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A Third Source of Developmental Differences

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An illustrative list is presented of human and animal studies which each point to the existence of a third source, in addition to genetic and environmental factors, underlying phenotypic differences in development. It is argued that this third source may consist of nonlinear epigenetic processes that can create variability at all phenotypical-somatic and behavioral-levels. In a quantitative genetic analysis with human subjects, these processes are confounded with within-family environmental influences. A preliminary model to quantify these influences is introduced.

KEY WORDS: Developmental noise; epigenetic processes; neural networks; chaotic dynamics; biological patterning; biometrical models.

INTRODUCTION

Behavior geneticists (e.g. Scarr and Weinberg, 1983; Wilson, 1983; Plomin, 1986; Boomsma and Molenaar, 1987; Eaves *et al.*, 1988; Plomin *et al.*, 1988; Molenaar *et al.*, 1991) have increasingly concentrated upon developmental (biometrical) models involving genetic and environmental processes. That is, apart from various modulating influences such as assortative mating, the ultimate sources underlying intra- and interindividual phenotypic differences are considered to reside either in the genetic makeup or in the environment. The environmental influences are usually broken down into the subclasses of within-family (E1) and between-family (E2) influences. Plomin and Daniels (1987) indicate that for personality, intelligence (after childhood), and psychopathology, almost all nongenetic variance belongs to the within-family type. Yet any endeavor to characterize these within-family environmental influences further has yielded unsatisfactory results (cf. Plomin and Daniels, 1987). Why is it so difficult to identify particular instances of what appears to be the most important type of environmental influences?

In order to try to answer this question we note that genetic and environmental processes do not constitute the sole major sources of phenotypic developmental differences. In fact, throughout the history of quantitative genetics, several studies have appeared which clearly indicate the importance of an independent third source of phenotypic variation alongside of genetic and environmental influences. In the next section an illustrative list of some of these studies is presented, including early work by Sewall Wright (1920) and Mather and Jinks (1977). The remainder of the paper is devoted to the presentation of a theoretical model of this third source which can explain its ubiquity at all levels of phenotypic variation. That is, an epigenetic process model generating intraindividual variation at both somatic and behavioral levels is outlined. It is shown that the variation due to this epigenetic process, which can be considered to be of chaotic origin, is confounded with within-family environmental influences in standard quantitative genetical analyses. In the concluding section we discuss the prospects of disentangling these confounding sources of variation in applied biometrical modeling of human phenotypic data.

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EMPIRICAL EVIDENCE OF A THIRD SOURCE

In what seems to be the first published path diagram, Sewall Wright (1920; reproduced in Province, 1986, p. 136) distinguishes three types of factors involved in the variation of fur coloration of guinea pigs: genetical, common environmental, and developmental. The latter type of factor is represented by D in the path diagram concerned and is supposed to be associated with developmental irregularity. The results reported by Wright indicate that more than 91% of the total variation of fur coloration in an inbred line of guinea pigs is accounted for by D. Hence it appears that in this case epigenetical processes constitute a major source of phenotypical variation.

In discussing the nonheritable variation in the numbers of chaetae between the left and the right side of the thorax in members of an inbred line of *Drosophila melanogaster*, Mather and Jinks (1977, p. 6) conclude that this variation is not attributable to environmental influences (it is unlikely that environmental agencies systematically act differently on the left and right side). Instead, Mather and Jinks attribute this intraindividual variation to the vagaries of development, in cell development and so on, affecting the two sides of the thorax differently. Moreover, they show that 91% of the variation in chaetae numbers between flies is a reflection of this developmental variation within flies. [For similar results see Clayton *et al.* (1957) and Reeve (1960).] Waddington (1957) referred to the same phenomenon as developmental noise. In sum, there appears to be a distinct major source of phenotypic developmental differences, in addition to genetic and environmental influences, that resides in the intrinsic indeterminacy of the epigenetic process underlying the growth of chaetae.

Of more recent origin is a paper by Gaertner (1990) which presents a review of experiments, performed during a period of many years, designed to standardize laboratory animals. Using inbred strains to reduce genetic variability, environmental variability was severely reduced by highly standardized husbandry. Yet Gaertner points out that his sustained and concentrated efforts to reduce phenotypic variability in quantitative traits have hardly been successful. In particular, he concludes that 70–80% of the range of body weight in inbred mice appears to be due to a third component creating biological variability, in addition to genetic and environmental influences.

