PHENOCOPIES, HEREDITY AND EVOLUTION[1]

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I. ABSTRACT

We review studies dealing with the mechanisms of the production of phenocopies and associated phenomena from 1864 to the present day. The role of these studies in elucidating the nature of gene action and developmental pathways is considered. It is concluded that phenocopies are produced via a range of mechanisms from simple enzyme/substrate effects to more complex phenomena such as the recovery of cells from stress. There have been attempts to increase the penetrance of phenocopies and to promote their genetic assimilation by Waddington, Bateman and others. They showed that selection, in the presence of environmental stimuli, resulted in enhanced expression and penetrance of particular phenocopies and that genetic assimilation resulted in some cases. Further evidence from the studies of Jollos, Borzedowska and Ho suggests that cumulative cytoplasmic factors are involved in this process. These may be genetic in nature, however, the existence of higher order factors cannot be excluded. Since relatively few species exhibit preformistic development (*sensu* Buss), there are a range of possible internalization mechanisms whereby heritable change may arise. These include seeding processes, as discussed by Sonneborn, and gene duplication and amplification. In those organisms that have early sequestration of germ cells, internalization may also occur with the concomitant violation of Weismann's barrier through the mechanisms suggested by Pollard, Steele and others. Finally we suggest that phenocopy phenomena illustrate that our concept of heredity requires revision, and that their further investigation is an important component of the study of evolution.

II. INTRODUCTION

Central to modern evolutionary theory is a particular conception of organisms and their environment. Indeed some concept of this relationship is fundamental to *any* theory of evolution. Neo-Darwinism generally maintains that a changing environment interacts with populations via differential reproduction of organisms of different phenotypes, and the genetic composition of such populations consequently changes through generations. This is thought to represent the controlling dynamic of

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evolution. An appropriate response by populations is however dependent upon the nature of the relationship between genotype and phenotype and on the nature of the interaction between organisms and their environment.

These relationships have been increasingly questioned in recent years as a result of a resurgence of interest in alternative evolutionary perspectives. For example, the work of Richard Goldschmidt on macromutations seems to be debated as often now as it was decades ago (e.g. Løvtrup, 1979; Clarke & Ford, 1980; Gould, 1982; Raff & Kaufman, 1983; Wallace, 1985). The experimental evidence for the transfer of genetic material from somatic cells to germ cells (Steele *et al.*, 1984) and the debate concerning the validity of the concept of Weismann's barrier (Buss, 1983; Pollard, 1987) has led to a questioning of some traditional views of heredity, and development (e.g. Ho, 1986; Ho & Saunders, 1979, 1982). Lewontin (1983) argued that our perspective of organisms as entities at the mercy of the environment is "incomplete and inaccurate". Recently, Løvtrup (1987) maintained that neo-Darwinism has either been falsified or is not falsifiable, and needs to be replaced by a more comprehensive evolutionary theory, which although recognizing microevolutionary events, also recognizes macroevolution (Løvtrup, 1979). Hitching (1982) has described a 'New Biology', Macbeth (1976) a 'Third View of Evolution' and Goodwin (1984) argued that we need a generative, rather than a specifically evolutionary theory in biology.

In the pattern of such changing ideas about evolution, the phenomena of phenocopies, genetic assimilation and internalization are of particular interest. The term *phenocopy* (see glossary), was originally introduced by Goldschmidt (1929, 1935*a*, *b*), and refers to an environmentally-induced character which closely resembles a known genetic mutant. *Genetic assimilation* is a term first used by Waddington (1953) to refer to a process whereby an environmentally induced change in form, such as a phenocopy, might under the influence of natural selection acting upon modifier genes, become heritable over a number of generations. He produced experimental evidence for such a process. Recently, Ho *et al.*, (1983*a*) performed a further experiment of this general type and obtained evidence that such phenocopies can increase in penetrance even in the absence of selection. They used the term *internalization* to refer to all processes whereby an environmentally induced character can become heritable whether in the presence or absence of selection.

We review the history of phenocopy experimentation and the progress of associated evolutionary and developmental concepts (see Goldschmidt 1938, 1957; Goldschmidt & Piternick, 1957*a*, *b*; Oyama, 1981 for other reviews). We then discuss our interpretation of these phenomena for an understanding of internalization, the nature of heredity and the relationship between organisms and their environment.

III. PHENOCOPY PHENOMENA

Goldschmidt (1929, 1935*a*, *b*) produced the first evidence for the widespread occurrence of phenocopy phenomena. He found that exposure of *Drosophila* to heat at a level not normally experienced by the organism (35-37^{0}C) at varying stages of development could induce a wide variety of phenocopies. Further, variations in the genetic composition of flies and the intensity and duration of treatment with heat shocks resulted in qualitatively and quantitatively different phenocopies. Phenocopies that appeared most commonly in the experiments included those that resembled bristle mutants (e.g. *forked*, *stubble*), wing (e.g. *scalloped*, *curly*, *arc*) and eye mutants (e.g. *lozenge*, *small eye* , *star*). Infrequently major body plan (e.g. *aristaless*) and colour modifications (e.g. *sooty*) also appeared. To Goldschmidt the study of such forms was a logical extension of his work on the enzymatic basis of sexual differentiation in the gypsy moth *Lymantria* (Goldschmidt, 1960). Through both these areas of research Goldschmidt attempted to elucidate the physio-chemical nature of gene action and heredity.

Goldschmidt (1938) discussed a number of earlier workers who had investigated environmentally induced forms resembling naturally occurring variants (Dorfmeister, 1864, 1869; Standfuss, 1896, 1906, 1907 and Merrifield, 1889, 1894; quoted in Goldschmidt,1938). These authors experimentally investigated the colour patterns of Lepidoptera wings. For example, Standfuss showed that it was possible to change the wing pattern of one race of butterflies to that of a pattern characterizing another race of the same species by subjecting the pupal stage to a temperature shock. For example, pupae of central European *Vanessa urticae*, when treated with low temperatures, produced adults indistinguishable from *V. polaris* found in Lapland. When the Swiss *Papilo podalirius* were subjected to high temperatures, individuals similar to *P. zanclaeus*, which is found in Sicily, were produced.

Beebe (1907) produced phenocopies of the dove *Scardafella inca* which resemble naturally

occurring varieties by rearing individuals at humidity levels corresponding to those of the habitat of each of the wild varieties. Tower and Beebe, like Standfuss and Dorfmeister before them, did not attempt to determine whether the same physiological mechanism was involved in the production of both the altered form and wild individuals, rather their interest centered on various phylogenetic and Lamarckian speculations (Goldschmidt, 1938).

As pointed out by Goldschmidt (1938) these workers were concerned with producing individuals which possessed characters similar to those of related species or races. Recently Stebbins & Basile (1986) proposed the term *phyletic phenocopies* for these forms which closely resemble the phenotype of a related taxon. They suggested that a study of the mechanisms of phyletic phenocopy production may aid the study of "...the developmental basis of evolutionary change, and occasionally ... the nature and action of the genes involved" (Stebbins & Basile,1986: 422). They discussed the experiments of Kollar & Fisher (1979) on the induction of teeth in hens and Rosenblum & Basile's (1984) induction of leafy shoots in plants that ordinarily develop a laminar vegetative body (a 'phyllomorph'). Other interesting examples of recent work on phyletic phenocopies, highlighted by Stebbins & Basile, include digit reduction in amphibians (Alberch & Gale, 1983, 1985) and phenocopies of the green alga *Volvox carteri* which resemble other Volvocale species (Kirk & Harper, 1986). Shapiro (1981, 1982) recently illustrated phyletic phenocopies in a number of butterfly species. Two *Nymphalis* species showed "ancestral" characters when subjected to cold shock (Shapiro, 1981) and *Pieris occidentalis* from lowland California exposed to a temperature of 6°C for 10 days or a short daylight regime (10L:4D) produced the vernal-alpine "*calyce*" phenotype.

