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CANALIZATION: GENETIC AND DEVELOPMENTAL ASPECTS

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INTRODUCTION

Canalization

The concept of canalization was used by Waddington (66) in the context of developmental biology. He emphasized two important points in the development of higher organisms. First, the end products of development, i.e. the adult tissues, are of sharply distinct types without intergradation. Second, the normal course of development is in his view a preferred path. Deviations of this path due to disturbances in the internal or external environment are corrected by regulatory processes. This occurs not only in the development of distinct types of tissue, but also on the organismic level in the realization of morphological patterns, in size and shape of organs and in matters of growth and determination of size of whole organisms. He depicted these phenomena in what he called the epigenetic landscape. Development starts in the egg. From there numerous developmental pathways branch out, leading to a great variety of distinct end results. These pathways are represented as a system of branching valleys in a descending slope. The developmental process is visualized as balls rolling through the valleys to their end point. The steeper the valley and the larger the ridges separating the valleys, the stronger the tendency of the ball, when it is pushed from its course along the valley bottom by internal or external disturbances, to go back to its original course. This

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picture suggested the concept of canalization of developmental pathways. Waddington envisaged that the epigenetic landscape is generated by the interaction of a large number of gene-controlled processes. This view was derived from his embryological work on mutants of 38 different gene loci which caused wing abnormalities in *Drosophila* (67). The interactions of their normal alleles are necessary for the development of a normal wing.

In developmental biology the concept of canalization has had only a limited appeal. This is perhaps due to the fact that regulatory processes have always stood in the center of embryological research and to the feeling that the concept of canalization could not add much to existing theory.

In evolutionary biology, the concept became important after Waddington's experiments (69, 71) with *Drosophila* on genetic assimilation of environmentally induced phenotypes. These experiments drew much attention because they showed how seemingly Lamarckian processes suggesting the inheritance of acquired characters could be explained according to genuinely neo-Darwinistic principles.

Canalization and the Inheritance of Acquired Characters

Waddington's experiments on genetic assimilation (69, 71) were generated by the concepts expounded in his paper, "Canalization of development and the inheritance of acquired characters" (68). With this title Waddington emphasized the relation between these two phenomena: "The first step in the argument is one which will scarcely be denied but is perhaps often overlooked. The capacity to respond to an external stimulus by some developmental reaction. . . . must itself be under genetic control" (68, p. 563).

This train of thought begins with the assumption of genetic variability for the sensitivity of characters to environmental factors: "The occurrence of an adaptive response to an environmental stimulus depends on the selection of a suitable genetically controlled reactivity in the organism. If it is an advantage, as it usually seems to be for developmental mechanisms, that the response should obtain an optimal value more or less independently of the intensity of the environmental stimulus received by a particular animal, the reactivity will become canalized, again under the influence of natural selection. Once the developmental path has been canalized, it is to be expected that many different agents, including a number of mutations available in the germ plasm of the species, will be able to switch development into it; and the same considerations which render the canalization advantageous will favour the supersession of the environmental stimulus by a genetic one" (68, p. 563).

Genetic variation in environmental response had been revealed by Goldschmidt (26, 27) and by Landauer (29) in phenocopy experiments, i.e. experiments in which normal genotypes were exposed during their development to extreme values of normal environmental factors or to environmental

factors—e.g. chemicals—that are not a part of their normal environment. Such treatment too often produces morphological abnormalities similar to abnormalities caused by known mutants (phenocopies). The kind of abnormalities, their expression level, and their frequency were revealed to be different in different stocks.

CANALIZATION AND GENETIC ASSIMILATION

The First Experiments

Wings of wild-type *Drosophila* possess a second cross-vein forming a connection between two longitudinal veins. Treatment of a population with heat shock (40°C) for 4 hr, 20–23 hr after puparium formation produces flies that have an incomplete cross-vein or none at all. This character, which is a phenocopy of several mutants found by *Drosophila* geneticists, was induced with a frequency of 40%. Waddington (69) started two selection lines. In the first line he selected as parents for the next generation flies that showed after the heat treatment the *cross-veinless* phenotype. In the second line he selected flies that had not reacted to the heat treatment with loss of the second cross-vein. The frequency of reaction increased in the line in which in every generation heat shock–induced *cross-veinless* phenotypes were selected and decreased in the line in which flies were selected that showed the normal phenotype after the heat shock. After 12 generations the difference in the induction of *cross-veinless* phenotypes between the two lines had become some 55%. In each generation, in addition to the pupae submitted to heat shock, a large number of flies were grown without heat shock. In the line selected for increased sensitivity in this control group, flies appeared without cross-veins. In these flies the erstwhile phenotypic reaction to the heat shock appeared now without environmental stimulus, i.e. appeared to have become genetically determined. Waddington (69) called this phenomenon of genetic fixation of a phenotypic reaction to an environmental factor *genetic assimilation*. He (69, 73, 77) gave an explanation in neo-Darwinian terms. The selection response was shown to be based on genetic variation present in the base population at the start of the experiment. Long inbred lines which are supposed to approach genetic homogeneity did not show any change in the frequency of the heat shock–induced *cross-veinless* phenotype under the same selection regime (2). Moreover, Bateman (2) repeated the first Waddington *cross-veinless* assimilation experiment with a different base population and obtained similar results. However, when she screened her base population for occurrence of *cross-veinless* phenotypes without applying heat treatment, she found that a low percentage was present spontaneously.

Waddington (71, 72) performed similar experiments with a far more spec-

tacular phenotypic reaction. Gloor (24) had obtained phenocopies of the *bithorax* mutant of *Drosophila* by submitting eggs in an early stage of their development to a treatment with ether vapor. This is a typical threshold reaction seemingly determined more by the structure of the developmental system than by a specific chemical action of the ether: heat shock applied at the same stage of development caused similar phenotypes (31). Extreme phenotypes obtained by these treatments showed a transformation of the third haltere-carrying thorax segment into a copy of a full-blown second thorax segment with a large mesonotum and a pair of wings, a phenotypic change of almost macro-evolutionary scope.

Twenty generations of selection caused a large increase in the frequency of the *bithorax* phenotype as a reaction to the ether treatment. Then *bithorax* phenotypes started to appear from control eggs not submitted to the environmental treatment. This happened in two selection experiments started from two different base populations.

In addition to *cross-veinless* phenocopies Bateman (2) induced by a heat treatment—2–4 hr at 40°C, 18 hr after puparium formation—three other aberrant phenotypes: absence of the anterior cross-vein, an extra cross-vein between longitudinal veins 3 and 4, and an extra cross-vein between longitudinal veins 2 and 3. Moreover, she induced phenocopies of the well-known *dumpy* mutant (1). By artificial selection for higher reactivity for each type of abberation in separate selection lines, she succeeded in increasing the frequency of phenocopies and obtained assimilation in each case.

Canalization and Assimilation: A Necessary Linkage?

