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The Phenocopy Concept: Illusion or Reality?

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The life history of scientific concepts, their birth, tumultuous adolescence and maturity, their ultimate senescence and oblivion, has received regrettably scant attention from those who fashion these concepts as their professional tools and who thereby become responsible for their use or misuse. Searching studies, such as FLECK[1] made of the syphilis concept, have done little to promote more general recognition that 'truth travels a variably curved path, and (that) scientists tend to travel in straight lines in the direction in which the thread pointed when they last had hold of it' (HARRISON[2]). The craving of the human mind for clear and sharp distinctions, the tendency to classify into either-or categories, continues to promote the use of spurious antitheses which by their apparent clarity hinder the progress of analytical thought and experimentation.

This danger is nowhere greater than in the field of biology. The physicist or chemist can in most instances safely operate with isolates, but the biologist must ever be on guard to remember the integrated nature of all living activities. The futile and naive controversies concerning the respective roles of form and function or abouth the relative importance of nature and nurture in the economy of organisms may seem amusing in retrospect; yet these pseudo-problems do, in fact, continue to plague clear thinking.

WOODGER's[3] complaint that 'physiologists persist in confusing isolation in thought with isolation in nature' remains especially valid in regard to discussions concerning the influence of heredity and environment upon the realization of phenotypic traits. This is illustrated by much recent writing on the problem of 'phenocopies'. Naive adoption by some, uncritical rejection by others make it necessary to scrutinize the meaning and validity of the phenocopy concept.

GOLDSCHMIDT[4] coined the term 'phenocopy' in 1935 as a designation for non-hereditary morphological variants of *Drosophila* imagines, which he had obtained by treating definite larval stages with heat shocks, and which closely resembled known mutant traits. Simultaneous work by JOLLOS[5] produced closely similar results and the same was true of 'X-ray morphoses' on which FRIESEN[6] reported in 1936. All of these studies agreed in revealing in great detail an astonishing degree of similarity between the spectrum of mutants and the range of experimentally-induced non-hereditary variations. It became clear at once that the probability of this parallelism being due to chance was negligible. It remained, however, to be determined if the results were meaningful in the sense originally implied by the term phenocopy. The implication was well expressed by GOLDSCHMIDT[7]: 'Mutant and phenocopy, then, look alike because changed genetic action as well as action by a phenocopic agent is limited to definite tracks.'

HENKE, FINCK and MA[8] were the first to suggest that it may be necessary to distinguish between 'true' and 'spurious' phenocopies. Their criterion for 'true' phenocopies was that the critical stage in development during which a particular modification can be produced experimentally should coincide with the time at which development of the homologous mutant deviates from the normal course of events. Later authors sought to base such distinctions on the condition that 'true' phenocopies should show a close and detailed parallelism with the corresponding mutants in the step by step alterations which lead to a particular variant. It seems to me, however, that neither of these two classifications carries much conviction. With reference to the time relationships, we know much too little about the real point of inception of mutant action to use it as a criterion for comparisons. But even when modifications clearly can be produced at a later stage than that at which the presumably corresponding mutant begins to deviate from the normal phenotype, it does not follow *eo ipso* that different developmental channels are involved. A particular mutant trait may, for instance, be brought about by insufficient production

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[1] L. FLECK, *Entstehung und Entwicklung einer wissenschaftlichen Tatsache* (Benno Schwabe & Co., Basel 1935).

[2] G. R. HARRISON, Atlantic Monthly, June 1955.

[3] J.H. WOODGER, *Biological Principles* (Kegan Paul, Trench, Trubner & Co., London 1929).

[4] R. B. GOLDSCHMIDT, Z. indukt. Abstamm. u. Vererblehre *69*, 38 (1935).

[5] V. JOLLOS Naturwissenschaften *21*, 831 (1933); Genetica *16*, 476 (1934).

[6] H. FRIESEN, Arch. Entwicklungsmech. *134*, 147 (1936).

[7] R. B. GOLDSCHMIDT, Scientific American, October 1949.

[8] K. HENKE, E. v. FINCK, and S.-Y. MA, Z. indukt. Abstamm. u. Vererblehre *79*, 267 (1941).

or complete lack of an essential compound at a specified developmental stage; a deficiency in the same substance, produced experimentally at a subsequent stage, may in genetically-normal sibs cause the regression or modification of parts that had already been formed and may subsequently, by identical physiological and morphological steps, be responsible for a phenotype closely mimicking the mutant trait. Such a situation would fulfill all the requirements that can reasonably be imposed on the phenocopy concept. Again, an agent acting from without might well achieve its mimicking end-result along routes that differ structurally from those followed by the mutant and yet do so by interference with the normal allele of the mutant. Contrariwise, it should be said that identity of the time of action or similarity of the developmental paths are by no means proof positive of an interference with the same genic activities. Mutant and modification might well produce homologous ends, and even along grossly similar routes, by an interference with quite dissimilar primary events[9].