The first studies of phenocopies (*sensu* Goldschmidt) were performed in the 1920s. Timofeeff (1926; quoted in Goldschmidt, 1938) reported that carefully timed temperature shocks of the pupa could induce structural changes in the wings of adult wild-type *Drosophila funebris*. The resulting phenotype resembled known genetic mutants such as *curly wings*. Jollos (1933, 1934) studied phenocopies (although he did not refer to them as such) in a manner similar to Goldschmidt. He found that qualitatively and quantitatively different phenocopies could be induced by varying the moisture content of the environment during heat treatment of *Drosophila* larvae. He also found that true genetic mutants and their associated phenocopies could result from the same treatment. Jollos (1934) tentatively proposed that in the production of phenocopies the heat treatment did not alter the genes, but "something else", which he proposed was a specific substance that was normally directly produced by the mutant gene; the "gene-product". Jollos (1934: 491) went further and hypothesized that because a mutant gene and a corresponding "gene-product" (in phenocopy production) resulted in a similar phenotype, the mutant "genes and the "gene-products" have the same or very similar structure". He also thought that the greater prevalence of phenocopy production compared with the true heritable genetic mutation was attributable to the "better insulation of the genes due to protection by the chromosome cover" (Jollos, 1934: 491).

Goldschmidt (1938, 1945) extended Jollos' hypothesis by suggesting that the observed pattern of phenocopy production indicated the existence of two quite distinct mechanisms of gene based phenotype production. The first class of mutant genes, Goldschmidt argued, are involved in the synthesis of specific substances, such as eye colour in *Drosophila*. This was regarded as the type of gene action that could not be mimicked by a phenocopy. By contrast, the second class of mutant genes was seen to control the relative velocities of chemical reactions during the developmental process. This type of gene action could be mimicked by an environmental stimulus and the resulting form classified as a phenocopy. Goldschmidt saw that this second type of gene action was a serious challenge to the prevailing one gene-one enzyme theory of gene action. He argued genes could control the *kinetics* of development, not just the production of specific substances.

Rapoport (1939), however, presented evidence that seemed to contradict the gene action dichotomy proposed by Goldschmidt. He found that chemical shocks could induce phenocopies that mimicked apparently qualitative changes in the organism. Phenocopies that had never been produced by thermal shocks, such as *yellow*, *aristapedia* and *eyeless*, appeared in treatments using silver lactate and various boron compounds. Rapoport (1939) interpreted this result as demonstrating that chemicals in the natural environment can play a significant role in non-hereditable form production. He considered that chemicals could have a far more profound influence on the form of organisms not by merely interfering with the kinetics of developmental processes, but also by actually causing qualitative changes in the character of the "morphogenic substances, the protoplasm and the genes themselves" (Rapoport, 1939: 417).

Child *et al.*, (1940) presented a detailed study of the sensitive periods of thermal phenocopy induction and found that heterozygous *Drosophila* were more sensitive to temperature than inbred homozygous individuals. In addition, Child *et al.* (1940) found that temperature shocks could cause

reversals of dominance whereby normally recessive mutant genes were expressed preferentially over normally dominant wild-type genes. This led Child *et al.* (1940) to conclude that the observed form of an organism is intimately linked to the nature of the 'external' and 'internal' environment in which development takes place.

A feature of the experiments of this time was the discovery that different environmental stimuli (e.g. heat, cold, humidity or chemicals) could produce identical phenocopies, but that the sensitive period for the induction of a particular phenocopy was different depending on the nature of the stimulus. In addition it was found that the sensitive period for induction of, for example, a bristle reduction phenocopy, was several hours in advance of the actual differentiation of the affected structure; in this case, the formation of the trichogenic cells. Child (1935) found that in *Drosophila melanogaster* heat treatment during the third larval instar could induce bristle reduction, whereas the sensitive period for X-ray induction is prior to this stage (Blanc & Braun, 1942).

Bodenstein & Abdel-Malek (1949) studied the induction of the aristapedia form from a developmental perspective. They concluded that the substitution of a leg-like structure for a normal arista did not serve to highlight the underlying mechanisms of genetic control of characters, but pointed to the plasticity and "potentialities" of the cells and imaginal discs that comprise an organism. To explain how different methods (e.g. X-rays, temperature, colchicine, nitrogen mustards etc.) could produce similar phenocopy effects Bodenstein & Abdel-Malek (1949: 110) suggested that "... the determining mechanism involved in these effects must be unspecific, a principle of rather general nature" rather than a specific gene action or gene product. Bodenstein & Abdel-Malek (1949) proposed that the developing organism is composed of a series of "growth fields", each of which has the potential to develop along a number of pathways to various end-point organs. While a growth field usually follows one path, to form an arista for example, a perturbation in the developmental sequence can divert the destiny of the cell cluster towards an equally rational outcome, for example a leg. In such a scenario genes may not play a dominant role in controlling form production, since the resultant form might be merely a consequence of the developmental properties of the system.

Sang & McDonald (1954) presented information that seemed to contradict Goldschmidt's argument that phenocopies could serve to elucidate the nature of gene action. They studied the effect of sodium metaborate on the eye development of wild type (+/+), heterozygous (+/ey) and homozygous (ey/ey) "eyeless" *Drosophila melanogaster*. They found differences between the modes of operation of the mutant gene and the metaborate, despite gross similarities in the resulting phenotype. They concluded that, as the eyeless phenocopy and the mutant eyeless condition were produced in a qualitatively different manner, the study of phenocopies could therefore only rarely provide an insight into gene action.

By the 1950s a substantial number of experimental studies on phenocopies had been completed. In a paper entitled "Problematics of the Phenocopy Phenomenon" Goldschmidt (1957) suggested that a number of significant experimental findings need to be explained in any comprehensive theory of phenocopy induction. These include the following:

1) There is the potential to produce phenocopies in all organisms.
2) A large range of treatments (e.g. heat, cold, chemicals, UV rays) produce phenocopies.
3) All known morphological mutants of all organs have been phenocopied in *Drosophila* by a variety of treatments.
4) Chemical treatments tend to produce more specific alterations in form than radiations or temperature.
5) All treatments achieve the highest penetrance at doses near the lethal limit.
6) The penetrance of a particular phenocopy tends to be proportional to the intensity of treatment.
7) The type of phenocopy and the penetrance and expressivity of any particular phenocopy is dependant upon the genetic constitution of the treated line.
8) Phenocopies tend to have specific sensitive periods for induction during development, this period usually relates to the onset of differentiation of the affected structure.
9) Phenocopying agents can, when applied to mutant individuals, affect the expression of the mutant character (i.e. decrease or intensify the mutant condition depending on the particular mutant in question).

Waddington (1953, 1956), Bateman (1959*a, b*) and Goldschmidt & Piternick (1957*a, b*) proposed models for phenocopy production based on the existence, in the experimental population, of sub-threshold alleles and modifier genes. In these models the action of the phenocopying agent and experimental selection cause the expression of the normally sub-threshold alleles, resulting in an aberrant form. Assimilation of the form in future generations was 'facilitated' by the modifier alleles.