The question arose whether the canalization concept is necessary for the explanation of the assimilation experiments. Bateman (1), a PhD-student of Waddington, explained the results of a repeat of Waddington's *cross-veinless* experiment in terms of a simple threshold model. She assumed in the population a normal distribution for the tendency to have a broken cross-vein. The percentage of the *cross-veinless* phenotype in the population will then be represented by the part of the distribution above the threshold. An increase of the frequency of this phenotype can occur as a consequence of a shift of the mean of the distribution, by an increase of its variance, or by a shift of the threshold. She depicts a model in which the position of the threshold is shifted to a lower value by the heat-shock. As a consequence part of the population-distribution now transgresses the new threshold, and *cross-veinless* phenotypes appear in the population. Selection of these phenotypes causes an accumulation of genes promoting this phenotype, thereby causing a shift to higher values of the population distribution for breakage of the cross-vein. After further progress, part of the distribution exceeds the threshold for normal temperature, and the *cross-veinless* character appears without heat-

shock. The same model was presented by Falconer (11). A probit analysis of the selection response of her two *cross-veinless* selection lines showed indeed that the selection response could be explained as a shift of the normal distribution for the tendency to cross-vein breaks without a change of its variance.

Curt Stern (61) suggested a similar explanation in a paper, "Selection for subthreshold differences and the origin of pseudoexogenous adaptations." He recognized that the phenomenon of genetic assimilation was deduced by Waddington (68) from the picture of his epigenetic landscape and that the experiments (69, 71) showing that genetic assimilation could occur were designed on this basis. But, as Stern stated, this does not mean that Waddington's basic assumptions were valid.

Suppose a population possesses genes that promote a certain phenotype in two environments, but their selection can only occur in the second environment, because a threshold prevents their expression in the first environment. Then selection for these genes causing their accumulation in the population will ultimately lead to their expression in that first environment. Stern presented a simple single locus model involving the recessive *Drosophila* mutant *cubitus interruptus (ci)*.

In a reply, Waddington (74) conceded that genetic assimilation can be explained by such a threshold model, but he regarded it as a "told to the children version." He maintained that the model could not explain why selection would go much further than is necessary in the environment that induces the new character, i.e. fixing it also in the original environment. Therefore, the threshold model would not be sufficient; canalization would have to be brought in. Bateman (2) already dealt with that argument by showing that such was not the case in her cross-veinless assimilation experiments, although she could imagine that when assimilation occurred in nature a phase of canalization by stabilizing selection might follow.

In 1961 Waddington (77) still maintained his viewpoint as is clearly revealed in a subheading "Genetic assimilation as a consequence of canalization" of a paragraph in which he attacked Stern (61) again. However, his arguments are related to a hypothetical evolutionary history of the callosities on the ventral surface of the ostrich. The sufficiency of Bateman's and Stern's model for the results of the assimilation experiments is not touched upon.

Genetic Variation in Phenotypic Reactions: The Anal Papillae

In his reaction to Stern's paper, Waddington (74) had already stated that Stern's model did not accommodate differences in reactivity between individuals. As we have seen, these were not required for the explanation of the experiments published before, but they are involved in Waddington's

experiments on the genetic assimilation of changes in size of the anal papillae of *Drosophila* larvae.

In the title of the 1959 *Nature* paper, "Canalization of development and genetic assimilation of acquired characters" (76), Waddington linked these two concepts again, although they were not integrated in the text. The first part of the paper dealt with an experiment on what Waddington named *canalizing selection,* i.e. artificial selection trying to change the sensitivity for temperature of eye-facet number in the *Drosophila* mutant *Bar*. The second part is on genetic assimilation of the purported increase in size of the anal papillae in larvae of *Drosophila melanogaster* by exposure to salt in the food medium.

Waddington gave two reasons for this experiment: (*a*) The previous experiments on genetic assimilation were all done with artificial selection on characters without clear adaptive significance, and (*b*) the characters used earlier were all threshold characters. It would be interesting if assimilation could be demonstrated with a quantitative, continuously varying character.

The anal papillae were described by Gloor & Chen (25) as regions of modified, strongly enlarged epidermal cells at either side of the anus. They suggested that the anal papillae were involved in osmoregulation. At pupation the papillae fold inwards, and the remnants of the papillae can be seen in the pupae. Waddington measured the size of (what he thought were) the remnants of the anal papillae in pupae. When he grew larvae on food with different amounts of salt (between 2% and 7%) he found that his measurements increased when the salt concentration increased. He interpreted this as an adaptive reaction: Larger papillae would perform better in osmoregulation. He did an experiment in which three different populations could adapt by *natural* selection to increasing concentrations of salt in the food medium. The increase of the salt concentration was gradual so that 20–30% of the eggs laid on the selection medium grew up to produce flies. After 21 generations of adaptation, the survival and the size of the anal papillae of the adapted populations and of their controls held on normal medium were determined on a range of salt concentrations. The results showed that survival was higher on salted medium, in particular at higher salt concentrations, in all three populations than in the corresponding controls, that the change of the purported length measurement of the anal papillae was larger in all three adapted populations than in the controls, and that at the lowest salt concentration (2%), the measurement in the adapted populations was still higher than in the control populations. Waddington's interpretation was that the outcome of the adaptation process was an increase of the strength of the phenotypic adaptive reaction of the length measurement, and fixation of part of the phenotypic response at the lowest salt concentration, i.e. genetic assimilation. However, this last point is perhaps not valid. If we extrapolate the curves of the

phenotypic reaction to 0% salt addition, it seems possible that there the curves of the adapted population would intersect the curves of their controls. This would mean that the adaptation was based only on genetic change that increased the strength of the phenotypic reaction of the anal papillae, an explanation not involving the concept of genetic assimilation.

A more serious objection is that of te Velde and Scharloo (53, 62–65) who discovered that Waddington measured not the remnants of the anal papillae in pupae but the remnants of the inactive epidermal zone *between* the anal papillae. A comparison, at the one hand, of the careful drawings of Gloor (25) of the photographs of larvae with papillae stained with silver-nitrate and of the scanning pictures made by te Velde et al (63) with, at the other hand, the drawings in Waddington (76, reprinted in 78) makes this very clear.

The anal papillae, measured on *Drosophila* larvae, increased in size with *decreasing* salt concentration. This is in agreement with results with other Dipteran species (e.g. *Aedes* and *Culex,* 79). The size of the inactive zone between the papillae which Waddington mistakenly measured as papillae-size is negatively correlated with the size of the papillae (64). Therefore Waddington's data are at best indirect measurements of the size of the papillae.

The phenotypic response of the anal papillae to decreased salt concentrations is twofold; one response is in size as a consequence of an increase in cell number, and the other is a rapid response of the ultrastructure of the papillae cells (3, 63). The papillae cells are not covered by the normally rather thick epidermal cuticula. The outward cell membrane is folded in large so-called apical lamellae which frequently are connected with mitochondria. This type of cells is well known, e.g. in many aquatic animals where they serve for the intake of ions. These lamellae are far better developed and in contact with more mitochondria when the salt concentrations in diluted food medium are low and an active intake of ions has to occur. Genetic variance occurs in the structure of the papillae which is related to the ultrastructure of the papillae cells. Normally the papillae are retracted at puparium formation. In populations grown on a low salt concentration (2%) an aberrant type was found in low frequency. In this type the papillae were not retracted during puparium formation. While in the normal retracted (R) type the papillae were present in the pupae as narrow ridges at either side of the interpapillar zone, in the stretched (S) type the papillae are still quite large organs. In populations adapting to salted media the S-type increased. Moreover, by artificial selection for this S-type on a low salt medium it was possible to increase S-type frequency to almost 100%, i.e. to obtain its fixation.

