced in the laboratory by controlled feeding. Hence the term *Reactionsnorm* was coined to describe the whole range of phenotypes (reactivity) of a single genotype under different environmental conditions. The existence of the norm of reaction has the immediate consequence that an organism is not uniquely defined unless its environment is specified. The environment exerts necessary formative influences on development. This simple truth has been obscured by the Weismannist[1] fallacy that organisms are uniquely determined by their genotype, and that environmental influences are merely 'disturbances' to be overcome on the way to realizing the ideal phenotype (*see* Ho and Saunders, 1982a).

Inherent in the concept of the norm of reaction is the notion of a reacting system. It is a physiological system governed by principles which ensure both stability and the capacity for organized change (Ho and Saunders, 1979). As evolution is in essence a series of organized changes in development, it follows that development contains the potential for evolution. So it was that Goldschmidt (1940) envisaged large organized changes in development could result from 'systemic mutations'—these 'hopeful monsters' becoming in one step the precursors of a new evolutionary lineage.

As time passed, Goldschmidt's systemic mutations—without the physiology—became accomodated (albeit uneasily) within the orthodoxy. Waddington (1957) attempted to re-instate developmental physiology by inverting Goldschmidt's argument, drawing attention to environmental effects on development which parallel the genetic, and reopening once again the enquiry concerning the precise role of the environment in evolution.

Like Goldschmidt, Waddington saw development as a totality of biochemical and cellular interactions. This dynamic system—the famous but imprecise 'epigenetic landscape'—is characterized by a topography: a set of equilibrium pathways ('valleys') separated by thresholds ('hills'), such that development is normally *canalized* or buffered against disturbance. Thus many genetic mutations or environmental fluctuations will not alter the end result of development appreciably. However, with large disturbances—genetic or environmental—the topography can change so

that alternative developmental pathways can be traversed, leading to different end-results.

The epigenetic system is not the additive outcome of individual gene action but a characteristic, at least, of the species[2] (Ho and Saunders, 1979). Proof of this assertion is provided by the phenomenon of phenocopies, a term coined by Goldschmidt (1935) to describe the mimics of morphogenetic mutants produced in normal wild-type strains of organisms by environmental agents. Phenocopies occur in all groups of organisms. Within a given species, the same kinds of phenocopies are found in all varieties or strains, indicating that different genotypes *per se* are irrelevant to the existence of underlying systemic constraints which define a dynamic *structure* of development. The latter ultimately determines the kinds of variations available to evolution. If we wish to understand evolution, the epigenetic system above all should be the object of our enquiry.

In order to explain phenocopies within the accepted orthodoxy, some neo-Darwinists, including Goldschmidt (1938) and Waddington (1957), postulated the existence of 'modifier' genes which as the name implies, modify the expression or penetrance of the phenocopy response, typically in a continuous 'polygenic' manner. The response itself is supposed to be controlled primarily by one or more 'major' genes. The hypothesis of modifier genes appears to be supported by the observation that different strains exhibit greater or lesser sensitivity to the agents inducing phenocopies (Goldschmidt and Piternick, 1957). Our own investigations to be described later indicate that this is an oversimplified explanation.

PARALLEL BETWEEN ARTIFICIAL AND NATURAL VARIATIONS

The phenomenon of phenocopies itself has a longer history dating back to the last third of the nineteenth century (*see* Shapiro, 1976). Extensive studies were carried out, for example, on wing pattern in butterflies in order to uncover the physiological basis of seasonal polyphenism—the existence in the same population of strikingly different seasonal generations. Temperature was found to be one of the controlling environmental factors and in many species of multivoltine Lycaenidae, it was possible to reproduce seasonal forms by appropriate temperature treatments at specific developmental stages. In some experiments (Standfuss, 1896; Fischer, 1901) phenocopies were obtained which resembled different geographic races or species in which the wing pattern was apparently genetically determined.

It turns out that parallels between artificially induced modifications and

naturally occurring variations are extremely common. A very large body of observations (first reviewed by Rensch, 1929) show that structural divergences known or presumed to be hereditary are often correlated with environmental differences, so much so that a number of ecological rules[3] have been formulated on this basis. More importantly, however, these same structural modifications can be artificially induced in related organisms by simulating the appropriate environmental conditions in the laboratory.

A well known study is that of Beebe (1907) who found that doves of the genus *Scardafella* increase in dark pigmentation in nature from Mexico to Brazil. The point of least pigmentation coincides with the area of lowest humidity and increases in either direction as the humidity increases. By exposing the lightly pigmented form to very humid conditions, he demonstrated that pigment was acquired gradually through a series of moults till finally a stage was reached which was darker than any known in nature. Another case is the honey bee *Apis mellifera* in Europe which exhibits a north–south cline in a number of morphometric traits. Alpatov (1925, cited in Robson and Richards, 1936) showed that cold temperatures in the laboratory induced the same effects as those found in nature. Recently, experimental transplants of red-winged blackbird eggs between nests in northern and southern Florida, and from Colorado to Minnesota, showed that a significant proportion of the geographical differences in nestling development is 'non-genetic'. The author (James, 1983) concludes that if 'natural selection is maintaining the cline of character variation . . . the genetic and non-genetic components of phenotypic variation must covary'.