In an interesting paper by Kurnit *et al.*, (1987) it is posited that chance plays a major during development. More specifically, computer simulations of a morphogenetic model for endocardial cushion outgrowth were performed under constant genetic and environmental conditions. Despite these constant conditions, significant variability in simulated embryonic development was observed. It is concluded that even if it becomes feasible to predict or control both genotype and environment during pregnancy, birth defects due to chance still will occur.

Each of the first three studies mentioned above presents convincing empirical evidence of a third source underlying phenotypical differences in inbred lines of animals. Note that this evidence has been obtained by means of straightforward biometrical modeling techniques. In our view quantitative genetical methods are indispensable in the study of autonomous epigenetical processes (contra Johnston, 1987; see also Burgess and Molenaar, 1993). The fourth study mentioned above has been included because it presents an elegant simulation study of one definite epigenetic process and hence paves the way for our theoretical considerations in the next sections. Together these studies provide converging evidence of the reality of a third source of developmental differences.

CHAOTIC PATTERN FORMATION

In order to substantiate the somewhat vague concept of an autonomous epigenetical process with highly variable output under constant genetic and environmental conditions, we take the bilateral variation in chaetae number (Mather and Jinks, 1977) as our starting point. As it is unlikely that environmental influences affect the left and right sides of the thorax in consistently different ways, these influences have to be taken to be constant in this respect. In a similar vein, genetic influences on chaetae number at either side of the thorax are constant within each fly. Consequently, the epigenetic process leading to the formation of chaetae within each fly evolves under constant genetical and environmental conditions and thus constitutes a deterministic process. How can such a deterministic growth process give rise to highly variable results?

A preliminary answer to this question can be obtained by taking note of a wealth of pertinent experimental and theoretical results obtained in developmental biology. In particular, we refer to dy-

namic models of biological pattern formation which explain emergent control and self-organization in, for example, embryonic fields (Meinhardt, 1982; Oster and Alberch, 1982). In the formation of the primary embryonic axis, processes are involved which are able to generate a pattern from homogeneous initial conditions and which control regeneration occurring after external perturbations. Meinhardt shows that a process model in which a short-ranging autocatalysis is coupled with a long-ranging inhibition can explain this emergent pattern and controlled regeneration occurring at various levels of embryonic growth. In fact, it is also able to generate mild variations in the formation of digits and segmants (Meinhardt, 1982, p.152 ff). The model concerned is represented by a nonlinear reaction-diffusion mechanism in which an activating substance stimulates its own production (autocatalysis) as well as the production of an inhibiting antagonist.

Although the structure of the reaction-diffusion mechanism discussed by Meinhardt is the result of genetic processes taking place in a particular environment, the actual formation (realization) of embryonic patternings is not coded in the genes or induced by environmental influences, but constitutes autonomic self-organization (for a similar point of view cf. Benno, 1990). This is especially evident from experimental results indicating the emergence of organizing centers which control the regeneration of perturbed structures. Due to the autocatalytic step in the reaction-diffusion, local morphogenetic fields are created which serve as dynamic prepatterns for controlled growth. And, importantly, the autocatalysis underlying self-organization is a nonlinear process. In their elaborate mathematical analysis of nonlinear reaction-diffusion systems, Nicolis and Prigogine (1977) have shown that the presence of nonlinearities in these systems is essential to the occurrence of self-organization under a wide range of conditions. Hence, the nonlinear mechanisms considered by Meinhardt give rise to self-organizing growth processes which could, at least in principle, explain the bilateral variation in chaetae number. Yet an appeal to these mechanisms does not completely answer our original question of how deterministic growth processes can give rise to highly variable results.

A reaction-diffusion is by definition a random process, i.e., a process which is described by a system of random differential equations (Soong, 1973). In contrast, we are looking for a deterministic epigenetic process giving rise to variable output under constant genetical and environmental conditions. It was already indicated in the above discussion of biological pattern formation that actual, autonomous epigenetic processes have self-organizing properties and therefore appear to be driven by nonlinear dynamics. Hence, the question now can be specified as follows: Can nonlinear deterministic processes yield variable output under the conditions concerned? The affirmative answer to this question has recently been obtained in mathematical systems theory (Moon, 1987): nonlinear (e.g., autocatalytic) deterministic systems can give rise to chaotic, i.e., highly variable, phenomenologically random output. Moreover, these deterministic systems are able to generate random growth processes of the kind we are looking for (Stanley and Ostrowsky, 1986). More specifically, Schierwagen (1990) discusses a chaotic developmental model of the nervous system which generates variable neuronal structures under constant conditions.