Goldschmidt (1957) and Goldschmidt & Piternick (1957*a,b*) extended Goldschmidt's (1938)

interpretation of phenocopy production to emphasize a view similar to that propounded by Bateman (1955) and Waddington (1953, 1956). Goldschmidt & Piternick (1957*a*: 165) stated that "... the different effects of treatment upon different genetic lines are due primarily i.e. apart from differences in the modifier system, to the presence of sub-threshold alleles ... of the mutants which are phenocopied; and that their action is raised above the level of visible effect by the treatment." Thus a phenocopy "... would not be a modification of development in the complete absence of the phenocopied mutant ... but phenocopy would rather mean a bringing to light of an otherwise sub-threshold mutant" (p165) and thus the observed form only "... appeared to be phenocopied" (p196). Hence for Goldschmidt & Piternick phenocopies are genetic mutants (albeit normally disguised) not developmentally convergent forms. However Goldschmidt & Piternick (1957*b*: 223–224) admitted that this interpretation does not adequately fit all their data.

Landauer (1958: 208) disagreed with the genetic assimilation models, directing his criticism at Goldschmidt (1957) and Goldschmidt & Piternick (1957*a, b*). Landauer (1958) contended that Goldschmidt's interpretation of phenocopy production was simplistic at best and that his need to "...appeal to special genes, whether they be called subthreshold mutants (isoalleles) or a combination of isoalleles and suppressors, places an insupportable burden on his hypothesis." Landauer (1958) argued that his work on fowls (Landauer, 1954, 1955, 1957) suggested a different interpretation for phenocopy production whereby they represent the end result of *interference* with the activity of a gene that under most circumstances would give rise to a normal phenotype. Thus organisms that resemble genetic mutants appear as a result of a 'weakness' in the constitution of the stock so that the pathway 'snaps' into an alternative developmental channel in response to a perturbation (analogous to the model proposed by Bodenstein & Abdel-Malek, 1949). He pointed out that this explains three of the most common features of phenocopy induction. Firstly, there is variation in the penetrance of some traits. Hence, direct gene controlled processes such as pigmentation are less susceptible to interference by phenocopy-inducing agents than are rate-dependant processes. Secondly, there is variable expressivity of any particular phenocopy in genetically different stocks. This is because different stocks have different degrees of "developmental integration" and thus differing susceptibilities to intervention by phenocopy-inducing agents. Thirdly, individuals appear within a stock having different grades of expression of the phenocopy.

Landauer (1958: 211) thus regarded phenocopies as indicating "...a failure of evolution to provide mechanisms which could cope with ... genic or environmental sources of dislocation" and that phenocopies provided an opportunity to "... shed light ... on the developmental functions of that awesome skeleton in the closets of genetical science - the normal genotype".

More recent models of phenocopy induction incorporate the more detailed understanding that now exists of the cellular and molecular mechanisms involved in gene expression and development. A variety of different mechanisms have been suggested and any or all may be involved in the induction of phenocopies, dependent of the nature and degree of the changes induced.

Capdevila & Garcia-Bellido (1974) have shown that the bithorax phenocopy can be induced by exposing eggs to ether at the syncytial nuclei stage of blastoderm formation. These workers also showed that exposure to the phenocopy agent resulted in alterations in the state of single cells which pass on this new state by cellular inheritance. When flies carrying mutant bithorax genes were exposed to ether they exhibited the same range of bithorax phenocopies as wild type flies and the mutant condition was not necessarily expressed. The authors suggested that this was evidence that the ether treatment acts by suppression of the bithorax system. Ho (1984), however, suggested that ether disrupts a prepatterning event during blastoderm formation. She discussed evidence that the ether affects the cytoplasm rather than the syncytial nuclei of the precellular blastoderm. At this stage the nuclei are totipotent and can incorporate into any cell type whereas the subsequently differentiated blastoderm cells are already determined.

Mitchell & Lipps (1978) proposed a molecular transcriptional model to explain the induction of heat shock phenocopies. Their experiments showed that heat shocks resulted in temporary suppression of transcription followed by a period of partial or complete recovery, although this does not explain the alteration in pattern. A category of proteins have been identified whose transcription and synthesis is accelerated by higher temperatures (Peterson & Mitchell, 1985). These proteins then bind to the DNA suppressing transcription of other proteins to varying degrees. Recent work (Pelham, 1985) has established that heat shock proteins are switched on by a heat sensitive protein (heat shock transcription factor) which binds to a site on the DNA upstream to the heat shock protein gene. This site is called a heat shock element and, together with the heat shock transcription factor, initiates heat shock protein

transcription (Pelham, 1985). The resulting proteins, of which there are many classes, seem to have a variety of roles in the regulation, inhibition and especially the repair of the transcription process during heat shocks and during exposure to some chemicals (Pelham, 1985; Peterson & Mitchell, 1985). According to Mitchell & Lipps (1978) heat shock phenocopies are produced when, during the recovery stage after a heat shock, transcription of certain proteins resumes too slowly or not at all, thus creating an imbalance of gene products affecting development. It has been shown that phenocopy production is reduced when individuals are given a mild heat shock prior to a major heat shock. The reduction is greatest when the pretreatment fully induces heat shock protein transcription without inhibiting cellular protein synthesis (Peterson & Mitchell, 1985). This suggests a possible, but as yet undemonstrated, role for heat shock proteins in minimizing damage during heat stress. It may be that abrupt changes in temperature do not allow sufficient time for the heat shock protein system to act to prevent the transcriptional imbalances during recovery.

However, Nijhout (1985) pointed out that this model does not explain the link between specific phenocopies and stimuli at specific sensitive periods. He proposed that in order to understand the origin of a specific phenocopy it is necessary to discover the role of gene action in the development of the affected character. It is then possible to postulate which transcription products are involved and how their absence or reduction in quantity will alter development.

Similarly Ananthan *et al.* (1986) reviewed research on the biological effects of environmental agents such as temperature, free radicals, ions, organic chemicals and heavy-metals. These effects include translation errors, protein denaturation, enzyme inhibition and modifications to cellular activity. Intracellular proteins that have been denatured by these agents activate heat shock protein genes (Goff *et al.*, 1984). Ananthan *et al.* (1986) proposed a model similar to those discussed above where a specific factor is activated by the denatured protein and then switches on a heat shock protein gene.

Ho *et al.* (1983*b*, 1987) believes that the link between specific gene action and phenocopies are very tenuous and that an alternative view where phenocopy induction is linked to the disruption of patterning processes (of which genes are necessary though not sufficient components) may be more tenable.

IV. GENETIC ASSIMILATION

The preceding discussion illustrates that there was a considerable body of information available as to the range and mode of action of phenocopies. Furthermore, there has been substantial debate over the processes underlying their induction and the significance of the fact that phenocopies often resemble naturally occurring forms. Waddington (1953, 1956) performed experiments using phenocopies to, firstly investigate the action of natural selection on the causal processes of development (i.e. epigenetics; see glossary) and secondly to consider the possible evolutionary significance of phenocopies.

Waddington (1957) conceptualized the potentialities of development in terms of the visual metaphor of the 'epigenetic landscape'. He argued that a fundamental feature of organisms is their ability to maintain developmental pathways in the face of varying environmental and genetic perturbations. He called this property of developmental buffering *homeorhesis,* which can be represented on the epigenetic landscape as *canalization.* Alternative pathways down the same developmental valley within the landscape Waddington called *chreods.* He considered the broad outline of a chreod to be due to internal developmental constraints, but that the form of a specific chreod resulted from past selective forces.