Many examples are known in which parallels between natural and artificial variations are attributed to special or local environmental conditions.

Woltereck induced helm modifications in *Daphnia* which paralleled those existing in natural races under similar environmental conditions. Races that lived in poor environments displayed those characters that can be invoked, in part, in any race cultured in a poor nutrient. Conversely, the characteristic features of races in rich environments were produced in almost any race maintained on a rich nutrient. Nonetheless, those respective features of the natural races appear more or less 'fixed' as they can persist side by side for a long time under identical culture conditions in the laboratory.

In summary, the naturally existing variations—many of which are regarded as clear adaptations by Darwinists and neo-Darwinists alike—are adaptive to the very environment capable of eliciting the parallel artificial modification. This strongly suggests that the environment plays a central role in the origin and evolution of the adaptation itself. In retrospect, this

should occasion little surprise. An adaptation is but a particular functional relationship between the organism and its environment. Functions in turn arise naturally out of organism–environment interactions (Ho and Saunders, 1982b). So it is entirely plausible that adaptations should originate from interactions between organism and environment. The missing link is how the somatic changes resulting from those interactions could become hereditary in the sense that they seem to anticipate the environmental conditions for which they are an adaptation.

THE EXPERIENCE AND ASSIMILATION OF NEW ENVIRONMENTS

The link between environment and heredity in the origin of adaptation is explained by Waddington (1957) as follows. A population of organisms experience a new environment and respond developmentally in a novel fashion, that is, a phenocopy appears. As the population is heterogeneous in modifier alleles, individuals will respond to varying degrees. If the response is adaptive, there will be selection for the modifier alleles which increase the initial intensity of the response and then (or at the same time) regulate it so that the same degree of response will occur within a range of intensity of the environmental stimulus. In other words, the response is canalized. Later, genetic assimilation takes place: the response now occurs in the absence of the environmental stimulus.

Canalization is explained as the result of selection for modifier alleles. As for genetic assimilation, Waddington was unclear as to what sort of mechanisms were involved. It could be the end-point of selection for modifier alleles which lowers the threshold for the response so much that the latter occurs spontaneously. Alternatively, genetic assimilation could be due to a chance mutation in a single (major) gene which fixes the response genetically, as it were.

There has been a recent revival of interest in genetic assimilation as a model for the evolution of new species (*see* for example, Shapiro, 1976; Matsuda, 1982), based to varying extent on Waddington's strictly neo-Darwinist hypothesis. Matsuda (1982) proposes the genetic assimilation of environmentally induced heterochronic modifications in talitrid amphipods and salamanders by the fixation of hormonal states normally subject to environmental control. He rejects the existence of modifier genes in favour of single mutations for genetic assimilation, thus reviving Baldwin's (1896) theory of organic selection. The latter states that after somatic modifications were produced by the environment, *coincident* genetic

mutations also arose which gave the same phenotypes. If those phenotypes were adaptive, they would be favoured by natural selection. The major flaw in the argument is that there is really no connection between the environmentally induced modification and genetic assimilation — unless the former causes coincident mutations to arise more frequently in some way. Furthermore, as both the somatic modification and the coincident genetic mutation result in the same phenotype, how could natural selection distinguish between the two?

Waddington's hypothesis has the virtue that it does connect the initial environmentally induced modification to the final genetically assimilated adaptation. Moreover, it is amenable to experimental testing whereas organic selection is not, for it is impossible to predict in advance whether coincident mutations will occur in any one experiment.

Among the first experiments on genetic assimilation, Waddington (1956) succeeded in producing assimilated lines of the phenocopy *bithorax* in *Drosophila* by means of generations of ether-treatment and selection. These lines, when analyzed, seemed to involve both single major gene mutations as well as polygenic modifiers. The same applies to subsequent work on other phenocopies (Bateman, 1959a,b).