Many instances are on record in which the time sequence of events shows a satisfying correspondence between mutant and experimentally-produced modification. Cases of impressive similarity in the manner by which mutant and modified phenotype are reached have also been described (HADORN[11]). Such examples may serve to strengthen our conviction that we are dealing with closely-related phenomena, but the decisive testimony must be furnished by evidence of a genetic nature. It is evidence of this kind which remains now to be examined.

Whenever an external agency impinges upon an organism, genetic forces of adjustment come into play. It is unlikely that such reactions occur at any time on the level of primary gene effects; in most instances it is, in fact, obvious that interpositions of this nature take place in reactions or events which are under indirect genic control, such as differential growth processes or enzyme-catalyzed steps of differentiation. Whatever is the precise mechanism by which experimentally-induced modifications occur, there is convincing evidence that the interference may be, and often is, in the same path by which recognized mutants produce homologous phenotypical results. This evidence is of the following kinds:

(1) Mutant genes and modifying agents which have similar phenotypic effects may, in conjunction, show *additive* activity. Such superposition has, for instance, been demonstrated by GOLDSCHMIDT[4] in the case of scalloping of the wings of *Drosophila*.

(2) Animals which are heterozygous for a recessive mutant gene frequently give an enhanced response to modifying agents that produce phenotypic effects homologous to the trait shown by the mutant homozygotes. A clear demonstration of this kind was given by SANG and MCDONALD[12] in their treatment with sodium metaborate of *Drosophila larvae* heterozygous for the recessive gene for eyeless. GOLDSCHMIDT and PITERNICK[13] and others have reported similar results. Recent (unpublished) experiments with fowl have shown: (a) that the treatment of chicken embryos with nicotine produces a shortening of the neck, due to abnormality of the cervical vertebrae, and that after high doses this defect is frequently accompanied by a shortening of the upper beak, hypoplasia of skeletal muscles (legs) and general edema; (b) that embryos heterozygous for a recessive lethal gene with very similar phenotype, the so-called 'crooked-neck dwarf' lethal, give a significantly higher response to treatment with nicotine than do related but non-heterozygous embryos; and (c) that embryos of a family of Brown Leghorn fowl in which abnormalities of the cervical spine are found on occasion (presumably as a mutant with very low penetrance) also react to nicotine with a higher incidence of the homologous malformation than do Brown Leghorn embryos of a family in which spontaneous occurrence of such defects had not been observed.

(3) The last-named of our observations on the results of treating chicken embryos with nicotine already belongs to the group of experiments on mutants with very low penetrance. GOLDSCHMIDT and PITERNICK[13] have reported several instances in which *Drosophila* mutants of this kind, e.g. *podoptera*, gave a high response to the experimental induction of comparable modifications. Another interesting example may be taken from the work of BERTSCHMANN[14] on wing-scalloping in *Drosophila*. She worked with a stock which produced occasional individuals with slight scalloping, *provided* that the cultures were kept at low temperature. Starting from such individuals, she obtained by selection 100% penetrance and a high degree of expressivity of the scalloping phenotype, a trait which on analysis proved to be polygenic. When the unselected stock was treated with nitrogen mustard it produced a high incidence of wing scalloping – and, as in the controls, the reaction was temperature-dependent, low temperature favoring higher penetrance. BERTSCHMANN was clearly justified in concluding that a 'latente genetische Bereitschaft' for response to the external sources of variation (temperature and nitrogen mustard) was present. Another instructive

[9] Doubts in regard to the distinction between true and spurious phenocopies have already been voiced by GOLDSCHMIDT[10] and NACHTSHEIM[24].

[10] R. B. GOLDSCHMIDT, *Theoretical Genetics* (Univ. California Press, Berkeley and Los Angeles 1955).

[11] E. HADORN, *Lethalfaktoren* (Georg Thieme, Stuttgart 1955).

[12] J. H. SANG and J. M. MCDONALD, J. Genetics *52*, 392 (1954).

[13] R. B. GOLDSCHMIDT and L. K. PITERNICK, J. exp. Zool. *135*, 127 (1957).