Comparison of the two types indeed showed that the S-type was better adapted to high salt concentrations and the R-type to diluted media. The size of the papillae decreased in both types with increasing salt concentration to the same extent. However, in the R-cells the apical lamellae were well

developed and reacted strongly on changing salt concentrations. In S-cells the lamellae were practically absent on normal medium and increased only slightly on diluted medium.

On normal medium the two types performed equally well. They maintained the osmotic value of their hemolymph equally well with moderate changes of salt concentration. When the salt concentration increased to higher values, the internal osmotic value could be better maintained by the S-type while on diluted medium the R-type did better. This was expressed in survival, in development time, in feeding rate, and in competition (53, 62).

CANALIZATION AND QUANTITATIVE GENETICS

Introduction

In evolutionary biology the concept of canalization became known by the results of the assimilation experiments. The importance of these experiments for an explanation of the evolution of pseudo-exogenous adaptations was widely accepted. The concept of canalization was, as it were, hitchhiking on the assimilation concept to recognition in evolutionary genetics. However, we have seen that several investigators (2, 61) showed that the concept of canalization was not necessary to explain the results of the assimilation experiments. A simpler explanation, in terms of expression of subthreshold genes, is sufficient.

How could it be shown that canalization really exists, that the organization of development could affect the expression of genetic variation in quantitative genetic characters and thereby the course of selection response?

Variation at Either Side of the Wild-Type

Waddington (70) attempted this in a paper "On a case of quantitative inheritance on either side of the wild-type" in which he reported on crosses with *Drosophila* stocks which showed either an interruption of the second cross-vein or extra venation attached to this vein. He used two stocks derived from his assimilation experiments which differed in the size of cross-vein interruption and two stocks with a different addition of extra vein material to the second cross-vein. With the normal phenotype included, he had five different levels of cross-vein expression. When he crossed the two lines with different levels of cross-vein interruption, the inheritance of the size of the interruption was additive. A similar result was obtained in crosses between the two lines with extra vein material. But when he made crosses between stocks "either side of the wild-type" the results were widely different from additivity. He concluded that "the wild phenotype could conceal within it a much greater range of dosages of vein producing genes than can any other phenotype" (77, p. 279). This implies that "the canalization of the normal vein pattern is such

that it is highly resistant to the disturbing effects of changes in the dosage of genes tending either to make more or to make less vein. In individuals in which the buffering capacity of the normal developmental course is exceeded, the phenotype does become altered, and reflects not too inaccurately the actual dosage of genes contained in them" (77, p. 279).

But there were complications: The quantity of wing-vein material used in the posterior cross-vein region could be arranged in different ways. There could be a complete cross-vein, or the same amount of vein material could be used for an interrupted cross-vein with a piece of extra vein attached. The character "amount of cross-vein" is not causally homogeneous; it is clearly the result of more than one participating developmental pathway, but each of the constitutive pathways of the character is, in Waddington's view, itself canalized. Here there is clearly no simple linear relation between effects of the genes involved and their expression in the amount of vein material; considerable interaction must occur between these genes.

Selection on Mutant Expression

Dun & Fraser (7, 8, 17–19), Rendel (40), Sondhi & Maynard Smith (56, 60, 34) and Scharloo (49, 51, 54) performed artificial selection on morphological characters which in wild-type individuals were constant or almost so. Instead of creating, as Waddington did, phenotypic variability by extreme environmental factors such as heat-shock or chemical agents, they introduced mutants producing variable phenotypes.

VIBRISSAE NUMBER IN MICE Dun & Fraser (7, 8) selected on the number of secondary vibrissae in mice. This set of vibrissae is arranged in three paired groups on the left and right side of the facial part of the head, one unpaired group under the chin, and a group on each forelimb. The total number of 19 is very stable; in a survey of 3000 normal mice, approximately one abnormality per 500 groups was observed. The sex-linked gene *Tabby* reduces vibrissae number to an average of 15 in *Ta*/+ females, and to 8 in *Ta*/*Ta* females and in the *Ta*/*Y* males. However, in contrast with the wild-type mice, in the *Tabby* genotypes considerable variation occurs. Artificial selection for vibrissae number could therefore now be applied on both *Ta*/+ females and *Ta*/*Y* males. The base population was formed by crossing three stocks with the mutant *Tabby* and two inbred lines. The selected populations—one selected for high vibrissae number and one selected for a low number—consisted throughout of some 30 females and 10 males. The mating system was such that in every generation there was segregation of +/*Y* and *Ta*/*Y* males and of +/+ and *Ta*/+ females. They differ only in the *Tabby* gene and its surrounding X-chromosome material. Such a scheme makes it possible to observe in every generation the effect of the genes accumulated by artificial selection on

the three levels of expression of the character, i.e. wild-type (i.e. males and females) with 19 vibrissae, *Ta*/+ females with 15 and *Ta*/*Y* males with 8 bristles.

The selection proved to be very successful when monitored in the *Tabby* genotypes. After seven generations the divergence between the two lines was at the *Ta*/+ level approximately 5 vibrissae and increased to about 8 vibrissae at the end of the experiment in generation 19. In the *Ta*/+ animals the change in the low line was twice the change in the high line; expressed in the *Ta*/*Y* males the response is approximately equal in the low and high selection lines while their divergence is smaller.

Also on the wild-type level there is divergence. The low line showed a small response in the first generations that accelerated in the second half of the experiment. In addition to a shift of the mean to 17 vibrissae, variability increased strongly: Instead of the almost invariable number of 19, the last generation showed a range of 4 vibrissae—15–19. The change in wild-type mice of the high line is small: The first sign was that animals with extra vibrissae started to appear more frequently; this happened in particular in the last generations.

Dun & Fraser concluded that there is genetic variation for vibrissae number behind the uniform genotype of wild-type mice which is revealed only in the mutant phenotype. This makes possible artificial selection which has an immediate response in the mutant types. Accumulation of the genetic change caused by selection on the mutant types is sufficient to change the invariable wild-type. The mutant types are far more sensitive for genetic change than are normal types, i.e. the development of the normal types is canalized. In their first publication Dun & Fraser explained the absence of phenotypic variability in wild-type mice, despite the presence of genetic variability, by assuming a sigmoid relationship between gene action and phenotypic effects. They assumed that (*a*) there is a basic genetic system, the vibrissae number system, forming a vibrissae substance varying in the population according to a normal distribution; (*b*) a separately determined genetic system governs the relationship between change in vibrissae substance and phenotypic change and (*c*) this relationship is a sigmoid function and the *Ta*-gene affects the mean and the slope of this function. Thus, each *Tabby* genotype has its sigmoid function, each with its own characteristic slope which would reflect the strength of its canalization, and its own mean. In a later paper (8) Dun & Fraser suggest that their results could also be explained according to a model suggested by Rendel, explaining the results of selection on the expression of the mutant scute in Drosophila (40). That model assumes (*a*) a polygenic system causing variation of a vibrissae forming substance, and (*b*) a genetic system determining one sigmoid relationship between the amount of vibrissae substance and vibrissae number with a steep inflection zone around the

normal vibrissae number representing the canalization of that phenotype. The *Tabby* gene would affect not canalization but only the amount of vibrissae substance. The selection would change only the amount of vibrissae substance.