Whatever the detailed mechanisms involved in canalization and genetic assimilation, the modifier genes model presupposes that the starting population contains variation in modifier genes, and that there is selection for the phenocopy, either naturally or artificially. The specific prediction which could be made is that canalization would not occur in a genetically uniform population or where there is no selection for the phenocopy. Thus, Bateman (1959a,b) reported no progress in the frequency (penetrance) of certain phenocopies of mutants affecting the *Drosophila* wing in inbred lines after a small number of generations of heat treatment and selection. But neither was there any progress in the massbred line until the high mortality of the phenocopies was reduced by shortening the period of heat treatment. No parallel checks on the mortality of phenocopies were done in the inbred lines. In one inbred line selected for a wing venation phenocopy for nine generations, progress was recorded in the last 3 generations. But that experiment was terminated with the remark, 'The fact that a parallel trend was also shown by the unselected line indicated, however, that the apparent response was in fact merely secular' (Bateman, 1959b, p.448). This happened to be the only instance in which an unselected control was maintained. The latter, as we shall see, is crucial to the interpretation of these experiments.

MATERNAL AND CYTOPLASMIC EFFECTS — AN ALTERNATIVE ROUTE OF INHERITANCE

One complicating factor which has been consistently overlooked in all investigations on phenocopies is the effect of the environmental stimulus on the hereditary constitution of the organisms. In particular, the possibility cannot be ruled out that cumulative cytoplasmic or maternal influences could lead to an increase in the phenocopy response in successive generations. This would indeed explain Bateman's (1959b) findings cited above, as well as some of her results in the genetic analysis of assimilated lines which clearly implicated maternal/cytoplasmic components.

Since the egg cytoplasm and cortical organization are conditioned by both maternal genes and environment (Cohen, 1979), it seems reasonable to conclude that the special environments experienced by the mother in successive generations may be passed on cumulatively in the egg. Only the modern counterpart of the medieval spermists — the '*omni ex* DNA' school[4] of geneticists and developmental biologists — would deny this possibility *a priori*.

Environmentally induced modifications of the cytoplasm which are transmitted across generations are by no means unknown. Many of these were studied extensively in *Paramecium* by Jollos (1921), Sonneborn (1970) and Beale (1957), in *Amoeba* by Danielli (1958) and in *Aspergillus* by Jinks (1957; 1958) and others. In all the above systems, considerable interactions between cytoplasm and nuclear genes were found, which often resulted in large phenotypic effects. (As we shall see later, cytoplasmic-nuclear interactions are central to the process of development.) Among higher organisms, Harrison (1928) induced heritable changes in the pigmentation of the cabbage white butterfly pupae by means of orange light, and Fujii (1978) reported the transmission of serum calcium disorders to the offspring of parathyroidectomized female rats up to the F_4 generation. Recently, Damjanov and Solter (1982) identified cytoplasmically transmitted factors which modify the development and malignancy of teratomas in mice. Considerations such as these led us to suspect that cumulative cytoplasmic effects may be responsible for the canalization of novel phenotypic responses to environmental challenge (Ho and Saunders, 1979). This mechanism, operating independently of, or in addition to natural selection, could have important consequences on the rate of phenotypic evolution.

CYTOPLASMIC EFFECTS AND THE CANALIZATION OF THE *BITHORAX* PHENOCOPY

In order to test our hypothesis, we re-investigated the *bithorax* phenocopy studied by Waddington (1956) (Fig. 11·1). By exposing *Drosophila* embryos to ether, homeotic transformations of the *meta*thoracic to *meso*thoracic structures are induced which mimic mutants of the *bithorax* genes. We chose this phenocopy because it is intimately connected with pattern formation—the major problem in development. Elucidation of the mechanisms involved in its canalization and genetic assimilation would therefore be a significant contribution to our understanding of the relationship between evolution and development.

FIG. 11·1 The bithorax phenocopy. (A normal fly is depicted on the left, and an extreme phenocopy on the right.)

We followed Waddington's (1956) procedure as closely as possible but without selection in a massbred and an inbred line of the same genetic background. The results have been published in detail (Ho *et al.*, 1983a) so I shall only review the salient points here.

According to the modifier genes model, there should have been no increase in the phenocopy response in successive generations of ether treatment, as there was no selection for the phenocopy. Actually, a steady increase in the frequency of the phenocopy was found in both inbred and massbred lines. The question arose as to whether 'indirect' natural selection for the phenocopy was taking place. Our own observations as well as those of Waddington (1956) indicate that the bithorax flies were if anything

naturally selected against. Statistical analyses of our data further demonstrated that generations of ether treatment increased phenocopy frequencies in a direct and cumulative way, independently of the effects on viability.

Direct evidence for cytoplasmic effects was obtained in that after the first six generations of ether treatment, embryos from a cross between treated females and control males showed the same increased tendency to phenocopy as embryos of the long-term treated line; whereas the reciprocal cross gave embryos which were no more responsive than controls.

A striking feature of our findings is the great similarity in the results obtained in the two different lines in all aspects. This strongly implies a basic identity in the epigenetic processes underlying the total response to long-term ether treatment in both lines.