Using the concept of epigenetics Waddington (1957) attempted to provide a mechanism by which a novel developmental response to an altered environment could become hereditable. Such responses would otherwise seem to require a Lamarckian explanation. Waddington envisaged that selection for modifier genes would lead to canalization of new forms. After many generations of selection the response would occur progressively earlier in development. Eventually certain 'major gene' mutations, occurring at random, could 'fix' the response in the developmental process so that it would occur even in the absence of the original environmental stimulus. At this point the response is said to be 'genetically assimilated'.

Implicit in Waddington's model was the presence in a population of substantial genetic variation. Moreover, a specific prediction arising from this presupposition is that genetic assimilation should not occur in a genetically homozygous stock (an assumption not tested by Waddington, but considered by later workers). Hence for Waddington (1953) the mechanism of assimilation was not clear. At that time, he suggested it could have represented a major gene mutation similar to the Baldwin effect (organic

selection; Baldwin, 1896, 1902; Lloyd-Morgan, 1900) or the end result of selection for modifier genes (see Ho, 1984).

Waddington (1953) first carried out an experiment on the genetic assimilation of the *Drosophila* wing phenocopy *crossveinless*. The phenocopy was produced by giving pupae a thermal shock. This experimental line was continued for twenty three generations, giving each generation a heat shock. By positive selection in each generation (selectively breeding from flies showing the altered phenotype) he was able to greatly increase both the penetrance and degree of expression of the phenocopy. Eventually he was able to show that the altered phenotype persisted even in the absence of the original environmental stimulus, in other words, genetic assimilation had indeed occurred.

Waddington (1956, 1957) carried out a further experiment on the *bithorax* phenocopy using a mass-bred, wild-type stock. In each of two replicate experiments, two lines were started using the offspring of the treated flies, one in which only flies showing the bithorax phenotype were mated (positive selection) and the other in which only flies showing the normal phenotype were mated (downward selection). Ether treatment of the eggs was carried out in every generation in all lines and the process of selection repeated up to generation 29. In addition, some eggs in every generation of the upward selection line were allowed to develop untreated to test for possible assimilation. Interestingly although the two replicates were drawn from the same stock, they behaved differently. In replicate one, the F_1 generation contained 24.5 percent phenocopies, whilst there were 48.8 percent phenocopies in the replicate two F_1 generation. Waddington was unable to explain this. After a number of generations of upward selection, it was observed that some flies with the bithorax phenotype emerged even in the absence of ether treatment. (Note: no check was made to see if bithorax offspring were produced in the absence of ether treatment in the *downwards* selection line.) By mating these nontreated, but bithorax adults together and thus selecting for spontaneous appearance of the bithorax phenotype, three true breeding bithorax lines were produced which consistently gave rise to 70 to 80 percent bithorax offspring. The phenocopy had become genetically assimilated. Genetic analysis showed that in two of these lines (which showed only a slightly altered phenotype) a single dominant mutation seemed to be responsible, while in the third (which showed the full bithorax condition) a polygenic system was apparently involved. In this third case there was also an effect due to a recessive X-chromosome condition.

Waddington (1956: 12) remarked " the fact that such a bizarre phenotype as bithorax can be assimilated, with high grade expression ... suggests that the genetic assimilation mechanism is a very powerful one, which could have far-reaching effects during evolution". He also stated "It seems likely that any modification produced by the environment could, if it were favorable to the animal, be genetically assimilated in a relatively short time" (1956: 10). After some indecision, Waddington (1975: 89) stated that genetic assimilation was in no way a case of organic selection (Lloyd Morgan, 1900; Baldwin, 1902) because organic selection "...differed from the notion of genetic assimilation primarily because it considered the initial adaptation to the new environment to be a non-genetic phenomenon on which selection has no effect."

Bateman (1955, 1959*a*, *b*) carried out similar experiments with wing phenocopies, obtaining comparable results to those of Waddington. Subsequent genetic analysis showed that polygenic effects were involved in the genetically assimilated stocks. Bateman (1955, 1959*b*) maintained inbred control lines in some of these experiments (Waddington had not done this) and reported no progress in the penetrance of the phenocopies in these lines. This result was consistent with Waddington's modifier genes model. However, as Ho *et. al.* (1983*a*: 358) pointed out, "neither was there any progress in the massbred line until the high mortality of the phenocopied flies was reduced by shortening the period of heat treatment. No parallel checks on the mortality of the phenocopied flies were carried out in the inbred lines." Furthermore, in one experiment on the assimilation of the *posterocrossveinless* phenocopy, two inbred control lines were included. To one line positive selection was applied whilst to the other there was no selection. Bateman (1959*b*) reported a lack of response in both lines. However, from her data it is evident that both showed an almost identical pattern of phenocopy penetrance to that of the massbred selected line until the tenth generation when the inbred control lines were discontinued. No tests were made to see if the phenocopy form would appear in untreated offspring of the inbred controls, moreover, there is positive evidence for cytoplasmic effects in Bateman's own analysis (Ho *et al.*, 1983*a*).

V. CYTOPLASMIC FACTORS

Jollos (1934) reported a remarkable experimental observation that has been overlooked by nearly all subsequent workers in this field. He reported that some "non-hereditary modifications" produced by subjecting *Drosophila* to heat shock during development showed a tendency to linger for a few generations even in the absence of the original environmental stimulus. These modifications were heritable only through the female line and would persist for a maximum of six generations. He therefore concluded that they were inherited through the egg cytoplasm. He called such modifications *dauermodifikationen* ('semi-permanent modifications'), a term which he also used for similar modifications which he had described in protozoa (Jollos, 1913, 1921; cited in Jollos, 1934). The modifications which he reported as showing this tendency to persist in *Drosophila* were as follows: aeroplanoid wings, dwarfism, rudimentary wings and higher resistance to the heat treatment. A similar result was reported by Harrison (1928) who induced changes in the pigmentation of the cabbage white butterfly by exposing pupae to orange light. This induced change persisted for several generations again in a way consistent with its being passed on through the egg cytoplasm.

The obvious implication of these observations is that inheritance through the egg cytoplasm could be involved at least in the early stages of genetic assimilation (Ho & Saunders, 1979). Waddington (1975) and Bateman (1955, 1959*a*, *b*) did not test for this possibility, however the comprehensive experiments of a Polish worker, Borzedowska, and the recent experiment of Ho and her co-workers have now shown conclusively that this is in fact the case.

Borzedowska (1963) experimentally investigated the production and subsequent genetic assimilation of the wing phenocopy, *extracrossvein*. This phenocopy was induced by applying heat shocks of 35°C soon after pupation. This treatment was continued for ten consecutive generations during which time selection for the altered phenotype occurred. After nine generations of thermal treatment and selection, a stock was returned to normal conditions (20°C) for two further generations. Under these conditions a small percentage of the experimental animals exhibited partial transmission of the phenocopy. These individuals formed the basis of the experimental lines which were then subjected to selection alone for further 19 generations.

Throughout the experiment there was a substantial increase in both the expression and penetrance of the *crossveinless* condition. For example, in the final generation a number of individuals appeared which had two, and in some cases three extra crossveins. The total frequency of phenocopied individuals progressively increased, finally reaching 98.06 percent. An important feature of these results was the significant difference in the behavior of the two sexes after the cessation of the heat shock, due to sex-linked gene expression; females exhibited a stronger response in both expression and the degree of penetrance of the phenocopy. In the 30th generation the total frequency of *crossveinless* individuals was 100 percent in females, in comparison to 95.96 percent in males. This difference was more marked when complete extra crossveins were considered, for example 99.33 percent of females and only 73.11 percent of males.

