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GENETICS AND EMBRYOLOGY[1]

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THE rapid rise of genetics has led to difficulties in relating its findings to those of embryology. The view has been expressed that progress in genetics is endangered by explorations in border fields. The problem has a general significance and seems to warrant discussion before a group of zoologists.

INBREEDING AND OUTBREEDING

In treating these difficulties it may be allowable to take a theory of one of the necessary conditions of evolution and to apply it to the analogous realm of scientific progress. Among the numerous constructive contributions of genetics to the discussion of the factors of evolution is the support it gives to the view that periods of isolation alternating with periods of intermingling among groups are necessary for progress. Inbreeding and outbreeding are both essential. New hereditary constitutions are swamped by outbreeding, and their recessive genes are incapable of expression under the condition of free contact within a large population.

With progressive isolation, small, homozygous, closely knit populations are produced. Each establishes its own clear and definite racial qualities. Many of the groups die out because of inherent weakness. Others have innate vigor and continue to flourish under isolation for a considerable period of time.

For the latter, two roads are open. If they continue in isolation their homozygous constitution leads to stagnation and their incapacity for rapid response to changing environments dooms them to extinction. If, on the other hand, after they have established themselves under isolation and are no longer in

[1] Address of the vice-president and chairman of Section F—Zoological Sciences, American Association for the Advancement of Science, presented at the Zoologists' dinner, Atlantic City, December 29, 1932.

danger of being swamped by outbreeding, they break through the barrier separating them from their neighbors, they give and receive valuable contributions. A great variety of new gene combinations becomes available. A new period of isolation may establish some of these as promising steps in further evolution. So, with alternating cycles of inbreeding and outbreeding, rapid evolution is made possible.

This theory may be extended to the analogous conditions of scientific progress. The enthusiasm of a group in a special field may lead to isolation. Isolation favors the development of clearly defined concepts and of well-organized programs of investigation along the lines prescribed by these concepts. This favorable result, however, may be followed by the development of routines of procedure, leading, if the isolation be long continued, to eventual stagnation and sterility of the field in question.

From this sad fate the special science may be rescued by the stimulus of excursions into neighboring realms. The explorers may bring back many "wild and unusual" data that refuse to be confined within the corrals of the defenders of the home territory. Much confusion results. The situation is "viewed with alarm" by the home guard. But from these outside contacts new concepts arise which gain precision and clear definition in a new era of organized investigation. Thus alternating periods of isolation and of contact favor scientific progress.

History of Genetics

Among the difficulties connected with the establishment of genetics were (1) the preoccupation of biologists with the interests and enthusiasms aroused by the publication of Darwin's "Origin of Species," (2) the introduction of a method of investigation unfamiliar to biologists, and (3) the inadequacy of knowledge of the processes of sexual reproduction.

Upon the rediscovery of Mendel's laws a small, compact body of active investigators extended his method to an ever-increasing number of species and to all details of somatic characteristics, including form, size, function and even behavior. The concept of stable units of heredity, or genes, was firmly established as a general law, and coordinately with it the law of segregation of the units of a pair in the formation of the gametes and the independent assortment of different pairs. Independent assortment was found to have exceptions. Linkage groups were recognized, and within a group there was discovered a certain percentage of breaking of the linkage, constant for any two pairs of genes. These percentages were shown to have a definite quantitative relation, such that the values in any linkage group could be arranged in linear order. And so, on and on, analyses of heredity data were made in terms of symbols, the genes, in a manner analogous to the atomic system of the chemists.

The investigators in genetics formed a compact body, the members of which had the same desire for demonstration of the universality of application of the gene concept. A very close inbreeding of ideas occurred, making the concept more and more well defined, unquestioned and all-sufficient. This isolation was of great importance from the standpoint of adequate development and demonstration of the gene concept. If the majority of geneticists had devoted their time from the very start to seeking points of contact with prevailing views in other biological fields, instead of devoting themselves to their knitting, the progress of the science of heredity might have been swallowed up in a morass of conflicting and imperfectly grounded speculations.