A clue to some of the epigenetic processes affected by long-term ether treatment was provided by analyses of the spatiotemporal characteristics of the phenocopy response (*see* Fig. 11·2). Long-term ether treatment results in an increased tendency to phenocopy and an extension of the critical period for phenocopy induction into both earlier and later embryonic stages. These effects are in turn due to the cumulative influence of ether on a 'prepatterning' event in the early embryo which establishes the main body segments of the adult (Ho *et al.*, 1983a,b,c). Once again, the results obtained in the massbred and inbred lines were very similar.

THE ROLE OF MODIFIER GENES

Our results do not rule out the existence of modifier genes which may have contributed to the increase in phenocopy response in successive generations. Rather, we draw attention to other mechanisms such as cytoplasmic inheritance—for which positive evidence was obtained—that may have profound effects on the rate of phenotypic evolution, independently of modifier genes.

The reason we cannot unequivocally rule out modifier genes is simply that our inbred line was tested by isoenzyme electrophoresis and found to contain residual genetic variation (Ho *et al.*, 1983a). Therefore, it may also have contained variation in modifier genes. As we have indicated, however, the most notable feature of our results is the consistency in the response of the two different lines in all aspects. Even though the inbred line was not isogenic the amount of genetic variation had been much reduced by the inbreeding regime. To explain the results in terms of selection for modifier alleles, one must assume that almost precisely the same alleles were still

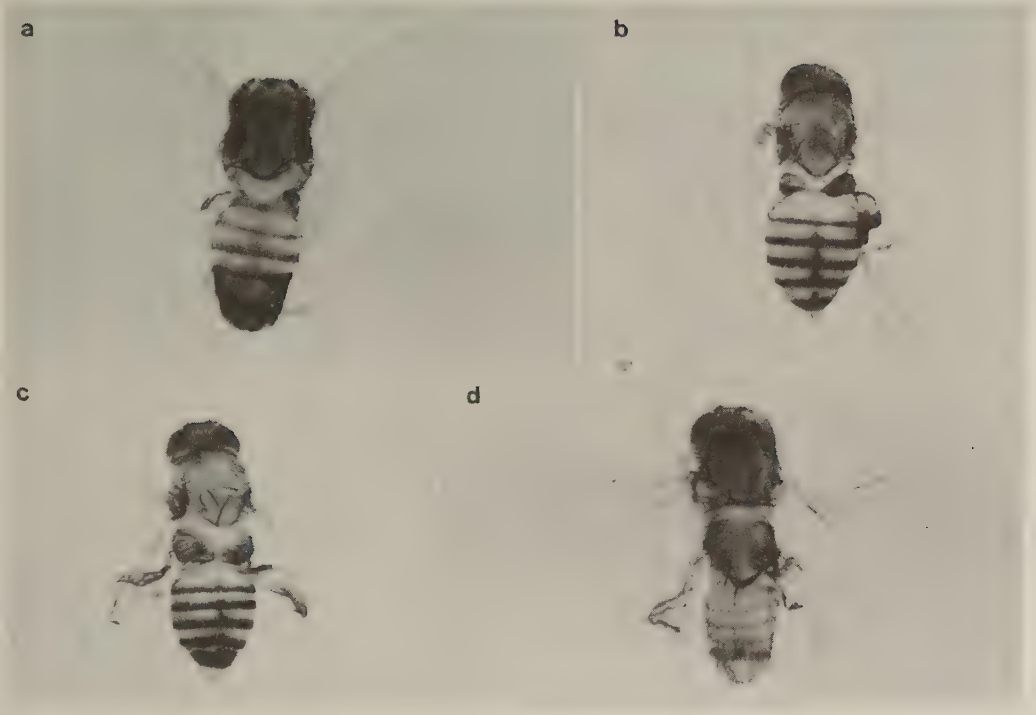

FIG. 11·2 Dorsal view of some of the bithorax transformations induced by ether treatment. A continuous range of tranformations is typically observed. The detailed spatial extent of the transformed spots (with respect to various landmarks on the thorax) differs according to the precise timing of ether treatment. (From Ho *et al.*, 1983b.)

present in the inbred as in the mass bred line. The only reasonable alternative is to recognize the existence of systemic, organismic properties common to both lines, which do not depend on specific alleles in specific genes. These properties are in part dependent on cytoplasmic constitution which may in turn be subject to environmental modification. We conclude that in our experiment, at least, the effect of any modifier genes would have been small compared to that of cumulative cytoplasmic influence.

Modifier genes have been invoked in order to explain the phenomenon of genetic assimilation within an accepted framework. Although modifier genes have been identified for certain mutations, their role in phenocopy responses is far from clear. The major factors influencing the penetrance and expression of the bithorax phenocopy, for example, turn out to be the timing of ether-treatment in relation to a more or less invariant critical period for phenocopy induction, and the tendency for the females in some lines to deposit a high proportion of fertilized eggs which have been retained in the body and are therefore no longer sensitive to ether (*see* Ho *et al.*, 1983b). None of the factors specifically modify the amount of penetrance or the intensity of expression.