In 1962, Fraser & Kindred (18) accepted Rendel's model because in the last generations of their selection experiment the *Ta*/+ females in the high line start to overlap with the frequency distribution of the normal phenotype. The *Ta*/+ females and the wild-type animals can be distinguished by the effect of *Tabby* on coat color. The variation of the *Ta*/+ females went down strongly because the frequency distribution shrinks at its lower end and animals seem to accumulate in the wild-type class of 19 vibrissae. Progress in this genotype then begins to halt while progress in the *Ta*/*Y* males continues. This shows that the strength of canalization is not controlled by the *Tabby* gene but is determined by the phenotypic value of the character vibrissae number.

SCUTELLAR BRISTLES IN DROSOPHILA Rendel (40) performed a similar experiment with the sex-linked mutant *scute* of *Drosophila melanogaster*. *Scute* is located almost at the distal end of the X-chromosome. Homozygous and hemizygous it causes a decrease of bristle number in several bristle groups and in particular of the scutellar bristles. Wild-type flies have 4 bristles on the scutellum in a fixed pattern of 2 anterior bristles and 2 posterior bristles. This is virtually an invariable pattern; it is diagnostic for the genus *Drosophila*. In wild populations, either in mass culture in the laboratory or in nature, only occasionally are flies observed with 3 or 5 bristles. The *scute* mutant lowers the mean in females to two and in males to one bristle. In addition, in flies carrying the mutant the number of scutellar bristles is variable; in males it varies from 0 to 3 and in females from 0 to 4.

Rendel practiced selection for a higher number of scutellar bristles on males. In his selection line, females were heterozygous for the *scute* mutant. So, in each generation there was segregation of *scute* and wild-type genotypes of both sexes, in which Rendel could monitor the phenotypic effect of genetic change caused by the selection on the *scute* males. The experiment consisted of 10 lines. In each line the 5 *scute* males with the highest number of scutellar bristles were mated with 5 randomly selected heterozygous females with 4 bristles.

The mean number of scutellar bristles in *scute* males is approximately 1 in the base population; this increases to 2.8 after 22 generations of selection. In *scute* females the original mean is a bit higher than 2 and increases to 3.3. At the start the sex-difference is around 1 bristle; it is halved at the end at generation 22. In *scute* males the variation in bristle number is rather constant. In the 0 bristle class and in the 1 bristle class there are almost an equal number of flies, while only in later generations of selection do flies with 4

bristles start to appear in an appreciable number. In *scute* females the variation in bristle number declines because the frequency distribution shrinks at the lower values and the flies accumulate in the class with the normal wild-type number of 4 bristles. Notwithstanding the strong progress in the *scute* animals, the mean of 4 bristles of the wild-type males and the +/*sc* heterozygote females scarcely changes. Only in the last 5 generations do flies with more than 4 bristles appear with a certain regularity; in the males there are even some with 6 bristles.

This pattern is similar to that found for the vibrissae in the *Tabby* mice. Selection on a mutant character toward the normal type is very successful until the expression of the mutant comes near to normal expression: Progress then stops and the variability declines. The genetic change causing large phenotypic change in the mutant phenotype scarcely changes the phenotypes of the nonmutant genotypes segregating in the selection line. For scutellar bristle number also, it was concluded that the normal phenotype is highly canalized.

Rendel interpreted his experiments according to a model depicted in Dun & Fraser (8) which can be summarized in the following points (*a*) Bristle formation is dependent on a gene product or morphogenetic substance (later called "make") which is normally distributed in populations, (*b*) the relation between the amount of morphogenetic substance and bristle number is not linear but is sigmoid, with a region around the normal, wild-type number where change of morphogenetic substance does not cause a phenotypic effect. Beyond this region, both at lower and at higher bristle numbers, change is easy and there is always phenotypic variability.

Rendel designed a method to measure the strength of the canalization of the different bristle classes based on the theory of the normal distribution and the assumption that the morphogenetic substance has a normal distribution in populations. When the amount of morphogenetic substance in an individual transgresses a threshold, 1 bristle is formed, and when it transgresses a second threshold, 2 bristles are formed, and so on. When a change in the amount of morphogenetic substance does not lead to a change in bristle number, clearly in that region the distance between the thresholds separating bristle classes must be large. The problem of quantifying the strength of canalization of a bristle class is measuring the width of a bristle class, i.e. the distance between two thresholds that form the boundaries of that class. When the morphogenetic substance has a normal distribution in the population and the frequencies of flies with different bristle numbers are known, the distance between the thresholds and the mean of the underlying normal distribution can be expressed in its standard deviation as probits. This makes it possible to compare the relative width of the different bristle classes within a population. Comparisons between populations or between different generations of a selection

experiment can only be made when the variance of the morphogenetic substance is not different. In many publications this seems to be taken for granted. Rendel could calculate that to move from 3 bristles to 5 it takes eight times the genetic change that it does to move from 1 bristle to 3 bristles.

OCELLI WITH BRISTLES IN DROSOPHILA The sex-linked mutant *ocelli-less* in *Drosophila subobscura* affects the ocelli and 3 pairs of adjacent bristles on the top of the head. (34, 59, 60). In the base population the homozygous *ocelli-less* mutant removed all bristles and ocelli in some flies, while the other flies could show a diversity of combinations. In some flies the ocelli were slightly displaced from their normal position, and the size of bristles and ocelli could be affected. Between the presence of bristles and ocelli, a correlation of $r = 0.46$ was found. This was used as a justification to express the degree of expression of the *ocelli-less* mutant by counting the presence of an ocellus or a bristle as a unit. A fly deprived of any ocelli or bristles got a 0 score, a fly with 3 ocelli and 6 bristles scored 9.

Sondhi applied artificial selection for a lower and for a higher number. There were two high selection lines and two low selection lines. The lines of each pair of lines had a different mating system; in one line inbreeding was prevented, in the other line matings were between siblings. In the base population founded by outcrossing of some laboratory stocks, the mean score in females was 1.76 and for males 1.44. Each line consisted of 5 pairs of flies. In the low lines the selection response was small; less than 1 bristle. In the high lines there was an immediate response which was more rapid in the inbred than in the outbred population. After 13 generations, in both lines progress stopped and so was the selection. In the upward selection in the last generations some flies were seen with extra ocelli and the frequency of extra bristles had increased.