The next stage was not so profitable. Viewing with satisfaction the tremendous advance in "atomic explanation" of heredity and the ever-increasing number of individual cases to which it could be applied, geneticists tended to continue in isolation indefinitely on the ground of lack of sufficient data for an adequate approach to other biological fields. The fact that at its beginning the view of general significance of Mendel's results was a wild speculation, seemingly contrary to the bulk of previous observation, was forgotten. The need of stimulation by foreign ideas was lost sight of in the desire for retaining clearness of objective. In other words, the inbreeding was giving rise to a homozygous population of ideas, and genetics seemed destined to the stagnation and sterility of an exercise in gene sorting.

The Cytological Frontier

From this stagnaton genetics was rescued temporarily by the older and continuing developments in cytology. The contacts here illustrate both extremes of functional relationship.

In the one extreme, two separate fields of biological inquiry develop independently to such an extent that the observations in one fit naturally into a common system with the other. No test is necessary. No independent demonstration is needed. For example, the law of segregation of genes fits naturally into the cytological observation of the separation of the chromosomes of a pair during maturation.

In an intermediate situation, observations in one field seem so obviously to be explained by those in another, yet without direct confirmation, that after the relation between the two is suggested, the confirmatory test may be applied at once. Test follows suggestion inevitably. For example, the law of independent assortment of different pairs of genes suggests independent assortment of different pairs of chromosomes. But such assortment of chromosomes could not be demonstrated until marked pairs were available for cytological observation. (Explanation of linkage of genes as due to location within a single chromosome also followed as a natural consequence of this hypothesis.)

At the other extreme, daring hypotheses of relationship are necessary. They must be *working* hypotheses, recognized as capable of test and therefore stimulating the invention of devices for test. A good example is the daring speculation on the cytological basis of linkage breaks, the view that the percentage of breaks in linkage is explained by the percentage of crossing over between the members of a pair of chromosomes. Its natural sequel is the view of a linear order of gene-containing chromatin granules within a chromosome and the accompanying exercises in chromosome map construction. When first formulated this hypothesis certainly was a daring speculation, but being accepted as a working hypothesis it was capable of test and has been substantiated in many different ways. The stimulus it gave to constructive work in genetics does not need review before this audience.

The success of the first excursions into the cytological realm led to similar ones concerned with deficiencies, translocations and many other phenomena of genetics. Continued success led to practical obliteration of the frontier, and now the terminology of the older genetics is fused with that of cytology, and the old symbols for units of heredity have become parts of chromosomes. Genetics, having incorporated cytology into its realm in this manner, entered upon a new program of sorting and rearranging genes and groups of genes. In place of the algebraic symbols of the old genetics, there were substituted the chromatin granules and chromosomal rods of the new, and these can be sorted and rearranged even more readily than the old symbols. The enthusiastic endeavors and striking advances in this field are commonplaces of recent genetics.

The cross between genetics and cytology was fertile, and the new period of inbreeding in the united realm has standardized concepts and procedures. The concepts are morphological ones and have now reached sufficient maturity so that they may be safely outcrossed with physiological and embryological ones.

THE EMBRYOLOGICAL FRONTIER

The wide gap between the genes as postulated units of heredity and their somatic expression in the adult can be bridged only by observations of embryological events. As at other frontiers, besides independent cultivation of the two fields in the hope that some obvious connection will appear, there should be coordination of experimental procedures, including materials from both sides.

Genetics supplies a technique for controlling hereditary constitutions. Experimental embryology supplies a technique for control of internal and external environments. From the embryological side a comparison can be made of two reaction systems, alike in all respects but one, and that one a single gene or a single component of the environment. A program of procedure based upon this possibility should throw valuable light upon the nature of genes and upon the physiological processes initiated by them. Study of time, place and nature of the changes in relative rates of physiological processes is of the greatest importance. In such a study the succession of events in the regeneration of an organ is a valuable aid and often yields clearer results than does a study of the more complex transformations of normal embryology.

Genetics made early contacts with embryology in the field of dominance, but for a long time evaded the issue by means of the presence-and-absence hypothesis. Multiple allelomorphs, the multiple effects of a single gene difference, the multiple factors concerned in a single somatic character, the effects of deficiencies and numberless other observations suggest intimate functional relations with embryology.

As examples, two embryological investigations will be outlined: the first is far from the frontier and concerns some observations on the development of serpulid worms; the second is near the frontier and concerns some observations on the bar-eye series of multiple allelomorphs in Drosophila.