Borzedowska (1966*a*) subjected the same treatment stock to 20 generations of further selection in an attempt to investigate whether this difference between the sexes would continue. She found that this trend did in fact persist, with males exhibiting incomplete penetrance even after a period of prolonged selection.

Genetic analysis of the assimilated forms (Borzedowska, 1966*b*) involved a series of reciprocal crosses, between treatment and control lines. It was found that treated P_1 females had, in general, a greater capacity to transmit the condition to their offspring than did treated P_1 males. In the F_1 a greater penetrance was found in females. In the F_2 generation, resulting from crosses between heterozygotes, similar degrees of transmission were found in all combinations of crosses except in those involving treatment females and control males. In this situation a higher penetrance was observed. Individuals with an extra crossvein appeared in the F_3 generation from mating F_2 flies which had no phenotypic changes. This result, in combination with those from F_1 backcrosses, indicates a non-Mendelian mode of inheritance.

Genetic analysis by further breeding experiments confirmed the feature had been genetically assimilated, with numerous loci on most or all chromosomes being involved (Borzedowska, 1970, 1972). Her conclusion was that, in this case, genetic assimilation resulted from an interaction between cytoplasmic and chromosomal factors (Borzedowska, 1972) .

Ho *et al.* (1983*a*) repeated Waddington's (1956) experiment on the assimilation of the bithorax

phenocopy. They followed Waddington's original procedure as closely as possible but designed their experiment specifically to test for the possible role of maternal cytoplasmic factors. Their experimental design differed from that of Waddington's in the following ways. Firstly they used both a massbred and an inbred stock (these were of the same genetic background). Secondly, they did not select for the phenocopy. In fact Ho *et al.* (1983*a*) argued that the evidence suggested that bithorax flies were in fact less viable than normal flies. Hence the bithorax condition was, if anything, being selected against.

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 applied. However, a steady and significant increase in the frequency of the phenocopy was observed in both the massbred and inbred lines. Furthermore, the phenocopy effect was observed to linger after cessation of ether treatment and was only gradually lost from the population; that is it behaved in a similar manner to the phenomena described by Jollos (1934). As Ho *et al.* (1983*a*) pointed out, these observations are consistent with the presence of cumulative cytoplasmic effects.

Further evidence for cytoplasmic effects was found when after the first six generations of ether treatment, embryos from a cross between treated females and control (untreated) males showed the same increased tendency to phenocopy as embryos of the long-term treated line. Reciprocal crosses produced embryos which were no more responsive than controls.

A striking feature of the results is the great similarity in the response of the massbred and inbred lines. Ho (1984: 276) concluded that " this strongly implies a basic identity in the epigenetic processes underlying the basic response to long term ether treatment in both lines." The possibility that modifier genes played some role in the increase in the phenocopy response cannot be entirely ruled out. This is because the inbred line was tested by isozyme electrophoresis and found to contain residual genetic variation (i.e. it was not isogenic; heterozygosity of the inbred line was 0.5% cf. massbred line 11.5%). However she argued that "... to explain the results in terms of selection for modifier alleles one must assume that almost precisely the same alleles were still present in the inbred as in the massbred 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. 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" (Ho, 1984: 278). It is important to realize however that although the phenocopy was shown to linger for a few generations after cessation of ether treatment, genetic assimilation was not shown to have occurred in this experiment. It is tantalizing to speculate whether genetic assimilation would in fact have resulted had the experiment been continued for a larger number of generations. It would also be of great interest to repeat this experiment and to include negative selection lines. If phenocopies appeared in the offspring of inbred, negatively selected lines cumulative cytoplasmic factors are most likely to have been responsible, rather than selection acting upon pre-existing genetic variation.

VI. INTERNALISATION

The evidence reviewed in the previous sections has shown that a phenocopy can increase in penetrance and expression primarily by the accumulation of cytoplasmic factors, with or without selection for the altered form. Further, genetic assimilation can involve interaction between the cytoplasm and the chromosomes. In other words, there is evidence that a phenocopy can become heritable (i.e. become internalized) by processes other than random mutations. Perhaps the primary reason why the possibility of a range of internalization mechanisms has not been widely considered in modern biology is the almost universal acceptance of Weismann's theory (Weismann, 1893) that no environmental influence on somatic tissue can be transmitted to the germ line.

However as Buss (1983) pointed out, in many organisms (all plants and the majority of animal phyla) the germline-soma barrier (the so-called 'Weismann's barrier') simply does not exist because germ cells are produced directly from somatic cells even in fully-grown organisms (somatic embryogenesis). In many other cases the germ tissue is not determined until relatively late in development (epigenetic development) and thus may record prior environmental influences. Only in vertebrates and a few other animal groups does early and irreversible sequestration of the germ tissue (preformistic development) occur. As we discuss below, there is evidence for mechanisms whereby this barrier can be penetrated even in this latter case. Hence an understanding of heredity substantially

depends on the nature of the processes by which somatic and germ cells interact. A range of internalization processes is therefore conceivable.

There are many possible processes of internalization (Pollard, 1987) such as seeding processes (Sonneborn, 1963, 1970), gene amplification and gene duplication (e.g. Schimke, 1980, 1983; Cullis, 1977, 1984), transposition and DNA methylation (Holliday, 1987). We discuss some of these below.

(1) *Seeding processes*

Seeding processes, or more correctly in this context 'cytotaxic processes', are those that exhibit an "ordering and arranging of new cell structure under the influence of pre-existing cell structure" (Aufderheide *et al.*, 1980: 253). While genes may specify the molecular components of cell surface structures experimentation has demonstrated that the assembly of such components into structures like cilia and the cytoskeleton proceeds largely independent of gene control. The heritability of such structures has in addition been found to reside within the structures themselves, rather than in the nucleic acid compliment of the cell.

Sonneborn (1970), in his studies of extra-chromosomal inheritance in *Paramecium*, was primarily interested in the assembly of genic products into organized structures, and the control of such assembly. *Paramecium* have a well structured cortex subdivided into longitudinal rows of 'unit territories'. These territories contain, in broad outline, a cilium, a basal body, a set of microtubules, a parasomal sac and a kinetodesmal fibre. Alternatively, unit territories exist (located in specific areas of the cortex e.g. the anterior part of the cell) that possess two basal bodies and cilia and, in addition to the features mentioned above, a fibre connecting the two basal bodies (Sonneborn, 1970). These two types of territory are arranged in a characteristic pattern around the *Paramecium*, and the components of each territory are always found in a fixed orientation with respect to the axes of the cell. Experimentation demonstrated that the spatial and temporal developmental events in the cortex were determined by the microgeography of the cortex itself, rather than being under any direct genetic control. Chen-Shan (1969) amputated one end of the cell to create a 'bald' (cilium and basal body free) area of cortex and found that the basal bodies and cilia that eventually filled in the bald area in succeeding cell divisions arose in an orderly fashion from the edges inwards and only immediately anterior to an existing basal body. That is, the microregion immediately anterior to the basal body was the only one which can mediate basal body assembly. Beisson & Sonneborn (1965) demonstrated the importance of cortex microgeography in the self regulation and development of cortex structure by the 180^0 rotation of a small patch of unit territories relative to their neighboring territories on a *Paramecium*. They found that in succeeding cell divisions the inverted patch spread along the length of the cell surface and 800 subsequent generations of progeny of this aberrant individual retained this inverted row. The inverted rows were inherited sexually and asexually, independant of changes in the cytoplasm or genetic compliment of the cell. This modification was by no means 'adaptive', indeed it caused the individuals to swim in a cork-screw manner. Sonneborn (1970: 353) said of the experiment: "There is no escape from the conclusion that the site of initiation of basal body assembly, its path of migration to the surface of the cell, and the orientation of associated structures around it are indeed determined by the molecular geography *within* the unit territory and *not* by any other outside influence, either nuclear or cellular." Grimes (1976) also found that cortical structures positioned in a 'foreign' environment undergo morphogenesis 'true to type' and such altered morphotypes are hereditary in subsequent generations.