Modifier genes (especially the 'polygenic' ones) are frequently assigned by genetic analysis to 'all chromosomes'. If they are ubiquitous, then they obviously cannot serve as an explanation for specific responses. As a category of explanation they have little or no predictive power: any experimental result will be consistent with the action of *some* putative modifier genes. This too conveniently obscures the underlying developmental physiology that should be the object of our enquiry.

GENES AND CYTOPLASM IN PATTERN FORMATION

In this and subsequent sections, I try to relate heredity and development at the level of mechanisms affecting pattern formation in particular. This in turn enables us to see how evolution and development may be connected through heredity. I stress that the argument is a general one and the concentration on pattern formation in *Drosophila* is simply in order to illustrate how in a specific case the generalities can be given substance.

So much is known concerning the genes affecting development that one might easily be led into thinking that development can be understood when all the genes affecting all the characters of an organism have been identified and mapped to parts of chromosomes. Yet, as many biologists including Goldschmidt (1938) realize, the enumeration of genes without the elucidation of the underlying physiology contributes little to our

understanding of development. There is another reason why development is not reducible to genes, however, even if it were possible to catalogue all the genes *and* their physiological functions. This will become evident as we concentrate the mind as before on pattern formation in the *Drosophila* thorax, of which a great deal is known in genetics terms.

The bithorax gene complex (BX-C) (Lewis, 1982) is a cluster of tightly linked loci cytogenetically defined by a series of homeotic mutants. The latter lead to specific transformations of compartments or segments in the thorax and abdomen. For example, mutations in the *bithorax (bx)* and *postbithorax (pbx)* loci respectively transform the anterior and posterior compartments of the *meta*thorax to homologues of the *meso*thorax. (Both these mutations are mimicked in the bithorax phenocopies described earlier.) Many BX-C mutants exhibit 'polarity' effects—causing the transformation of particular segment(s) to a more posterior or more anterior segment. The loci themselves are interestingly arranged roughly in the order of the body segments each affects. This suggests a model of gene function involving the activation of increasingly more genes in each segment as one proceeds caudally (Lewis, 1982). Thus in the mesothorax no genes are active as it represents a ground state; whilst in the last abdominal segment all the genes are active.

The expression of the BX-C complex is in turn affected by at least ten other loci which are not linked with the cluster (*see* Garcia-Bellido and Capdevila, 1978; Lewis, 1982; Duncan, 1982; Struhl, 1982). Mutations in some of those loci cause homeotic transformations of all or nearly all body segments, and furthermore, interact with mutations in the BX-C complex in a multiplicative way. For example, mutants of the *extra sex comb (esc)* locus cause all body segments including the head to transform to the last abdominal segment, typically A8; whereas deletion of the entire BX-C results in the transformation of the segments posterior to the mesothorax—including the A8—to mesothorax. In the double mutant, all the segments are transformed to the mesothorax—as the last abdominal segment is mesothoracic in the single BX-C deletion mutant. This implies that a hierarchy of gene function is involved in segmental determination (*see* Struhl, 1981). In analogy with the well known prokaryotic operon model, the BX-C complex appears to contain the operator and structural genes, whereas the other loci seem to code for various inducers or repressors (Lewis, 1982; Struhl, 1981; Ingham, 1981).

The genetics of segmental determination in *Drosophila* is fascinating, and continues to be most extensively and thoroughly investigated. As more and more loci affecting segment specification are discovered, however, the simple analogy with prokaryotic gene function inevitably breaks down. One result of this is an 'explanatory' system which tends to identify increasingly

small bits of anatomy with increasingly minute bits of DNA—an atomistic extension of the 'one gene one character' concept of the early Mendelians (*see* for example Duncan, 1982). In the simple analogues of the operon model, spatial organization is largely explained in terms of differential gene activation in different segments due to hypothetical gradients of repressors or inducers[5]. One fundamental question which is never really addressed is how the spatial distribution of hypothetical repressors/inducers becomes established in the first place, since the genes coding for these molecules must have been present in every nucleus of the embryo (cf. Løvtrup, this volume). The epigenetic origin of spatial organization is a major problem in developmental biology that is not reducible to differential gene activation. The solution, I believe, lies in the maternally imposed organization of the mature oocyte.

This idea is not new, and could be traced back to a theory espoused by Boveri, Loeb and Morgan (*see* Davidson, 1968; Cohen, 1979) which proposed that the egg cytoplasm is responsible for the form of the embryo in the rough, and that the role of mendelian factors is merely to determine the details of the individual development. In different forms, the morphogenetic role of cytoplasmic and cortical organization has been emphasized by many other eminent embryologists within the first half of the present century (Wilson, 1925; Spemann, 1938; Dalcq, 1938; Horstadius and Wolsky, 1936; to name but a few).