Sondhi (59, 60) designed a model to explain "the bounded distributions of phenotypes in terms of genes which in their primary effects on an ocelli-bristle-forming substance are additive throughout their range." He assumes that this morphogenetic substance is normally distributed in the population. When this distribution does not transgress a threshold T_1, all flies will lack bristles and ocelli; when it straddles the threshold, part of the flies will miss all bristles and ocelli, but the part with amounts of substance higher than the threshold will possess ocelli and bristles in various combinations. When the distribution extends beyond a threshold T_2, flies with all 6 bristles and 3 ocelli will appear; when a third threshold T_3 is passed, extra ocelli and/or bristles will appear. However, it is not clear how Sondhi explains the constancy of the wild-type flies. He depicts for the *ocelli-less* stocks—the foundation population and the downward and upward selected lines—frequency distributions of the ocelli-bristle forming substance with equal variances which only differ in

their means. For the wild-type population he gives a distribution with very low variance, thereby not extending beyond the thresholds T_2 and T_3. This implies that the *ocelli-less* mutant affects both mean *and* variance of the distribution, although Sondhi only remarks that "the amount of precursor in flies carrying the $+^{oc}$ gene is unlikely to be absolutely constant." He then states that "canalizing selection during the past evolution of the species would ensure that the same adult phenotype would develop, provided that the amount of precursor is within this range," i.e. the range between T_2 and T_3. In this model canalization seems to act independently of the components of the model.

This is in contrast with the models designed by Dun & Fraser (8, 18) and Rendel (40) where canalization is related to the degree of phenotypic expression of the character and is presented as the slope of the function relating phenotypic change to genetic change in that region or in the probit-width of the wild-type class, i.e. the distance between the thresholds which set the limits of phenotypic wild-type class; 4 scutellar bristles in *Drosophila* and 19 vibrissae in the mouse.

WING-VEIN INTERRUPTION IN DROSOPHILA The selection experiments described above all dealt with characters consisting of discrete elements which were added to obtain a total score; vibrissae in mice, bristles or bristles and ocelli in *Drosophila*. In the mouse it was a character composed of elements of similar structure located in groups on different parts of the body; in *Drosophila* with the *scute* mutant it was similar structures in a fixed pattern on the scutellum; and with the *ocelli-less* mutant with dissimilar structures but with a partly common underlying developmental system.

Scharloo (49, 50, 54) selected on the relative length of the fourth wing-vein in the presence of the mutant ci^D *(cubitus interruptus dominant)* in *Drosophila melanogaster*. The mutant ci^D is a recessive lethal gene on the tiny fourth chromosome ($< 0.2\%$ of the total genetic map) and has as dominant morphological effects terminal interruptions of the fourth and of the fifth longitudinal wing-vein.

This character was chosen because the location of ci^D on the fourth chromosome made it possible to introduce the mutant into the genome of populations with the help of marked balancer chromosomes without disturbing the rest of the genome. A character is thereby created in the population that did not exist before. Therefore, it could not have been shaped in a long history of natural selection, and this has consequences for the structure of its genetic variation (55).

The mutant was introduced into three wild-type populations of different origin. The character relative length of the fourth longitudinal wing-vein was expressed as the percentage ratio of the length of this vein to the length of the

third longitudinal vein, both measured distally of the first cross-vein. From each base population a low line was started in which flies with the shortest veins were selected, and another line was begun in which the flies with the longest veins were used as parents for the next generations. Each selection line consisted of 3 bottle cultures. From each bottle the wing-veins of 20 flies from each sex were measured, and from each sample the 4 with the most extreme measurements were selected. A rotational mating system was used to minimize inbreeding.

In all lines there was an immediate response. Realized heritabilities in the three pairs of lines were between 0.3 and 0.4. In the low lines there was a regular shift of the frequency distributions to lower values. Variability decreased when the fourth vein became shorter and the end of the vein approached its attachment to the second cross-vein.

In the high lines the response pattern was unexpected. In all three lines selected for a longer fourth vein, bimodal frequency distributions appeared. After passing this phase there was a concentrated unimodal distribution and some slow progress until a plateau was maintained. The appearance of bimodal frequency distributions was described earlier by Clayton et al (5) as a consequence of segregation of genes with large effect. But there were several arguments that this could not have occurred here: (*a*) In all three high lines the bimodal distributions occurred in the same range of phenotypic values between 70 and 80. (*b*) When selection was reversed (back-selection) from the unimodal distribution around 80, the bimodal distribution reappeared in the same range as in the upward selection and became unimodal again after shifting below the 70 value. (*c*) In this phase of bimodality, a high frequency of asymmetric flies appeared with a long fourth vein in one wing and a short vein in the other wing. (*d*) When the length of the fourth vein was shifted by temperature (in the lines involved it becomes longer when the larvae and pupae are reared at lower temperature) through the same range where change by selection generated bimodal frequency distributions, the bimodal distributions appeared again. This occurred when the distribution shifted through this region from below, i.e. when the back selection line with a unimodal distribution below 70 was submitted to low temperature. It occurred too when the high line with the unimodal distribution above 80 was grown at higher temperatures. When bimodality reappeared there was again a high frequency of asymmetry. (*e*) When the mean vein-length is plotted against temperature there is a thresholdlike relation when the frequency distributions go through their bimodal phase.

These points together prove that properties of the developmental system determine the pattern of response to disturbances. The disturbances can be caused by genetic differences as in selection, by such environmental differences as temperature, or by developmental error—the lack of precision in

development which causes fluctuating asymmetry. This is the kind of situation predicted by Waddington in canalized systems; buffering against disturbances generated by genetic variability, by environmental factors, or by developmental noise.

To explain these phenomena, the following model was made: (*a*) In the wing-anlagen of larvae there is a vein-forming substance. The level of vein-forming substance varies between individual larvae according to a normal distribution. It is subject to change by genetic and environmental factors, and by developmental error. (*b*) Vein formation depends on the competence of the cells along the track of the fourth vein to react to the vein-forming substance with the formation of vein-material. (*c*) There is a gradient of competence in the wing-anlagen. This gradient decreases from wing-base to wing-tip. When the level of vein-forming substance increases, vein length increases from wing-base to wing-tip. The slope of the gradient explains that the interruption of the fourth wing-vein in ci^D is always terminal. (*d*) The gradient is not linear but shows a thresholdlike change in the region 70–80. This explains the bimodality. When wild-type is approached, i.e. when the fourth vein becomes nearly complete, the gradient is supposed to become steeper.

The increasing steepness of the gradient when the fourth vein approaches completion is based on the following observations: The variability of the frequency distributions decreased when the mean vein length increased between the zone of bimodality and completeness of the fourth vein. Low temperature had little effect though it was very effective in lengthening the fourth vein when ci^D stocks had lower mean values i.e. lower than the region of bimodality. Plateaus in the selection lines were established at similar values just higher than 80, and there was little progress thereafter even with long continued further selection, i.e. in one line 30 and in the other 2 lines 10 generations.

The lack of response to selection in the direction of wild-type (i.e. complete) fourth vein was not due to exhaustion of genetic variability. The three possible crosses among the three high lines were made to create new genetic variability. From the F_3, selection was started for a shorter and for a longer vein, respectively. While the short vein lines made good progress showing the presence of genetic variability in the F_3 base populations, the progress in the high lines was very small indeed, and only in one line were a few flies obtained with a complete fourth vein. (54).

The important features of these experiments are: The developmental system of wing-venation influences the effect of factors that change this continuously variable character; genetic factors, environmental factors, and accidents of development (developmental error or developmental noise) are affected to the same extent; when the mutant phenotype is approaching wild-type, change becomes progressively more difficult.