Investigation of the nature of the development of the ingestatory apparatus of *Paramecium* also confirmed that the cortical requisites for development are largely self-reproducing. Sonneborn (1963) created 'doublet' paramecia which had double vestibules (an open depression of the cell surface at the entrance of the 'mouth') and gullets 180^0 apart. The doublet form was found to be hereditary, and if one gullet was lost (Sonneborn, 1963) or injured by ultraviolet irradiation (Hanson, 1962) a double body-single vestibule/gullet condition was also hereditary. Furthermore, if a piece of tissue from the right side of the oral region was taken from one cell and implanted close to the gullet in another cell, a single cell-double vestibule/gullet line could be formed (Hanson *et al.*, 1960; cited in Sonneborn, 1970). The above observations suggest that the number of vestibule/gullet structures formed in any line is not determined by nuclear DNA action. Rather it is determined by the presence, number and position of vestibule/gullet junctures, and gene action cannot restore or repair the original condition in the face of the self-replicating juncture system (Sonneborn, 1970).

Sonneborn (1970) drew an analogy between the observations described above and the *in vitro* assembly of organelles and viruses. Flagella-like structures can be precipitated from a solution of flagellin following the addition of small pieces of organized flagella. In the same way, pieces of mutant flagella ('curly') added to a solution of wild type 'wavy' flagellin produce curly flagella (Oosawa *et al.*,

1966; cited in Sonneborn, 1970). This process, named 'seeding' by Sonneborn, demonstrates that the "... presence or absence of an existing molecular assembly, a nucleation center, can determine under certain conditions whether molecules remain in solution or assemble into structures." He further stated that "... the conformational state of the nucleation center can determine the conformational state of the molecules that grow on it, thus determining the gross form of the resulting structure" (Sonneborn, 1970: 349). These phenomena illustrate that changes in organismic structure caused by a variety of non-genetic means can be heritable.

More recent work on cell organelle assembly and cytotaxis has been reviewed by Aufderheide *et al.* (1980) and Grimes (1982) among others, and readers are referred to these reviews for details and references. For the purposes of this review it is only necessary to examine some of the major findings of molecular and pattern determination studies.

The cell surface controls developmental responses at multiple levels within the cell. It has been found that in cases of deflagilation or decilation the cell surface can respond in one of two ways to obtain components needed for the regeneration of these structures. The cell surface can induce the synthesis of de novo molecular components (Synthesis-dependant assembly) or alternatively (for example, if cyclohexamide is present to inhibit protein and RNA synthesis) can draw on a 'flagellar precurser pool' of tubulin (Synthesis-independent assembly) to form structures.

Once in control of the necessary molecular components, the next level, assembly and patterning of individual structures, appears also to be controlled in large degree by the cell surface. Structures can be patterned concurrently with assembly, as in the propagation of ciliary units directly under the control of adjacent and pre-existing ciliary units (see earlier discussion of the experiments of Beisson & Sonneborn, 1965 and Chen-Shan, 1969). Alternatively, structures can be assembled then moved into position; thus showing assembly followed by patterning. Possibly the most prominent case of this type of local patterning is of trichocyst assembly. Trichocysts are assembled deep in the cytoplasm then transported to the cortex to occupy vacant sites in the plasma membrane (Aufderheide, 1978*a, b*).

The highest level of cytotaxis involves the patterning of organelles and other structures over large intracellular distances. The structures of interest at this level are those that are non-randomly positioned in the cell e.g. contractile vaculoles and the gross orientation of blocks of similar structures such as cilia. As hypotheses, two extreme positions are tenable, with a range of options between the two. It could be that cytotaxis at this large-scale patterning level is merely an extrapolation of the mechanistic processes seen at the next lower level, or alternatively, the large-scale events could be entirely removed from 'lower' processes and exhibit higher-order properties different in nature and principle from lower processes.

Examples of large-scale patterning are abundant and include such features as doublets and duplicated oral apparatus (see for example Sonneborn, 1970; Aufderheide *et al.*, 1980; Frankel, 1982; Frankel & Nelsen, 1986). Two major conceptual approaches have been adopted to understand large-scale patterning (Aufderheide *et al.*, 1980). The first, influenced by the existence of 'determinative regions' envisages an 'intra-cortical communication system' of localised nearest-neighbour interactions (Sonneborn, 1975). The second, influenced by the relational aspects of morphogenic fields and global polarity, envisages positional information being 'read' from cell surface structures, and morphogenic fields delineated by major structural discontinuities or topographic boundaries (see Aufderheide *et al.*, 1980: 292).

Frankel & Nelsen (1986) attempted to understand how large scale patterning can develop. They studied the transient phenocopy *janus* in the ciliate *Tetrahymena thermophila*, which characteristically produces a mirror-reversal of the large-scale pattern of part of the cell surface. Many workers maintain that the phenocopic agent brings about the altered phenotype through a stable inactivation of the relevant wild-type gene. However, Frankel & Nelsen (1986) found that the configuration of the phenocopy was the result of geometric rather than genic stimuli. In fact, they argue that the common link between the phenocopy and the genetic mutant is not genetic (i.e. both inactivate wild-type genes) but "a final morphogenetic response rather than a specific internal pathway that brings about that response" (Frankel & Nelsen, 1986: 83).

Some may argue that seeding processes of the type described above are of limited interest in the larger evolutionary sphere partly because of their demonstration only in lower eukaryotic organisms, and because of their relevance only to organelle-templated organelle biogenesis. However, as Hjelm (1986) recently pointed out, the phenomenon of cytotaxis observed in ciliates may eventually be recognised as a universal biological principle controlling pattern determination in all organisms, and be intimately involved in processes such as carcinogenesis.

Most cells contain cytotactically inherited information regarding the biogenesis of organelles, including the cytoskeleton, cell surface differentiation and plane of division. The modification of such information by carcinogenic substances could lead to neoplastic transformation and the subsequent proliferation of cancerous cells. As early as 1942 Mottram (cited in Hjelm, 1986) proposed that some cases of tumourigeneis could involve aborted cell divisions that resulted in enlarged cells with abnormal nuclei; a state directly comparable to doublet creation in ciliates by antiserum treatment. These early observations are consistent with recent reports on the response of cells to carcinogens (see Hjelm, 1986 for details). Research on the carcinogenic effects of mineral fibres, such as asbestos, has also demonstrated that the chemical composition of the agent is not the important criterion when considering carcinogenic potential, but the fibre dimensions. The attachment of mesothelial cells to long thin fibres may disrupt cell division by changing the cytotactic characteristics of the cell, as may thin films of plastic (see Hjelm, 1986 for details).

Thus seeding processes can have a major role to play in the pattern determination of a cell. The extrapolation of the role of seeding processes from protozoan biology to a more global evolutionary sphere is not justified by experimental evidence to date. However if, as Hjelm (1986) asserts, cytotaxis is a general property of organisms (see also Frankel, 1982), then internalized, non-genic cell modifications could play a significant role in the development of new morpholgies and also point the way forward for research. Organisms must be regarded as having self-regulatory and self-transformatory capabilities that are not necessarily rooted in the structure of DNA. Importantly, the process of cytotaxis demands scrutiny of the often tacitly held assumptions that all hereditary information is held in the nucleic acid compliment of the organism.