The following general picture emerges from the above considerations. Developmental differences can be generated by three kinds of sources: genetical, environmental, and epigenetical. The latter epigenetical influences are the result of autonomous developmental processes with emergent self-organizing properties and obeying nonlinear dynamics. The structure of such autonomous developmental processes can be represented by nonlinear reaction-diffusion systems or nonlinear deterministic systems of differential equations. The structure of each developmental mechanism, in particular the parameters in the corresponding nonlinear model system, will be determined by genetical and environmental influences and hence will vary between subjects. For a fixed subject in a given developmental phase, however, the genetical and environmental influences are constant and hence the structure of the developmental processes associated with this subject also is fixed. Yet the constant nonlinear dynamical mechanisms within a fixed subject still can yield highly variable chaotic output, constituting a third source of developmental differences.

CHAOTIC PATTERNINGS AT HIGHER LEVELS

Until now the discussion has focused on epigenetical processes underlying developmental dif-

ferences in physiological phenotypes like neural growth. In order to be of interest for behavioral geneticists, however, we have to provide at least one plausible scenario according to which this source of variation at the physiological level can become manifest at behavioral levels. Such a scenario presents itself by noting that biological pattern formation, as discussed in the foregoing section, not only pertains to embryonic growth but also relates to the formation of neural networks during later developmental stages (Meinhardt, 1982, p. 172 ff). At each developmental stage epigenetical processes are involved in the formation of these neural networks (cf. Benno, 1990) and are capable of generating substantial variation in network structure at the physiological level (cf. Edelman, 1987, Fig. 5.4). Such differences in homologous network structures can give rise to differences in their functioning and, thus, become manifest at behavioral levels. Moreover, at the functional level itself neural networks obey nonlinear dynamics, for instance, because of the presence of synaptic thresholds, and therefore their operation can reproduce the self-organizing properties of epigenetical processes.

In his recent book on neural Darwinism, Edelman (1987) denotes the formation of neural networks by the primary repertoire, whereas the organization of functional dependencies within and between groups of neural elements is called the secondary repertoire. An impressive body of biochemical and physiological evidence is presented which shows and partly explains the ubiquitous variability in the structure of both repertoires. According to Edelman, this endogenous variability has genuine adaptive value in that it is a prerequisite for the action of selection mechanisms which constitute the basic nonlinear forces in the creation of repertoires. Furthermore, Edelman (1987, p. 329) concludes that "the individualistic flavour and the extraordinary richness of selective repertoires suggest that, in each brain, epigenetic elements play major and unpredictable roles. Categorical genetic determination has no place in such systems; neither has instructionist empiricism." In a nutshell, then, the structural and functional diversity of neural networks is due to autonomous epigenetical processes with variable output, even under constant genetic and environmental influences. This diversity of primary and secondary repertoires, implying variation at all behavioral levels, constitutes the third source of developmental differences.

A PRELIMINARY BIOMETRICAL MODEL

In standard behavioral genetical analyses of human phenotypes, the third source is confounded with causes of within-family environmental variation and, consequently, can be identified only if a sufficient number of identification constraints is introduced. These constraints serve to delineate autonomous developmental processes insofar as is necessary to distinguish them from nonshared environmental influences. This can be accomplished only if there exists a suitable working model of the developmental processes concerned. In the foregoing sections we considered one such working model based on Edelman's theory of neural group selection. Edelman's epigenetic model still requires considerable elaboration in order to enable the derivation of definite identification constraints, yet it is one of the most promising working models currently available and therefore will guide us in a preliminary attempt to include the third source in developmental biometrical models.

As an illustration, consider the genetic structural model of longitudinal data discussed by Boomsma and Molenaar (1987). This model includes genetic and environmental autoregressive processes underlying the time course of a univariate phenotypic trait. In particular, within-family environmental influences are modeled by such an autoregressive process, implying that the lagged autocorrelation between these environmental influences at different time points is less than one. Let the phenotypic trait at stake be a psychophysiological variable that is associated with the primary repertoire, such as degree and type of lateralization of the brain as reflected by differences between homologous EEG derivations. It then can be expected that the pertinent epigenetic processes underlying lateralization reach their end state at some early age, after which a relatively stable pattern of lateralization is maintained across most of the life span. Such structural differences between homologous neural networks in the left and right hemispheres of the brain may give rise to stable lateralized EEG components. In the longitudinal genetic EEG model these stable components could each be represented by a common within-family environmental factor having lagged autocorrelations of one (Eaves *et al.*, 1986). The presence of third source influences then could be inferred from systematic left–right differences between these common within-environmental EEG factors.