[14] M. BERTSCHMANN, Z. indukt. Abstamm. u. Vererblehre *87*, 229 (1955).

example comes from our own work on rumplessness of fowl (LANDAUER[15–17]). This skeletal trait is known in three mimicking genetic forms, viz. as dominant (Rp-1) and recessive (rp-2) gene substitutions and as a more or less rare 'sporadic' variant which presumably has a polygenic background. The incidence of sporadic rumplessness varies in different stocks from a fraction of one to as much as 4 or 5%, but is typical for each stock and its residual genotype. The genetic nature of sporadic rumplessness was demonstrated by the effect which genotypes with dissimilar incidence have on penetrance and expressivity of the recessive mutant (rp-2). When the rumplessness-inducing effects of insulin were tested on various stocks, it was found that the incidence was directly related to, i.e. increasing with, the frequency of occurrence of sporadic rumplessness. We are, therefore, again dealing with a situation in which the response to external modifying agencies depends intimately on the extent to which the genotype favors homologous variation.

(4) Further evidence for the relatedness of genic activity and of response to modifying agents with homologous phenotypic effects comes from observations on the role which genetic modifiers may play in both situations. It has been shown repeatedly in our work with chickens that the accumulation of modifying genes which reduce penetrance and expressivity of a mutant lowers in a similar manner the response to external forces which are accountable for homologous modifications of development. Even after the phenotypic expression of a gene in *Drosophila* had become completely suppressed by genetic modifiers, its presence in the genotype played an important role in determining the measure of reactivity to an external force favoring a corresponding developmental modification (GOLDSCHMIDT and PITERNICK[13]). Again, it has been shown that sexual differences in the expressivity of a particular trait are in a similar manner reflected in sexual dissimilarities of response to forces from without (SANG and MCDONALD[12]).

(5) An interesting example of modifying influences exerted by the maternal environment can be found in the beautiful studies of MCLAREN and MICHIE[18] on vertebral variation in mice. They established the following facts. Two inbred strains of mice differ in the mean number of lumber vertebrae, C3H/Bi having mainly five and C57BL/How mainly six of these vertebrae. Reciprocal hybrids resemble in both sexes the maternal strain; the trait is, therefore, not sex-linked. Transfer of F_1 zygotes from reciprocal crosses to the uteri of females of either one or the other of the two strains produced young with vertebral counts typical for the host-mother inbred stock. In the last analysis the two strains clearly differ in genetic factors which are, however remotely, responsible for dissimilarities of uterine functions during pregnancy, thereby determining the number of lumbar vertebrae in their progeny. It is equally evident that after egg transfer the uterine environment modifies development of reciprocal F_1-hybrids in such a way as to produce an exact duplication of the features which are typical for the host strain.

Available evidence demonstrates, therefore, that (1) genic and external factors may produce additive effects; (2) organisms which are heterozygous for a recessive mutant gene frequently give heightened response to modifying factors with homologous developmental effects; (3) mutants with low penetrance, similar to single doses of recessive genes, often promote reactivity to external modifying action; (4) modifying genes may produce closely similar results in combination with mutants or external sources of homologous variation; (5) the uterine environment of a host-mother may serve as a source of homologous variation. In all situations in which intimate relations of this kind can be observed between genic activity and the manner in which external agencies interpose their effects on development, we can confidently conclude that the concept of phenocopy, as indicating the use in both instances of closely similar development tracks, *if probably only part of the way*, is born out by experimental evidence. The tracks must, in any event, be sufficiently related to accommodate in similar manner the directing motions of the variety of genic modifying forces to which reference has been made.

It is obvious, of course, that one should avoid using the term phenocopy indiscriminately. It is true, as GOLDSCHMIDT has pointed out, that in many instances of experimentally-produced variants without hereditary counterpart, homologous mutants may be found subsequently. Remarkable examples of this kind have been reported in the field of vitamin deficiencies in man (SYNDER[19]). The question remains if, in addition to cases in which a verdict of 'not proven' is in order, there are situations in which the concept 'phenocopy' is inapplicable. I believe this to be true whenever the events by which a developmental variant is produced clearly are of a nature that has nothing in common with the manner in which any gene-directed process might lead to similar endresults. A case in point are, I believe, the malformations produced by German measles and other viruses. FRANCESCHETTI, BAMATTER, and BOURQUIN[20] and TÖNDURY[21] have referred to the rubeola-induced defects of human fetuses as phenocopies. But since it has been shown by HAMBURGER and

[15] W. LANDAUER, Growth Symposium *12*, 171 (1948).
[16] W. LANDAUER, Amer. Naturalist *89*, 35 (1955).
[17] W. LANDAUER, Amer. Naturalist *91*, 79 (1957).
[18] A. MCLAREN and D. MICHIE, J. Embryol. exp. Morphol. *6*, 645 (1958).
[19] L. H. SNYDER, Science *129*, 7 (1959).
[20] A. FRANCESCHETTI, F. BAMATTER and J. B. BOURQUIN, Helv. paediat. Acta *2*, 339 (1947).
[21] G. TÖNDURY, Arch. Julius-Klaus-Stiftung *27*, 170 (1952).