(2) *Environment, DNA and Weismann's Barrier*

Since the advent of recombinant DNA technology a variety of processes that allow DNA to interact with and be modified by it's environment have ben discovered. Some of these phenomena appear to contradict both the Weismannian doctrine of absolute separation of germline and soma and the stipulation of the Central Dogma of molecular biology that so-called biological information is transferred in a linear and uni-directional fashion from DNA to proteins. A complete coverage of this is not attempted here and for greater detail and references readers should see recent reviews by Ho (1986), Pollard (1984, 1987, 1988) Cullis (1983, 1988) and Holliday (1987). Below we mention briefly those points that are most directly concerned with the possible production and internalization of phenocopy type phenomena.

Gene amplification is a process that results in an increase in the amount of a certain gene product by multiple copying of the DNA sequence that codes for it. The extra copies of the sequence can be found either as extra-chromosomal elements known as double minutes or be ligated onto the existing chromosomes giving an altered structure that appears as a homogeneously staining region. The significance of gene amplification is that the amplified sequences usually contain the codes for a gene product that represents an appropriate response to an external stimulus. For example, Schimke (1983) found that exposure of mouse cell cultures to the toxin methotrexate resulted in amplification of the sequences coding for the dihydrofolate reductase protein which de-toxifies methotrexate. Similar although more complex changes have been observed in plants as in the studies of Cullis (1977, 1984) and others who found dramatic changes in the nDNA and rDNA of flax in response to different growth conditions.

Moveable or transposable genetic elements are short sequences that move within and between chromosomes and are another means by which DNA can undergo spontaneous alteration. There are several categories of transposable elements some of which may derive from the double minutes produced during gene amplification. Other categories use RNA intermediates to move between chromosomes and some of these behave in a very similar way to retroviruses using reverse transcriptase to transcribe themselves back onto the DNA. These retrotransposons may have the ability to move between cells.

Both the rate and induction of transposition appear to be sensitive to external stimuli. It has been shown, for example, that environmental stress in maize (McClintock, 1984) and in Drosophila cell cultures (Strand & McDonald, 1985) will effect transposition. Futher, the retrotransposons may have the ability to move into the germ cells carrying sequences altered in somatic tissues in response to external stresses.

DNA methylation has come to be increasingly implicated in the control of gene expression (see Holliday, 1987 for further reference and details). Generally it seems that DNA sequences with methylated cytosine are inactivated. Cells appear to have the ability to both selectively methylate and demethylate sequences thus de-activating or activating them respectively. These patterns of DNA methylation are passed on through successive cell generations. This may be a mechanisms by which cells

become differentiated during development (Holliday, 1988).

There is evidence to suggest that DNA damage may cause abnormal loss of methylation and consequent epigenetic defects which are then passed on to subsequent cell generations. Should such changes occur in the germ cells they would be passed on to at least a proportion of offspring which would represent direct inheritance of an acquired character. Methylation of DNA then, represents a mechanism that could account for both the production of a phenocopy and its internalization.

The phenomena discussed should not just be considered in isolation but are all operating at the same time with consequent opportunity for complex interactions between them. Generally they represent a substantial basis for accepting that environment-DNA interactions are commonplace. Far from being largely fixed and conservative as the Central Dogma supposes, the DNA is, infact, highly dynamic in a manner similar to that proposed by Ho (1986). It also seems quite possible to envisage a number of mechanisms by which environmentally induced DNA changes might be passed into the germline and thus inherited by the offspring of effected individuals. Such a violation of Weismann's barrier could occur even in organisms with early sequestration of germ cells and when these mechanisms are experimentally confirmed a re-evaluation of the currently accepted view of the hereditary process will be necessary.

VII. DISCUSSION

We have attempted here to outline the progress of research into the nature of phenocopies and their induction. Furthermore, we have discussed studies dealing with related genetic and developmental phenomena such as genetic assimilation and internalization mechanisms. We will now consider the relationship between the results of these studies and various conceptions of phenocopies and heredity and their relationships to evolution.

Phenocopies

Throughout their extensive history phenocopies have been used in various conceptions of development and evolutionary change. For Goldschmidt phenocopies represented a means to explore the physiochemical basis of gene action and phenotypic change. For Waddington and others phenocopies were a tool to study the nature of developmental processes and the linkage between the genotype and the phenotype. Most of these attempts to place the phenocopy phenomena within an appropriate theoretical framework were, we maintain, unsuccessful. However, in the last fifteen years a perspective that may more readily encompass and make intelligible the mass of experimental data (some of which are discussed in this review) on phenocopies and related phenomena has emerged — structuralism.

Piaget (1974) and Webster & Goodwin (1982) have attempted to apply a structuralist orientation to understanding the nature of causality in development. In a structuralist perspective, an organism is regarded as a self-organizing, transforming system, which responds to the conditions imposed on it by following a set of rational pathways through ontogeny. Under this framework, phenocopies become readily explicable. Genetic mutants manifest themselves by a disturbance of the 'normal' course of development (e.g. through the presence or absence of a particular gene product) causing a different developmental pathway to be stabilized. In the same way the environment external to the organism imposes limits which can alter the course of development. According to Piaget's theory (1974), the environmental perturbation does not reproduce exactly the effect of a genetic mutant (*contra* Goldschmidt, 1938), but interferes with some 'downstream' process which may be far removed from the primary gene action (Goodwin, 1982). This type of relationship between genetic factors and biological form is radically removed from the view of many biologists. Most regard 'information' within the genes as coding directly for a particular character i.e. the genetic programme is seen as sufficient to account for development (Wolpert & Lewis, 1975).

By contrast, Webster & Goodwin's (1982) perspective, while also a structuralist view, employs a field concept to describe and analyze development. 'Biological fields', like those of physics, have inherent properties that enable them to establish smooth spatial patterns even under conditions of severe perturbation. Phenocopies thus appear as morphological manifestations of an internal change in boundary conditions of the field. Both genetic changes and external environmental agents can perturb the system altering generative parameters. This may consequently result in organismic development

proceeding along a different pathway and towards an altered, but rational biological structure. It is, therefore, not surprising that it is possible to experimentally produce copies of naturally-occurring forms, i.e. phyletic phenocopies (*sensu* Stebbins & Basile, 1986).

Moreover, this viewpoint is compatible with the results of Capdevila & Garcia-Bellido (1974) on the bithorax phenocopy where the alteration of the state of a single cell early in development results, through clonal inheritance, in an altered adult structure. The evidence provided by Ho *et al.* (1983*a*) showing that ether affects the cytoplasm of the pre-cellular blastoderm also suggests the existence of field properties that predetermine the fate of different regions of the cytoplasm. Furthermore Mitchell & Lipps' (1978) theory that heat shock phenocopies are caused by gene product imbalances might also be indicative of an disrupted biological field, which consequently results in an altered adult form.

Heredity

Waddington, Bateman and others in the 1950s endeavored to establish the basis for the heritability of various phenocopied characters. They tacitly assumed that genes and selection were involved in the assimilation of these factors. However, the experiment of Ho *et al.* (1983*a*) has shown that cumulative cytoplasmic factors can bring about progressively increased penetrance even in the absence of positive selection. Lambert *et al.* (1986) also suggested that similar cumulative factors may be involved in the induction of melanism in some moth species.