This is only one preliminary, though already quite intricate, attempt to model a particular aspect of the third source. We expect, however, that deliberate elaboration of Edelman's epigenetic model will enable one to carry out more sophisticated tests of the presence of third source influences in developmental behavior genetic analyses. In the first instance these tests will require the availability of time-series data (as in our EEG example). An elaborate discussion of the biometrical modeling of human time series data from the perspective of identifying third source influences will be given in a forthcoming paper.

DISCUSSION

Substantial effects of nonshared environmental influences are a common finding in behavior genetical studies. In fact, the importance of these effects greatly outweighs the between-family environmental influences on most behavioral characteristics (Plomin and Daniels, 1987). Yet any endeavor to identify further possibly important types of within-family environmental influences has yielded unsatisfactory results (birth order, for example, can account for only a fraction of the variance due to these influences). Consequently, the real nature of these influences remains more or less a mystery. In our opinion, an important reason why the sources of these influences are still unknown is because a significant part of nonshared environmental influences may not be due to environmental differences at all, but result from intrinsic variability in the output of deterministic, self-organizing developmental processes. That is, in regular biometrical modeling of phenotypic variation the E1 influences are confounded with influences due to a distinct third source, which resides in the chaotic time course of autonomous epigenetic processes and is independent of genuinely genetic and environmental influences. The basic tenet of this article is to present this point of view, reinforced by recent progress in the analysis of biological pattern formation.

Reiterating, chaotic epigenetic processes are capable of creating variability under constant genetic and environmental conditions. If such a process were simulated twice on a computer, where all starting conditions as well as the genetical and environmental influences are identical between simulation runs, then one would still obtain different outputs. In this respect the third source differs from other endogenous influences which also are distinguished as nonshared environmental factors in a standard behavior genetical analysis. One such alternative influence is genetic balance, which might underly inbreeding depression and heterosis. According to Mather (1973, p. 130) inbreeding depression is commonly reflected in increased variability among repetitive parts such as bilateral characters in animals and floral morphology in plants, while heterosis is similarly reflected in an increased uniformity among repetitive parts. The degree of genetic balance is a characteristic of the genotype and consequently cannot account for developmental differences obtained under identical genetic and environmental conditions, as in the above simulation example. Similar remarks can be made with respect to endogenous influences such as genomic imprinting (Hall, 1990) and mitotic crossing-over (Côté and Gyftodimou, 1991).

The autonomy of the third source, i.e., its independence of genetical and environmental factors, opens up interesting possibilities to identify third-source influences in behavior genetical analyses of human phenotypes. A general approach might be to estimate the individual E1 factor scores underlying a multivariate phenotype (cf. Boomsma *et al.*, 1990) and then apply mathematical techniques drawn from chaos theory to this set of E1 scores. For instance, one could thus determine the eventual fractal dimension of E1 in a longitudinal behavior genetical analysis.

One plausible interpretation of the third source can be given in terms of Edelman's theory of neural group selection. We considered the main outlines of such an interpretation, at least as we see it, and proposed a preliminary genetical structural model based on this interpretation in order to assess the impact of the third source on human phenotypic data. These endeavors require more elaboration, however. A more definite approach along these lines to the biometrical modeling of EEG will be presented in a forthcoming paper. We expect that this work will yield important contributions to the identification of endogenous sources underlying E1.

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REFERENCES

Benno, R. H. (1990). Development of the nervous system: Genetics, epigenetics and phylogenetics. In Hahn, M. E.,

Hewitt, J. K., Henderson, N. D., and Benno, R. H. (eds.), *Developmental Behavior Genetics: Neural, Biometrical, and Evolutionary Approaches*, Oxford University Press, New York, pp. 113–143.

Boomsma, D. I., and Molenaar, P. C. M. (1987). The genetic analysis of repeated measures. I. Simplex models. *Behav. Genet.* **17**:111–123.

Boomsma, D. I., Molenaar, P. C. M., and Orlebeke, J. F. (1990). Estimation of individual genetic and environmental factor scores. *Genet. Epidemiol.* **7**:83–91.

Burgess, R. L., and Molenaar, P. C. M. (1993). Human behavioral biology: A reply to R. Lerner and A. von Eye "Sociobiology and Human Development: Arguments and Evidence." *Hum. Dev.* **36**:45–54.

Clayton, G. A., Morris, J. A., and Robertson, A. (1957). An experimental check on quantitative genetic theory. I. Short-term responses to selection. *J. Genet.* **55**:137–151.