Cytoplasmic and maternal determinants of development are indeed well-known (Raven, 1961; Sander, 1976; Wolsky and Wolsky, 1976; Wessels, 1977; Cohen, 1979). In all organisms except mammals, embryogenesis right up to late gastrulation—the 'phyletic' stage—is completely under the control of the egg cytoplasm (Davidson, 1968). During that time there may be transcription of the zygotic genes but certainly no expression of their products. From our point of view, the most important aspect is that crucial determinants of polarity and symmetry are laid down in the egg cytoplasm or cortex during oogenesis (Wessels, 1977; Raven, 1961). The cortical and cytoplasmic organization of the mature egg contains not only all the instructions necessary for making the phyletic body plan, but also a precise time-schedule for doing so (Wessells, 1977).

It would be wrong, however, to imagine that the body plan is preformed in the cytoplasm or cortex. This is not necessary; nor is it desirable in most cases. Indeed, the prodigious regulatory capabilities of the majority of eggs and early embryos show that the picture of detailed preformation is decidedly false. Mosaicism (or apparent detailed preformation) becomes established always after a determination event that sets up a kind of 'prepattern' (Stern, 1953) of the major body regions (*see* Ho *et al.*, 1983b,c). The time at which the event occurs varies. In so-called

'determinate' eggs, such as those of the ascidian *Styela,* and the annelid, *Tubifex*, this occurs probably during oogenesis or immediately after fertilization. In other eggs capable of varying degrees of regulation, it occurs later during embryogenesis. It will be instructive to consider prepatterning once again, in *Drosophila*.

As mentioned earlier, we have analyzed the spatiotemporal characteristics of the bithorax phenocopies induced by ether in some detail. The results suggest to us that ether disrupts a prepatterning event which occurs at the surface of the embryo during the precellular blastoderm stages. Other evidence for the existence of such an event include the following. Ligation of *Drosophila* embryos before, but not after, blastoderm formation results in gross disturbances to segmentation (Schubiger, 1976) implying that segments are determined at blastoderm formation. Clonal analyses also indicate that segments are distinct at or soon after the blastoderm stage, at which time, the anterior and posterior compartments of the thoracic segments are also determined (Steiner, 1976; Wieschaus and Gehring, 1976; Garcia-Bellido *et al.*, 1976). That the prepattern affects primarily the cytoplasm rather than the underlying nuclei is corroborated by the finding that blastoderm nuclei are totipotent, and can become incorporated into any kind of differentiated cells, whereas blastoderm cells are determined in their developmental fate (Illmensee, 1978). This result also implies that nuclear genes become involved—activated or otherwise—in pattern formation secondarily, through cytoplasmic-nuclear interactions. The latter in effect transfer a global prepattern locally to individual nuclei or groups of nuclei which only then could express their genes differentially.

The case is sufficiently strong that a prepatterning event is required for segmental determination. Segments are therefore not specified by a preformed maternal organization. What is laid down during oogenesis, so far as spatial organization is concerned, are the principal axes of the future organism: the antero-posterior and dorso-ventral polarities, both of which can be drastically disturbed by mutations in maternal effect genes (Wieschaus, 1980).

As Davidson (1968) points out, cytoplasmic localization of developmental 'determinants' is universal, though the precise nature of the determinants remain to be elucidated. The necessity for cytoplasmic localization in metazoan development may also be viewed in the light of the particular problem involved in 'translating' linear instructions—presumably coded in the genes—into a three-dimensional spatial domain which is the organism. It may be that what is required is no more than an initial symmetry breaking in the form of a polarity, such as the animal-vegetal polarity of the amphibian egg, or the antero-posterior

polarity of the *Drosophila* egg. This, together with a train of cytoplasmic reactions, activated by sperm-entry (*see* Jaffe, 1979) then generate a prepattern of the main body regions in the early embryo. The overall dynamics involved in prepattern generation are unknown (although we were able to make inferences concerning the reactions at a local level affecting metathorax prepatterning (Ho *et al.*, 1983b,c)). They could be the sort envisaged in Goodwin's field theory description (this volume). Whatever the precise nature of prepatterning, it is clearly pre-requisite for pattern formation.