CONCLUSIONS In the classic experiments on artificial selection of quantitative characters in *Drosophila* and mice (4, 5, 9, 10, 32, 33, 48, 51), the scale of measurement was more or less linear at least on a logarithmic scale. Factors that changed the character had similar effects on different parts of the scale, and if selection was not proceeding smoothly, this was a consequence of genetic processes (delayed recombination, involvement of factors with large effects) and not of limitations set by physiological or developmental processes.

In all the experiments in which quantitative characters, affected by mutant-expression, were artificially selected to normal type, progress of selection became more difficult in the neighborhood of wild-type. This was seen as the consequence of canalization of the wild-type phenotype and as a confirmation of Waddington's theory. Moreover, also at other levels of expression there were regions in which change is more difficult or where it is facilitated. Therefore, these experiments showed clearly the influence of developmental systems on the expression of genetic variability and thereby on selection response.

In the experiments with the mutants *scute* and *ocelli-less* in *Drosophila,* and the *Tabby* mutant in mice, it was possible to break through the wild-type barrier and to proceed at the other side of wild-type. This did not go far but did suggest that there was one continuous function, relating factors that changed a character and their phenotypic effects. This function would encompass a range beginning far below the wild phenotype where change was easy; then change would become progressively more difficult when passing wild-type, followed again by a region of facilitated change beyond the bounds of normality. This function represented canalization; change of canalization would occur by change of the developmental processes underlying this function. This hypothesis is depicted in the model of the *scute* experiments first given by Dun & Fraser (8) and adopted by Fraser & Kindred (18) for the mice vibrissae. It was later accepted by Waddington and is now featured in several reputable text books. The function would have got its shape by the action of natural selection of the stabilizing type. However, Fraser & Kindred (8) suggested as an alternative possibility "regularities in the basic pattern of development of the tissue concerned," foreshadowing the discussion on the role of developmental constraints in evolution.

The original concept of canalization implied that buffering would involve all factors pushing development out of course. Only in ci^D is there unequivocal evidence that the effects of genetic differences and nongenetic factors, i.e. environmental differences and the developmental indeterminacies generating asymmetry, are similarly affected. The other side of wild-type of the fourth vein interruption is, of course, extra venation. This occurred in two of the three lines selected for a long fourth vein. The extra-venation appeared before the fourth vein was complete and was in one line widespread and

caused by a *plexus* allele. Although the variation at the other side of wild-type, i.e. extra venation, was affected by the same genetic differences acting at the other side, i.e. on the length of the interrupted fourth vein, they are not acting according to a single continuous curve, as envisaged in Rendel's model.

Complications

Discussion concerning the significance and general validity of models with a single canalization function for scutellar bristle number and other characters arose from theoretical considerations and further analysis and experiments.

Alan Robertson (47) suggested that the probit transformation could not be used in a biologically meaningful way because of the nature of some characters. This would mean that some of the conclusions drawn about genotype-phenotype relationship were no more than statistical artefacts. In the models a morphogenetic substance shows continuous variation in its concentration between individuals. It determines the phenotype on a measurable scale; as the concentration transgresses a threshold, the score is 1; when it transgresses the next threshold the score becomes 2; and so on. Robertson states that such a model is only meaningful when the scores are really cumulative or sequential.

SCUTELLAR BRISTLES Scutellar bristle number is not such a cumulative character. There are four separate sites which are not filled up in any sequence; the total score is the sum of four independent events. Robertson suggested a model that starts with probabilities for the presence of a bristle on each separate site. The frequency distribution of individuals with particular bristle numbers can then be calculated. This model implies that we are not dealing with one frequency distribution of morphogenetic substance underlying the total number of scutellar bristles, but with four separate frequency distributions. Each site would have its own frequency distribution of morphogenetic substance and its own threshold which has to be transgressed for the appearance of one bristle on the particular site. Determining bristle class width in probits for total scutellar bristle number is then a meaningless procedure. Class width would have no significance because according to this "per site" model it does not indicate the amount of change of an underlying variable necessary for change of bristle number. Crucial here is the independence of bristle formation at the separate sites and the possibility of calculating frequency distributions for total bristle number from the probabilities of bristle formation for the particular sites.

Rendel (41) compared calculated distributions with distributions observed in four *scute* stocks. In one stock with the single site probability model, flies with 5 bristles were predicted and were not found.

Canalization of total bristle number would imply negative correlations

between bristles on different sites. In a stock canalized around 2 bristles by a special mode of stabilizing selection (45), the correlation between the presence of anterior and posterior bristles was -0.6. Latter (30) found negative correlations in a wild-type stock selected for high bristle number. However Latter & Scowcroft (30a) found that at the normal 4-bristle level the genetic correlation could be zero or positive. While these observations show the occurrence of mutual dependence of the bristle sites in the presence of scute, it does not exclude the possibility of a partly independent determination.

Alan Robertson (47) suggested that scutellar bristle number could better be considered as two pairs of bristles, an anterior and a posterior pair, each at least partly under specific control. He mentioned that extra bristles tend to appear first at the anterior sites, and bristles tend to be lost first at the posterior sites.

Scowcroft et al (57) indeed showed that a single developmental scale does not provide an adequate description of the phenomenon of canalization of scutellar bristles. The effect of the addition of extra *scute* loci was earlier reported by Fraser & Green (16) and by Rendel et al (44). They had found that additional *scute* loci always caused an increase in bristle number. Rendel et al observed that this effect became smaller measured on the underlying probit scale when approaching wild-type. They interpreted this as the "switching off" of the *scute* gene as the normal level of morphogenetic substance comes closer.

Scowcroft et al studied the effect of varying the number of *scute* loci from 1 to 3 in males and from 2 to 4 in females. They constructed special chromosomes with 3 different *scute* alleles, one mutant allele with extreme expression, one mutant approaching wild-type, and an allele that produces a normal number of scutellars. Scutellar bristle number always increased with increasing dosage of the *scute* locus, although the increase became smaller when coming closer to wild-type. A separate analysis was made of the change at anterior and posterior bristle sites. The responses to increasing dosage are strikingly different when measured at the anterior or at the posterior bristle sites. Starting from a low bristle number in the extreme mutant the gain is predominantly at the posterior sites, starting from wild-type level and increasing the dosage of the normal allele the number of anterior bristles increases without change of the posteriors. Moreover, the posterior bristles are more strongly canalized than the anteriors. Finally, the posterior bristles play a different role than anteriors in defining the 3 to 4 and the 4 to 5 thresholds which would determine the canalization at 4 bristles. In fact, the lower border of the 4-class is determined by posterior bristle number while the upper border is determined by the number of anterior bristles.

This is confirmed in experiments of Scowcroft (56) who applied selection for anterior or for posterior bristle number and for total scutellar bristle

number. He used two populations, one straddling the 3/4 threshold and the other the 4/5 threshold. The changes of total bristle number round the 3/4 threshold involve addition or loss at the posterior site while changes around the 4/5 threshold are changes of anterior bristles. The independent control of posterior and anterior bristles is also revealed by temperature experiments; while the anterior bristles decline in number at higher temperatures, the posterior bristles increased (23, 37).