Côté, G. B., and Gyftodimou, J. (1991). Twinning and mitotic crossing-over: Some possibilities and their implications. *Am. J. Hum. Genet.* **49**:120–130.

Eaves, L. J., Long, J., and Heath, A. C. (1986). A theory of developmental change in quantitative phenotypes applied to cognitive development. *Behav. Genet.* **16**:143–162.

Eaves, L. J., Hewitt, J. K., and Heath, A. C. (1988). The quantitative genetic study of human developmental change: A model and its limitations. In Weir, B. S., Eisen, E. J., Goodman, M. M., and Namkoong, G. (eds.), *Proceedings of the Second International Conference on Quantitative Genetics*, Sinauer, Sunderland, MA, pp. 297–311.

Edelman, G. M. (1987). *Neural Darwinism: The Theory of Neuronal Group Selection*, Basic Books, New York.

Gaertner, K. (1990). A third component causing random variability beside environment and genotype: A reason for the limited success of a 30 year long effort to standardize laboratory animals? *Lab. Anim.* **24**:71–77.

Hall, J. G. (1990). Genomic imprinting: Review and relevance to human diseases. *Am. J. Hum. Genet.* **46**:857–873.

Johnston, T. D. (1987). Developmental explanation and the ontogeny of birdsong: Nature/Nurture redux. *Brain Behav. Sci.* **11**:617–663.

Kurnit, D. M., Layton, W. M., and Matthysse, S. (1987). Genetics, chance, and morphogenetics. *Am. J. Hum. Genet.* **41**:979–995.

Mather, K. (1973). *Genetic Structure of Populations*, Chapman and Hall, London.

Mather, K., and Jinks, J. L. (1977). *Introduction to Biometrical Genetics*, Chapman and Hall, London.

Meinhardt, H. (1982). *Models of Biological Pattern Formation*, Academic Press, London.

Molenaar, P. C. M. (1986). On the impossibility of acquiring more powerful structures: A neglected alternative. *Hum. Dev.* **29**:245–251.

Molenaar, P. C. M., Boomsma, D. I., and Dolan, C. V. (1991). Genetic and environmental factors in a developmental perspective. In Magnusson, D., Bergman, L. R., Rudinger, G., and Törestad, B. (eds.), *Problems and Methods in Longitudinal Research: Stability and Change*, Cambridge University Press, Cambridge.

Moon, F. C. (1987). *Chaotic Vibrations*, Wiley, New York.

Nicolis, G., and Prigogine, I. (1977). *Self-Organization in Nonequilibrium Systems: From Dissipative Structures to Order Through Fluctuations*, Wiley, New York.

Oster, G., and Alberch, P. (1982). Evolution and bifurcation of developmental programs. *Evolution* **36**(3):444–459.

Plomin, R. (1986). *Development, Genetics and Psychology*, Lawrence Erlbaum, Hillsdale, NJ.

Plomin, R., and Daniels, D. (1987). Why are children in the same family so different from one another? *Behav. Brain Sci.* **10**:1–60.

Plomin, R., DeFries, C., and Fulker, D. W. (1988). *Nature and Nurture During Infancy and Early Childhood*, Cambridge University Press, Cambridge.

Province, W. B. (1986). *Sewall Wright and Evolutionary Biology*, University of Chicago Press, Chicago.

Reeve, E. C. R. (1960). Some genetic tests on asymmetry of sternopleural chaetae number in Drosophila. *Genet. Res.* **1**:151–172.

Scarr, S., and Weinberg, R. A. (1983). The Minnesota adoption studies: Genetic differences and malleability. *Child Dev.* **54**:260–267.

Schierwagen, A. (1990). Microenvironmental constraints and morphological differentiation in mammalian central neurons. In Maynard Smith, J., and Vida, G. (eds.), *Organizational Constraints on the Dynamics of Evolution*, Manchester University Press, Manchester, pp. 167–189.

Soong, T. T. (1973). *Random Differential Equations in Science and Engineering*, Academic Press, New York.

Stanley, H. E., and Ostrowsky, N. (1986). *On Growth and Form: Fractal and Non-Fractal Patterns in Physics*, Reidel, Dordrecht.

Waddington, C. H. (1957). *The Strategy of the Genes*, Allen & Unwin, London.

Wilson, R. S. (1983). Human behavioral development and genetics. *Acta Genet. Med. Gemmellol.* **32**:1–16.

Wright, S. (1920). The relative importance of heredity and environment in determining the piebald pattern of guinea pigs. *Proc. Natl. Acad. Sci. USA* **6**:320–332.

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