SERPULID DEVELOPMENT

In all genera of serpulids the worm secretes a calcareous tube. There are two semicircles of gills, one on each side of the head. Each gill has a central shaft with thread-like pinnules on each side and with a terminal thread. The gills have a food-collecting as well as a respiratory function.

In the adults of one group of genera, of which Protula is an example, all the gills retain this typical gill structure.

In the adults of a second group, of which Apomatus is an example, the second gill from the dorsal side in each row has a terminal enlargement in place of the terminal thread. This acts as a plug, or operculum, for closing the mouth of the tube. Both of these modified gills retain the lateral pinnules and continue to have a respiratory and food-collecting function as well as the opercular one.

In the adults of a third group of genera, of which Serpula is an example, in place of one of the second gills there is an operculum without gill function. Its stalk is without the lateral pinnules, and the terminal enlargement is a simple cup with a notched edge. In place of the other second gill, there is a bud of embryonic cells, the rudimentary operculum.

In the adults of the fourth group of genera, of which Hydroides is an example, the relations of the structures are like those of the third group, but the functional operculum is a much larger and more complicated organ, having its terminal enlargement made up of two distinct cups, each with its own spines.

In their individual life cycles members of the fourth, or Hydroides, group pass through definite stages resembling closely the adult stages of the other groups. Emphasis is to be laid on the fact that these stages are passed through after the worms have completed the egg and free-living larval stages and after they have entered upon the sedentary mode of life in a calcareous tube. In each stage the function of the organs of the opercular series is the same as that of their adult homologues. The stages are not the transitory phases of a construction process. Furthermore, one stage is not, in any obvious sense, a necessary mechanical step in the building of the succeeding one. In fact, the transformation involves a radical destruction of parts of the preceding stage and the construction of strikingly different new parts. In some of the steps of the transformation the whole old organ is destroyed and is replaced by a new one.

The process warrants further description of the development of Hydroides. Soon after the free-swimming larvae settle down and secrete a limy tube, they form a right and a left row of gills, of the first, or Protula, type, without opercular modifications. After several days in this condition, the cells at the base of the terminal thread of the second left gill divide rapidly, forming an opercular enlargement, and the terminal thread is absorbed, but the stalk retains its lateral respiratory pinnules. The organ now has the general plan of the second gill of the second, or Apomatus, type. The opercular swelling continues its growth and forms a single cup with a serrated edge. The lateral pinnules are absorbed and the organ is a functional operculum of the adult third, or Serpula, type. In the meantime, the second right gill stops its growth, its terminal thread and lateral pinnules shrivel, and the stalk drops off at a definite cleavage plane near its base. The cells of the basal stub proliferate to form a small bud of embryonic character. At this point the whole opercular system, right and left, resembles that of adults of the genus Serpula. After a few weeks in this condition, a time during which new gills are added at the ventral edge of each branchial circlet, the functional operculum, in the position of the second left gill, drops off at a basal cleavage plane. The embryonic bud at the position of the second right gill now enlarges rapidly and develops into an operculum with a complicated double cup of the fourth, or adult Hydroides, type. At the same time, a bud of embryonic cells, the new rudimentary operculum, is developed in place of the lost functional operculum of the third, or Serpula, type.

According to the findings of genetics, the gene complement of the cells involved in the development of the second gill, right or left, remains unchanged throughout the life cycle of Hydroides. It is stimulated to respond in one way at one time and in another way at another time, or the physiological processes initiated earlier in the life cycle and involved in the opercular modification, affect the cells in these positions in different ways at different times. That the structural differentiation at any stage is not merely part of a sequence of necessary events in the erection of the adult structure is evidenced by the fact that regeneration of the removed functional organ (whether gill without opercular modification, gill with opercular modification, Serpula type of operculum or Hydroides type of operculum) gives a replica of the removed organ *without recapitulatory stages*. Thus an organ may be repeated in duplicate by appropriate stimulation either of the basal cells of the removed organ or of the prepared embryonic bud of the opposite side. Transplantation technique should yield further important results.

A stage in such a series is not the natural developmental consequence of the immediately preceding type of development of an organ. The change is a deep-seated one, involving other parts of the body and concerned with the time at which the gene complex is stimulated to activity.