CANALIZATION AND GENETIC ASSIMILATION — SPECULATION ON MECHANISMS

Our investigations on the bithorax phenocopy showed that ether disrupts the early prepatterning event in the *Drosophila* embryo. The effect of ether is inherited cumulatively through the cytoplasm of the oocyte, and is manifest as an increase in the phenocopy response due to alterations in the timing and rates of reactions involved in prepatterning. At this moment, one can only speculate concerning the sort of cytoplasmic 'localization' responsible for the effect of ether. The seemingly continuous nature of the latter leads one to suspect that cumulative changes in the concentration of some membrane protein(s) or lipids — which take part in the prepatterning event — may be involved. Thom (1968) describes a molecular mechanism of facilitation or 'memory' which may be applicable here. In essence, a metabolic state S results in the synthesis of a molecular structure M. If M persists beyond the state S, it will subsequently facilitate a return to the state S. Such a mechanism could be the basis of the facilitation of the phenocopy response in successive generations of ether treatment.

Cytoplasmic inheritance thus appears to be responsible for canalization; the mechanism of genetic assimilation, however, is still unclear. I am inclined to doubt that random or fortuitously coincident mutations could be responsible. I am not aware that ether treatment increases mutation rates in general, so that for instance, mutations to *white eyes* or *dumpy wings*, as well as to *bithorax* are above the spontaneous background level. If mutations in 'major' genes accompany genetic assimilation, they could well result from 'instructive' processes in the sense that the environmental stimulus increases the likelihood for the right mutations to occur. The instructive process does not have to be as unsubtle as 'reversed translation' (unlike systems described in the following chapter, no specific protein or RNA sequences are involved in the environmental signal). When we bear in

mind that gene expression depends on nuclear–cytoplasmic interactions, then it is not difficult to envisage a feedback to the genome from the cytoplasm due to alterations in concentrations of proteins and metabolites which favour alternative gene expression states by the same mechanism of molecular memory stated above. The latter, if sufficiently intense, could then be stably inherited (i.e. become genetically assimilated).

One clear example is antigen expression in *Paramecium aurelia* (Beale, 1957). These organisms exist in a number of stably inherited gene-expression states each characterized by the presence of a different antigen. The states are determined by cytoplasmic factors which in turn depend on environmental conditions. A given cytoplasmic state favours the expression of alleles at one particular locus. Certain cytoplasmic states in turn are controlled by the very genes whose expression are favoured by the same cytoplasmic states[6]. This system underlines the reciprocal interactions between nucleus and cytoplasm in the determination of phenotype.

Recent investigations using recombinant DNA technology have brought to light a plethora of cellular mechanisms affecting gene expression (Brown, 1981; Pollard, this volume). Many involve genomic changes such as gene loss, gene amplification and DNA rearrangement. Some are directed by the environment and serve useful functions while others occur apparently at random to no specific purpose. Genomic change is not necessary for stable alterations in gene expression, however. Binding of different repressors to a single operator gene can result in the mutually exclusive and stably inherited expression of alternative structural genes (Johnson *et al.*, 1981).

Genetic assimilation may therefore be achieved either by directed genomic change or the fixation of a cytoplasmic state favouring altered gene expression. In classical genetic analyses, these would be identified as 'major gene mutants' and maternal/cytoplasmic components respectively. Re-examination of earlier results (Waddington, 1956; Bateman, 1959a,b) indicate frequent instances of both. Yet another mechanism for genetic assimilation may be envisaged when we are dealing with sexual organisms which are as a rule genetically very diverse. This may be described as a kind of organic selection via cytoplasmic-nuclear incompatibility reactions. It is possible that as the environmental stimulus continues to modify the cytoplasm, the latter becomes incompatible with some of the nuclear genes or genotypes. Organic selection will then operate through the elimination of genotypes which give lethal or harmful combinations with the cytoplasm. A final state will be reached when all the incompatible genes are eliminated and nuclear-cytoplasmic compatibility is re-established. The assimilated phenotype will often end up as a compromised version of the original (e.g. the enlarged-haltere line obtained by Waddington (1956) from the

bithorax phenocopy). Nuclear-cytoplasmic incompatibilities are well known in interspecific crosses (Moore, 1955). These result in developmental arrest often at gastrulation—the stage at which nuclear gene products are first used. By implication, intraspecific crosses succeed because the genes and cytoplasm are compatible.

Genetically, assimilation by organic selection will probably appear to be the result of polygenic factors mapped to all chromosomes, though maternal/cytoplasmic components and selection of *pre-existing* rare variants may also be implicated.

The alternative hypotheses presented above for genetic assimilation could be tested. If an instructive event is involved, assimilation will take place as readily in an isogenic line as in a massbred line, whereas the reverse is likely to be true if organic selection via cytoplasmic-nuclear incompatibility is involved. The application of recombinant DNA analyses in conjunction with more conventional genetic methods will no doubt provide a definitive picture of genetic assimilation at the molecular level.

CONCLUSION

When a truly physiological approach is adopted, one invariably arrives at an extended framework encompassing not only nuclear genes, but cytoplasm and environment (cf. Goodwin, this volume). Only such a system is capable of epigenesis and evolution.