Fraser & his associates (12–15) have produced further evidence showing that the relation between genetic and environmental factors in changing scutellar bristle number and phenotype cannot be represented by one morphogenetic substance acting according to one single function controlling bristle number at either side of the wild-type. First, Fraser et al point out that it is possible simultaneously to add to and to subtract from the basic pattern of four bristles. Such flies are very rare in wild populations but are more frequently present in lines selected for scutellar bristle number. This situation is comparable to Waddington's and Scharloo's flies with simultaneous vein-interruption and extra-venation (70, 52). It could mean that the character is not causally homogeneous.

Secondly, Fraser et al studied the effect of genetic variation accumulated in lines selected for higher scutellar bristle number in the presence of *scute* on non-*scute* individuals and vice versa. Fraser & Green (16) used wild-type lines selected for higher numbers of scutellars. They substituted a *scute* mutant for the wild-type *scute* allele in their lines. There was no correlation between the number of extra bristles in the wild-type lines and the number of bristles after the introduction of the *scute* mutant. Moreover, they constructed Y-chromosomes with the extreme tip of an X-chromosome which includes the wild-type *scute* gene attached to it. When these chromosomes were segregating in their lines, flies were obtained with different numbers and different combinations of alleles of the *scute* locus. Positive correlations for bristle number between genotypes were found only when both genotypes had either more than four bristles or less than four bristles. Notwithstanding their identical genotypes (besides the situation on the *scute* locus) there was no correlation when one of the lines possessed more than four bristles per fly and the other had a mean below four. Their conclusion was that there were two different sets of genes changing scutellar bristle number, one set acting in *scute* flies, the other in wild-type flies.

This is in contrast with Rendel's results on which his model was based. There he reported an, albeit small, increase of scutellar bristles in the wild-type sibs of the *scute* flies on which selection was practiced. Fraser (13) repeated Rendel's selection for higher number of scutellars in *scute* flies. He had four replicate selection lines. The results are roughly in agreement; the selection on scutellar bristles in *scute* flies caused higher bristle numbers in

non-*scute* sibs. However, Fraser (14, 15) found in his wild-type lines selected for higher bristle number a recessive gene *extraverticals* (*x-vert*, probably an allele of *polychaetoid*) which contributed about 3 bristles to the selection response of 5 bristles. Backcrossing this gene into the base population decreased its effect: it is dependent on the genetic background. This gene and its modifiers are not expressed in the presence of scute; on the other hand the modifiers of scute are not expressed in the homozygous presence of *x-vert*.

Sheldon & Milton (58) selected wild-type flies for high scutellar bristle number and obtained two lines with about 11 and 13 scutellar bristles after 140 generations. Backcrossing of a *scute* mutant into both lines produced means of 8.1 and 6.8 respectively for the sc/sc^+ wild-type females and 3.2 and 3.1 respectively for the *sc/sc* females, compared to means of 4 and 1.2 for the same genotypes in the control population. The genes causing an increase of bristles in the non-*scute* selection lines do not have much effect in the presence of *scute*. The wild-type sibs of *scute* flies selected for a higher bristle number obtained means of only 4.07 and 4.3, although the mean of the *scute* sibs was not much different from the flies with *scute* in the background of the selection lines. Haskell (28) earlier reported that modifiers of *scute* expression in abdominal bristles did not affect the same character in wild-type flies.

Scharloo (1988) obtained canalization of 8 scutellar bristles in a wild-type stock by stabilizing selection. He found that it was not based on canalization of total bristle number but on an increased precision per bristle site.

VIBRISSAE IN MICE Alan Robertson's (47) objections against the use of probits for genephysiological interpretations of canalization apply not only to the scutellar bristle pattern but also to the ocellar apparatus and to vibrissae number in mice. In fact, Pennycuik & Rendel (38) found for vibrissae number a good fit between expectations, on the basis of Robertson's theory and observation. The frequency distributions of total vibrissae number in mice agreed with the prediction made on the basis of the vibrissae numbers on the five separate sites. The vibrissae number is clearly controlled independently on each site.

An indication that in this character different processes can be involved in changes in vibrissae number is the different embryological basis for an increase in vibrissae number in the high selection line of Dun & Fraser and for a decrease of vibrissae number in a back selection line. In the first there was a change of the number of hair follicles, in the second there was not a decrease of the number of follicles, but a suppression of the formation of hairs (Jacobsen quoted in Fraser—14).

This was confirmed in selection experiments by Kindred (28a). She selected for a higher number of vibrissae in wild-type (i.e. non-*Tabby* mice) from the experiment in which mice with the *Tabby* mutant were selected for

higher vibrissae number (7, 8, 18). In that experiment after 34 generations of selection the mean vibrissae number of the wild-type sibs of the *Tabby* mice was scarcely higher than normal (19.1 instead of 19). Selection for higher number from these wild-type sibs had an immediate response without any effect on the *Tabby* sibs which still segregated in the line. Selection caused changes of genetic variability acting on a different aspect of the development of the vibrissae number than when selection was performed on the mutant character with values below normal. This does not fit the earlier model (18) which has one continuous canalization function translating genetic differences in bristle number over the whole range of variation of the character at either side of wild-type.

WING-VEIN INTERRUPTIONS In the early experiments with selection to normal phenotypes of morphological mutants in mice and *Drosophila,* changing expression became more difficult when wild-type was near. This was found too in the selection for a longer fourth longitudinal wing vein of the mutant ci^D. However, selection toward wild-type and temperature experiments with a different allele, *cubitus interruptus dominant of Gloor* (ci^{D-G}), revealed a strikingly different pattern. The temperature experiments showed an almost perfect linear relation between temperature and the relative length of the fourth vein. The frequency distributions were unimodal throughout over a large part of the possible range. At the lower temperatures (20°, 17.5°, 15°) wild-type was approached easily, and overlap with wild-type was obtained as a continuation of the linear shift of the frequency distributions (50, 51). While with the ci^D wild-type could never, or only in a few flies, be obtained after 40 to 60 generations of selection, on the contrary, with ci^{D-G}, overlap with wild-type occurred after only 4 or 5 generations (55).

Scharloo (51) selected the same character in the mutant *Hairless*. *Hairless* (*H*) is a homozygous lethal, and has as a heterozygote dominant morphological effects: It removes bristles on the top of the head and causes interruptions of the fourth and the fifth longitudinal wing-vein. These interruptions are only present in part of the flies. Selection for shorter length of the fourth vein was done in five selection lines. In all lines after a few generations all flies showed the fourth vein interruption, and the veins became shorter rapidly. The variability of the length of the vein was quite large in the beginning, but it became smaller when the vein became shorter and its end was approaching the the second cross-vein. Then selection progress, i.e. change of the mean vein-length, became smaller.