The same genes stimulated by the different physiological agents of different periods of the life cycle give strikingly different somatic results at a particular point in the individual. In the same way, two exactly similar gene complexes of two groups of cells located in different parts of the same individual may at *one time* respond in different ways because the stimulating agents differ. The problem of differentiation of the individual, therefore, is one involving both time and place, though the gene complex is postulated to remain constant and independent of time and place.

From the standpoint of the recapitulation theory in its old form, the embryological facts find a ready explanation in terms of the supposed phylogeny of Hydroides. The problem may, however, be stated in other terms. How can one hereditary constitution give a juvenile organ different from its own adult organ but similar to the adult organ resulting from another hereditary constitution? The answer is not apparent, but two considerations may have a bearing upon the question: First, the differences in the opercula of the adults of the four groups of genera are similar to differences resulting from gene mutations in other animals. Second, changes in physiological processes resulting from environmental factors have been shown in many cases to resemble changes in physiological processes resulting from gene mutation without corresponding environmental change. May we not be justified in formulating the *working* hypothesis that gene mutation produces change in gene reaction similar to that produced in physiological processes by environmental change? And further, may we not be justified in hunting here for clues to the nature of gene action and even to the nature of gene structure?

Why does a gene complex express itself at the site of the second gill in different ways at different times in the life cycle? A gill without opercular modification, a gill with opercular enlargement, an operculum of the Serpula type and an operculum of the Hydroides type—these are not stages in the development of the adult Hydroides structure; but, as regeneration studies show, they are characteristically different and independent expressions of a single genotype at one and the same location in the developing individual. Certainly a more intimate study of the developmental processes in ontogeny will throw some light on the nature of the gene differences at the basis of differences in adult somatic expression. Such a study should pay special attention to regeneration and grafting technique in forms differing in a single gene or in a single environmental factor when all other hereditary and environmental factors are controlled.

MULTIPLE ALLELOMORPHS

The second embryological investigation concerns the changes produced by a single gene mutation.

The different members of the bar-eye series of allelomorphs in Drosophila have characteristic differences in size of the eyes and in number of ommatidia as determined by facet counts. From the genetics side much is known of the normal rates of mutation, quantity and position effects within the gene or its immediate neighborhood, and acceleration of the rate of mutation under x-ray and radium treatment and at high temperatures.

The number of eye facets is strikingly affected by the temperature of the developing individual, and each mutant has its own temperature coefficient. In most of the members of the series the number of facets decreases with increase in temperature, but in infra-bar there is a reversal of the temperature effect. The characteristic adult somatic differences of several mutants thus may be simulated by one mutant when raised at different temperatures.

By appropriate transfers of larvae from one temperature to another at different times in different individuals, it is possible to locate in the life cycle the exact period during which temperature has a differential effect. This effective period comes in the third instar of the larva, has definite limits and is of short duration. The temperature of the developing individual at other times in its life cycle has no effect upon the number of facets in the adult eye. If we assume that the different physiological processes involved in the development of the eye have different temperature coefficients the differences in adult facet numbers of individuals raised at different temperatures are explained.

Changes in the bar-gene may be postulated to produce changes in the relative rates of the processes acting during the effective period and thus bring about changes in the temperature coefficients of the mutants and may even reverse the effect. A study of the nature of the difference in developmental processes produced by the different genes of such a series of mutations is likewise an avenue of approach to knowledge of the structure of the gene and the nature of gene action.

Since the circumstances connected with the production of the bar mutants make it possible to formulate a theory of difference between the genes of the series in terms of structural change, quantitative in some cases, and since the resulting somatic effects may be measured in terms of temperature effect upon an individual with unchanged genes, it seems worth while to postulate, for purposes of experimental test, theories of gene action in terms of relative rates of the physiological effects they produce.

CONCLUSION

In conclusion, let us return to the original thesis, of the need of recurring cycles of inbreeding and outbreeding for sound scientific progress. Daring explorations on the embryological frontier of genetics, long considered by many geneticists as wild and dangerous speculations, are gaining ground as legitimate enterprises. Some of the results of these explorations are receiving attention. Both genetics and embryology are profiting by the functional contact. Fears that the "organizer" of the embryologist might destroy the gene or that the gene might inactivate the "organizer" are turning out to be groundless. Both concepts have passed their immature periods in isolation; they are now mature, and the union is a fertile one. It will serve no good purpose to brand the offspring as illegitimate.