In contemporary biology the terms 'heritable' and 'genetic' are generally treated as synonymous. In fact the Collins English Dictionary defines heredity as the "transmission from one generation to another of genetic factors that determine individual characters". However, as Maturana (1980) pointed out, biological characters are simply not transmitted from one generation to another since each organism arises developmentally *de novo* (Levin & Lewontin, 1985; Lambert & Hughes, 1988). Hence a persistent change in the environment can result in a heritable characteristic in an equilavent sense to a change in the organism's genetic constitution. Hence not all that is 'heritable' is necessarily 'genetic'. For example, under most conditions *Drosophila melanogaster* possess two wings and a single thorax. We say that this character is 'hereditary'. However, if conditions on Earth were to alter such that there was a high concentration of ether in the atmosphere we might now say that the bithorax condition was heritable. Hence a character is hereditable only under a set of defined conditions.

The preceding discussion suggests that treating the organism and the environment as separate interacting entities is inappropriate. 'Environments' can be seen to exist at many biological levels e.g. the environment of a gene is the chromosome, the environment for a cell is the tissue etc. (Weiss, 1950; Lima de Faria, 1983). Weiss (1950) considered organisms were like Chinese boxes in that within each level there is always a series of underlying levels. Organisms are arranged such that interactions occur between levels in all directions (Koestler, 1978).

For this reason we suggest the idea of *context* is more appropriate than that of *environment* when discussing causation in development. Context therefore, refers to all levels of organization. Within a particular context all systems operate and change. We agree with Lewontin (1983) that adult organisms create their own environments. However, we see the logical extension of this to include context *internal* as well as *external* to the organism. As an organism develops it creates novel contexts and increasing complexity. For example, the dynamics of the brain of a developing foetus has its own hormonal context which operates in a specific way and consequently affects sexual differentiation (Hughes & Lambert, 1987).

In a generally similar manner Ho (1986) recently suggested that heredity should be regarded not simply as the replication and transmission of DNA but a process which involves feedback interrelationships between organisms and environment at all levels. She concluded that since there is a reciprocal feedback between organisms and their environment (indeed a circular relationship) and since numerous examples of feedback to DNA can be illustrated, the concept of heredity needs to be reformulated as one process. As do we, Ho (1986) recognized a multitude of environmental levels (see Table I, Ho, 1986) which affect biological dynamics. These are, in our terminology, different contexts.

Evolution

Some biologists have argued that phenocopy phenomena are irrelevant to the study of evolution, irrespective of any debate over their causal mechanisms. For example, Løvtrup (1982*b*) questioned the importance of phenocopies for the study of evolution. He commented: "Others have adopted a quasi Saint-Hilarian stand, submitting that the environment can influence the course of epigenesis such that the organisms become adapted to it. As illustration the phenomenon of phenocopies is often mentioned. However, all known phenocopies are trivial from an epigenetic point of view; it seems ignorance about epigenetics can best explain that people advocate this mechanism" (Løvtrup, 1982*b*: 395-6). Løvtrup maintained that the answer to the problem of the mechanism of morphological evolution lay in the realm of macromutation. His contention that phenocopies represent trivial phenomena seems parallel to the argument that all known mutations also represent trivial phenomena; an argument that Løvtrup himself would apparently strenuously reject (Løvtrup, 1976, 1982*a*).

In a recent critique of those who support the concept of saltational evolution, Levinton (1986) also argued that major morphological changes induced by experiment or environment and mediated through minor changes in developmental timing, for example, do not point to a major role for developmental or epigenetic change in evolution. Thus, in Levinton's view, the phenomenon of atavism (e.g. the development of hind limbs in a whale), or equally the phenomena of phenocopies, are of peripheral significance to the study of evolution. Levinton (1986: 265) stated: "It is often easy to induce novel structures by simple experiments. But this act only induces what evolution has already created. It does not, and could not, propel the organism into a new morphological realm .... The reinduced structures have already evolved, and this evolution may have been through the cumulative action of many genetic modifications. The major work, in other words, has already been done. That is why the novel structure appears so suddenly." However, he provided no evidence that the remarkable range of phenocopies inducible, for example, in *Drosophila melanogaster* are all relics of some ancestral condition. It should be remembered that such phenocopies affect a great proportion of the external characters of the adult. Hence, to make the sweeping statement that all of these phenocopies represent some unknown ancestral condition seems rash and is, in fact, currently unsubstantiated.

Obviously phenocopies do not arise at random; their occurrence is predictable and they obviously develop in accordance with general developmental principles. However, the expectation that inducible forms necessarily represent ancestral stages is based on the tacitly held belief that biology exclusively reflects past historical contingencies. According to this view these biological principles are moulded through history and have no meaning independent of it. In contrast, we suggest that the studies reviewed here illustrate the presence of a range of emergent properties that are not legacies of history. Because organisms possess these emergent properties they have the *potential* to create novel form when in a novel context. Surely this is the stuff of evolution.

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IX. GLOSSARY

Canalization. The ability of a developmental system to resist perturbations.

Chreod. In terms of Waddington's epigenetic landscape this refers to alternative pathways within one developmental valley.

Environment. Commonly this refers to " the external surroundings in which a plant or animal lives, which tend to influence its development and behavior." (Collins English Dictionary 1979). We suggest that in biological discussions the term should be retained for this use only, and 'context' should refer to all levels at which the unit under discussion interacts.

Epigenetic. 'Epigenesis' was originally meant as the antithesis of preformation. Waddington (1957) regarded epigenetics as the causal processes in development, and used the term almost

synonymously with embryology or ontogeny (see Sibitani, 1983 for a useful discussion).

Genetic assimilation. Waddington (1975) defined this as, "the process .... by which phenotypic character, which initially is produced only in response to some environmental influence, becomes, through a process of selection, taken over by the genotype, so that it is formed even in the absence of the environmental influence which had at first been necessary." Waddington suggested that assimilation depends on the frequency of 'major' and 'minor' genes and formation of gene complexes. His experiments were designed to provide laboratory evidence for the power of selection. Consequently emancipation from environmental control was then argued to result from genetic assimilation.

Heredity. There are two current meanings attributed to this term. Firstly "the transmission from one generation to another of genetic factors that determine individual characteristics: responsible for the resemblances between parents and offspring. Secondly, the sum total of the inherited factors or their characteristics in an organism." (Collins English Dictionary 1979). We consider that the second meaning is preferable since genes singularly do not result in biological form. As discussed, a characteristic may br heritable in one environment but not in another. Therefore the result of gene action cannot be divorced from environmental context.

Homeostasis. Lerner (1953) referred to homeostasis as the tendency of a population to maintain, and if necessary restore, a particular distribution of gene frequencies. Other authors, including Dobzhansky (1955) regarded homeostasis as the tendency to keep fitness constant. Waddington (1975), however, has suggested that the phenomenon is really a maximization of fitness rather than holding it constant since , if in a given environment, fitness increased there would be no tendency to reduce it. Homeostasis can also be regarded as a developmental phenomenon in which a developmental pathway is maintained even in the presence of contextual perturbations.

Internalization. This term refers to all mechanisms by which heritable changes in organisms arise.

Penetrance. The proportion of individuals in any generation which exhibit a particular characteristic.

Phenocopy. This term was introduced by Goldschmidt (1929). It was defined as an environmentally induced form which mimics a genetic mutant. Lewontin (1974) pointed out that a confusion has persisted over this term. The phenotype of an individual is not the result of either the environment or the genotype. Instead it is the result of a unique genotype environment interaction. Recently Stebbins & Basile (1986) argued for the terminological distinctness of "phyletic phenocopies" meaning environmentally induced forms which are characteristic of other species.

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