Epigenesis depends on a series of nuclear-cytoplasmic and organism-environmental interactions. Cytoplasmic localization, crucial to embryonic development, is itself the result of an earlier ovarian epigenetic process. Davidson (1968) points out that the only preformation involved in development is the nuclear genome itself. So it is that in the neo-Darwinian scheme, in which development is seen as an unfolding of a preformed genetic programme, there can be no evolution except by fortuitous random mutation and natural selection. Within the framework presented in this chapter, development and evolution are formally and materially connected. Organisms develop in accordance partly with the assimilated experiences of their forebears and partly with their own experiences. Development evolves through the internalization of new environments. The material link between organism and environment, and development and evolution alike is the hereditary apparatus which realistically includes both cytoplasm and nuclear genes. The cytoplasm registers the somatic imprint of experienced environments which can be transmitted to the next generation independently of the nuclear genes. At the same time, it acts as a true

communication channel between the environment and the nuclear genome in the coordination of developmental and evolutionary processes.

There has been a recent explosion in molecular genetics research following initial breakthroughs in recombinant DNA technology. The genomic content of every organism is for the first time susceptible to being read base by base from beginning to end. Yet the first glimmerings have already yielded major surprises. Forever exorcized from our collective consciousness is any remaining illusion of development as a genetic programme involving the readout of the DNA 'master' tape by the cellular 'slave' machinery. On the contrary, it is the cellular machinery which imposes control over the genes. The central role of protein–protein and protein–nucleic acid interactions in the regulation of gene expression is reinforced many times over by the detailed knowledge which has recently come to light in both eukaryotic and prokaryotic systems. The classical view of an ultraconservative genome—the unmoved mover of development—is completely turned around. Not only is there no master tape to be read out automatically, but the 'tape' itself can get variously chopped, rearranged, transposed and amplified in different cells at different times.

The emergent picture is that of an extremely fluid genome amidst a potential chaos of mechanisms all threatening to subvert the existing order (Dawid, 1982). This only deepens the age-old enigma of the stability and repeatability of development itself. I believe the solution lies at least partly within the epigenetic framework.

Stability and repeatability reside in the dynamics of the epigenetic system in two senses. First, it is the automatic result of physicochemical reactions of which the system is composed—and the physicochemical environment in which the system is in turn embedded (cf. Saunders, this volume). Second, it is due to assimilated experiences held jointly in the nucleus and cytoplasm. These introduce regular biases into developmental reactions which may otherwise behave in a non-committal or unpredictable way (cf. Goodwin, this volume). Assimilated experiences therefore anticipate the environments to be experienced.

But the hereditary apparatus is not and cannot be completely prescient (*see* Plotkin and Odling-Smee, 1982). New or unforeseen environments will crop up during epigenesis of the present generation. The developing organism must react and adjust to existing circumstances. The multiplicity of mechanisms involved in gene expression poses the insurmountable problem of how the astronomical amount of 'information' required to control the mechanisms could be accommodated in the genome. The answer which organisms have discovered for themselves since the beginning of time is to rely largely on clues from the environment. So it is that the organisms adapt and the environment serves.

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Notes

(1) As Darwin was not a Darwinist and Marx not a Marxist, so Weismann too was not a Weismannist. He admitted the possibility of the inheritance of variations produced as the result of interactions involving physiological changes, as distinct from mere injuries (*see* Matsuda, 1982, for an interesting discussion on this point).
(2) Homeotic transformations—the substitution of normal structures by their serial homologues—are probably common to the entire arthropod phylum, as they have been observed in regenerating appendages in crustacea as well as in insects. St. George Mivart (1871) described varieties of pigeons which possess long feathers and partial fusion of the third and fourth digits in the legs. Both of those conditions are characteristic of the wings. This suggests the intriguing possibility that homeosis may reflect a fundamental organization of all developments.
(3) The best known are as follows: *Bergman's rule* states that in nearly related warm-blooded animals, the larger live in the north and the smaller in the south. *Allen's rule* states that the extremities (i.e. feet, ears, tail) of mammals tend to be shorter in colder climate in closely allied races. *Gloger's rule* states that southern races tend to be black, brown or grey and especially rust red whereas northern races tend to be pale.
(4) I am much indebted to Professor Lewis Wolpert for suggesting the term.
(5) Static gradients of hypothetical morphogens are a key feature of many current models of pattern formation. Since gradients are universal, the burden of explanation is placed entirely on different hypothetical threshold values of the same morphogen activating different genes, thus resulting in spatial pattern. The archetype is Wolpert's positional information model (*see* Saunders, this volume). The alternative view places greater emphasis on dynamic processes which establish a relatively more specific 'prepattern' of spatial organization (Stern, 1953).
(6) Recent investigations show that the alternative antigenic states involve transcription of different messenger RNA species (Preer *et al.*, 1981).

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