HABEL[22], TÖNDURY[23] and others that in these cases damage to the embryo is brought about by viral invasion and destruction of certain embryonic organs and tissues, it is difficult to conceive of any common developmental denominator between these events and comparable end-effects of mutant conditions, even if the latter should come about by cellular destruction and resorption. The same applies *a fortiori* to the results of destruction by surgery or X-rays of circumscribed embryonic areas. A more complex situation exists in the case of sex reversal of female fowl, to which NACHTSHEIM[24] has referred as phenocopies of males. As after viral infection, the primary event is the destruction of an organ (the ovary) by a process (tubercular infection or malignancy) which has no equivalent in genetic conditions, but the physiological events which are initiated thereby are those determined by the residual genetic constitution.

The question whether the reactions of organisms to external insults are, however indirectly, governed by their genetic constitution was not an issue in our discussion. That is an evident fact, based on the inseparability of action and reaction as a consequence of evolutionary integration. The question at issue was whether it is justified to use the term phenocopy with the implication that external sources of modification and genes with similar phenotypic expression reach their goals by developmental routes which are, at least in part, identical. It has been shown that a considerable amount of genetic evidence is available in support of this conclusion. It should be recognized that in any specific situation the phenocopy concept must remain shadowy until its relevancy is clearly established.

There is no proof that external sources of developmental variation ever produce their results by interference with primary gene functions. It would be a mistake to interpret the phenocopy concept in this manner, and any expectations based on such implications will surely prove illusory. Again, mere similarity between the end-results of mutant action and of those produced by external agencies is not enough to make the phenocopy concept meaningful to geneticists. But if we accept the concept in its restricted meaning, viz. as designating identical or closely similar phenotypes reached by paths which, part of the way, are shared with those of the homologous mutants, then existing evidence validates its reality, and in this sense work on phenocopies may become a valuable tool in our search for some of the developmental mechanisms by which mutant genes produce their phenotypic effects.

Zusammenfassung

Verfasser bespricht den Begriff Phänokopie und dessen Grenzen. Er kommt zum Schluss, dieser Begriff sei nützlich, sofern er sich auf experimentell produzierte Phänokopien bezieht, die mit den Erbphänotypen identisch oder ihnen sehr ähnlich sind und deren Entwicklungsgang zum mindesten teilweise mit demjenigen homologer Mutanten übereinstimmt.

[22] V. HAMBURGER and K. HABEL, Proc. Soc. exp. Biol. Med. *66*, 608 (1947).

[23] G. TÖNDURY, Geburtsh. u. Frauenheilk. *12*, 865 (1952); Helv. paedit. Acta *7*, 105 (1952).

[24] H. NACHTSHEIM, Exper. *13*, 57 (1957).

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Über die Orientierung bei der Kondensation von Benzil mit monosubstituierten Guanidinen[1]

Bekanntlich lässt sich die alte Biltzsche Synthese[3] von 5,5-Diaryl-hydantoinen und -2-thiohydantoinen aus Benzilen und Harnstoff bzw. Thioharnstoff in Gegenwart von Alkalien auch auf die Synthese von 5,5-Diaryl-glykocyamidinen (I, $R=R'=H$) anwenden, wenn Harnstoff bzw. Thioharnstoff durch Guanidin ersetzt wird[4]. Der einzige bisher ausgeführte Versuch zur Übertragung dieser Kondensation auf *substituierte* Guanidine, wie 1,3-Diphenyl-guanidin[5], blieb erfolglos; ebenso der Versuch der analogen Kondensation des N-Butylguanidins mit Glyoxal[6].

Wir kondensierten Benzil mit zwei monosubstituierten Guanidinen, dem N-Benzyl- sowie dem N-(β-Morpholinoäthyl)-guanidin durch Kochen der alkoholischen Lösung in Gegenwart von Kaliumhydroxyd. Bei Verwendung von

[1] 5. Mitteilung über Hydantoine, Thiohydantoine und Glykocyamidine[2].

[2] 4. Mitteilung siehe K. LEMPERT und J. BREUER Chem. Ber. *92*, 1710 (1959).

[3] Zusammenfassende Darstellung: E. WARE, Chem. Rev. *46*, 414 (1950).

[4] CH. HOFFMANN, Bull. Soc. Chim. France *1950*, 659. – C. V. DELIVALA und S. RAJAGOPALAN, Proc. Indian Acad. Sci. (A) *31*, 107 (1950).

[5] R. G. NEVILLE, J. org. Chem. *23*, 1588 (1958).

[6] I. S. BENGELSDORF, J. chem. Soc. *75*, 3138 (1953).