The length of the fourth vein in *Hairless* is sensitive to the rearing temperature. The fourth vein becomes longer at lower temperatures. When the vein-length was shifted through the same range as was transgressed by the selection, a similar variation pattern was observed: large variability when the

vein approaches completion and smaller variability in regions nearer to the attachment of the fourth vein with the second cross-vein. Change per degree Celsius is greater when the vein is nearing completion than in regions nearer to the second cross-vein (50, 51). Introduction of chromosomes of unselected stocks gives a similar picture; genetic change is easy in the neighborhood of wild-type and becomes more difficult in regions of more extreme expression of the mutant (51). Fluctuating asymmetry was approximately proportional to total variance.

Also in this case all factors which change this character—genetic factors, environmental factors, and developmental error—acted according to the same scale. However, the scale for the same character differs: there is a difference not only between mutants of different genes but also between alleles of the same gene locus.

CONCLUSIONS

The following conclusions can be drawn from the outcomes of the early assimilation experiments:

1. If artificial selection favors environmentally induced aberrant phenotypes, an increase of the frequency of the phenotypic reaction is obtained.
2. Selection for the induced phenotypes causes accumulation of genes which first, in conjunction with the environmental factor, increase the frequency of the phenotypic reaction and later realize the same phenotype on their own without environmental interference.
3. While specific environmental treatments induce often a variety of morphological aberrations, artificial selection for a specific type increases specifically that type of reaction and ultimately its assimilation.
4. Because of a lack of response to such a selection in genetically homogeneous inbred lines; it must be concluded that the response may be based on genetic variation present in the base population before the selection started.
5. Waddington (68, 69, 73, 74, 77) suggested that genetic assimilation has to be explained in terms of canalization and genetic variation of reactivity to environmental factors. Canalization would be a consequence on systems of interacting genes; genetic variation in reactivity implies gene-environment interaction. Bateman (2) and Stern (61) showed that a simple threshold model with additive effects of the genes involved and without gene-environment interaction could explain the experiments on genetic assimilation.

Change of a phenotypic reaction of a measurable character was shown in Waddington's experiments (75) on what he thought to be the size of the anal papillae in *Drosophila* larvae. Because he measured not the anal papillae in larvae but the remnants of the inactive epidermal region between the papillae,

he made at best an inverse measurement of an organ with adaptive reactions. The real reaction of the anal papillae is a decrease of their size with increasing salt concentration. Te Velde et al (64, 65) did not show genetic variation in this reaction which is based on changes in cell number. A rapid phenotypic reaction in the ultrastructure of the papillae cells (63) showed genetic variation in populations which had clear adaptive significance and could be fixed by artificial selection.

Introduction of morphological mutants revealed genetic variation for characters that were invariable in normal phenotypes. Artificial selection on such characters thereby became possible. The patterns of change and variation of the selected characters revealed the effect of the organization of developmental systems on the expression of genetic variability and thereby on selection response.

In the early experiments it was found for all characters that, when the character was approaching the normal, phenotypic change became progressively more difficult. In all experiments the modifier genes for mutant expression accumulated by selection toward wild-type could also change the character at the other side of wild-type, where variability increased again. This picture was interpreted as an expression of the canalization of the normal phenotype.

This suggested models in which the expression of the character was determined by two genetically independent components: a morphogenetic substance supposed to have a normal frequency distribution in the population, and one mapping or canalization function, in the *scute* and *Tabby* models ranging from extreme mutant expression, beyond wild-type, into a region with supernumerary structures. The function maps the effect of changes in morphogenetic substance into the character expression. The change in morphogenetic substance could be caused by genes or by environmental factors. The mutants were supposed to change the mean of the normal distributions of morphogenetic substance and not the mapping function. Artificial selection changing mutant expression would act via polygenic variability which is partly responsible for the normal distribution of morphogenetic substance. When environmental factors would only act via the morphogenetic substance, the mapping function would be a gene-environmental factor/phenotype mapping function (GEMP, 51). Then, the mapping function would represent real canalization, and the developmental system would govern the effects of genetic and environmental differences in the same way. Such a situation was revealed for the *cubitus interruptus* dominant mutant; genetic differences, environmental factors, and within-fly variance all acted according to the same mapping function (49, 51).

However, the concept of one mapping function characteristic for a specific morphological character could not be maintained in the light of further

analysis. In the first place, it was shown that such pattern characters as the scutellar bristle pattern and ocellar pattern in *Drosophila* and the vibrissae in mice could not be considered unitary characters. Their components, the separate bristles, vibrissae, and ocelli, were shown to have a partially independent determination which has important consequences for the interpretation of the experiments in which they are involved.

It has now been shown that there is not one homogeneous developmental process governing the expression of a character at either side of wild-type. In scutellar bristle number and for venation characters, effects at either side of wild-type, i.e. loss and addition, could occur simultaneously. This shows that the characters involved are causally not homogeneous. In this respect it is important that in the scutellar bristles modifiers effective below the normal score of 4 bristles are often not effective above the four level, and modifiers effective above the four level are suppressed below this level. A similar situation exists in vibrissae number in mice. Further, in the experiments on the interrupted fourth vein in *Drosophila,* the mapping function for the same character was strikingly different in different mutants. This shows that characters do not have specific mapping functions. Different mutants changing the same character affect and sensitize different steps in its developmental pathway, each with its own mapping function.

Does this mean that we have to abandon the canalization concept? Of course not. It is a fact that wild-type is relatively constant compared to mutants and to phenocopies. We cannot escape the conclusion that development in wild-type individuals is geared to produce constant phenotypes notwithstanding the presence of genetic variability and the omnipresence of environmental differences. This is the case not only in the constant morphological patterns which were the predominant object of research on canalization. It also occurs as we saw in this review, in adaptable physiological processes for the regulation of osmotic value of the hemolymph of Drosophila larvae. Forbes Robertson (47a) revealed it for developmental time and growth of *Drosophila* larvae. He showed that abnormal food conditions revealed large amounts of genetic variability for growth. They were not expressed under normal conditions, and the genetic variants involved showed interaction which generated normal growth and development.

However, we have to recognize that our understanding of this fascinating subject is still limited. Further research has to analyze the developmental processes underlying the characters involved. It is of course a sobering thought that we do not know how the mutants involved in the experiments described earlier realize their morphological effects nor what underlies the changes made by artificial selection.

There were promising starts some 30 years ago, for instance, the work of Forbes Robertson (47b, 47c) on the role of cell size and cell number in the

determination of body size in *Drosophila*. Another example is the work of Spickett (60a) on the changes in the development of sternopleural chaetae in which both pattern formation and the timing of differentiation of bristles were related. That timing cannot be neglected is indicated in the work of Poodry (39) who showed that there is a clear difference in the time of differentiation of anterior and posterior scutellar bristles.

Comparative analysis of patterns in different species as done by Garcia Bellido (21), studies on the genetic analysis with mutants changing complex patterns (6), and changing patterns by artificial selection (51, 52) can be important tools. We may hope that not too long from now we will be able to build a synthetic view from the building stones of quantitative genetics and developmental biology. Quantitative genetics will have to unravel their overall characters in components amenable for developmental and physiological analysis. Developmental biology will have to make the link between the theories of pattern formation (20, 36, 46) and the underlying molecular structures and processes (22).

This will ultimately give the basis for understanding the relation between constraints generated by inherent, structural properties of developmental processes and reversible developmental constraints as canalization built by natural selection (35).

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