THE
AMERICAN NATURALIST

VOL. XLVI February, 1912 No. 542

SOME ASPECTS OF CYTOLOGY IN RELATION TO THE STUDY OF GENETICS[1]

PROFESSOR EDMUND B. WILSON
COLUMBIA UNIVERSITY

THE consideration of genetic problems from the standpoint of cytological research sometimes encounters a certain opposition or prejudice which seems to me to be due to a misunderstanding of the position that is actually held by many cytologists. It probably grows primarily out of a conviction that the heredity of particular traits is not to be explained by referring them to the operation of particular cell-elements or "determiners," but results from an activity of the whole cell-system, or of the whole organism, regarded as a unit. With this view, as will appear, I am essentially in agreement. In the second place, the opposition is a kind of reaction or protest against the theory of pangens or biophores and the too elaborate logical constructions that have been built upon it, especially by Weismann. I also consider this theory untenable, or at least unnecessary. I will therefore attempt to outline a point of view from which I think genetic problems may reasonably be regarded from the standpoint of the cytologist.

The most essential result of modern genetic inquiry I take to be the proof of the independence of the so-called

[1] A paper read before the American Society of Naturalists at the Princeton meeting, December 28, 1911.

"unit-characters"—that is to say, that they may be independently combined, disassociated and re-combined in many different ways. The independence of these characters often seems to be complete; more rarely it is limited by definite phenomena of "coupling" or "repulsion." The interesting facts recently brought to light by Bateson and Punnett in case of certain unit-characters in plants, and by Morgan in case of sex-limited characters in flies, demonstrate that coupling or repulsion, as exhibited in the F_2 generation, are a consequence of an original association or separation in the grandparental gametes. In the cases referred to, characters that enter the F_1 zygote in the same gamete tend to "couple" (remain in association) in the gametes produced by this generation; while if these same characters enter the F_1 zygote in different gametes they tend to "repel" each other (remain separate) in the ensuing gamete-formation. This is almost a proof that the factors for coupled characters are borne by a common vehicle or substratum in the germ-cell, while in repulsion they are borne by separate ones. Not alone such facts, but the whole history of unit-characters points unmistakably to the conclusion that they are in some way connected with material substances or bodies; and that it is the combinations, disassociations and recombinations of the latter that explain the corresponding behavior of the former. For example, in sex-limited heredity the peculiar linkage of certain unit-characters with sex becomes readily intelligible, as several writers have recently pointed out, if factors necessary for the production of these characters are associated in the same material body with a factor that plays a certain necessary rôle in the production of sex. In this particular case, as it happens, we are actually able to see a material body (the "X-chromosome") which undergoes precisely such a mode of distribution with respect to sex and sex-limited characters as is demanded by the hypothesis. The question must here be squarely faced, in a very real and concrete form, whether unit-characters are in fact dependent upon separate material bodies or substances, and whether the chromosomes can be regarded as such bodies or the carriers of such substances.

Without entering upon the evidence in detail I shall take it for granted that both these questions may be answered in the affirmative. Accepting this (if only for the sake of argument) how can such a conclusion be reconciled with the "action of the whole," and how formulated so as to escape the pangen-theory? The latter theory has fallen into discredit for two reasons. One is because of the quasi-metaphysical assumption that the "physical bases" or "determiners" of unit-characters are organized, self-propagating germs. Let us lay this assumption altogether aside as incapable of verification, and think of the "determiners" in a more vague way only as specific chemical entities of some kind. The second and more serious objection lies against the notion that the "determiners" are to be regarded as "bearers" of the corresponding characters. This is a fundamental error, as may be made clear, I think, by a specific illustration. It has been proved that many "unit-characters" are not units, but require for their production the cooperation of several factors, as is shown with especial clearness in the heredity of color. In such cases, as is now generally recognized, we should not speak of "unit-characters" but of unit-*factors*. Different "unit-characters" come into view as particular unit-factors are added to or subtracted from a given combination in the zygote. The factor for gray color in mice, to take a familiar example, operates by inducing a reaction of the germ that can only take place in the presence of several antecedent color-factors lower in the scale; and the latter, in turn, are only operative in the presence of still another factor that is necessary to the production of any color. The first and most obvious suggestion given by such facts is that what is added to or subtracted from a given combination or state of equilibrium in the zygote is some kind of chemical entity which induces a specific reaction of the germ sooner or later in its development. But beyond this it is perfectly evident that however far backwards we may follow such a series of unit-factors, at every stage they play their specific rôle only in so far as they form part of a still more general apparatus of ontogenetic reaction that is constituted by the organism as a whole. *The whole of this apparatus, the entire germinal complex, is directly or indirectly involved in the production of every character.* We find it convenient, indeed necessary, to treat particular factors of reaction (*i. e.,* the "determiners") as if they were concrete and separate things. Such, in fact, they may be, as already indicated; but when we speak of them as "bearers" of the corresponding characters, we are using a figure of speech that may be highly misleading. The reactions (characters) which they call forth are not "borne by" them. They appear as responses of the germinal organization operating as a unit-system; and it is to this system as a whole that every character belongs, or by which it is "borne"—if indeed we may permit ourselves to employ the latter expression at all.

The point of view thus indicated may, I think, be made entirely clear by a chemical illustration. A number of writers, among them Adami, Guyer and Kossel, have of late called attention to the parallel that may be drawn between the physical basis of heredity and the complex molecular groups of the proteins and other organic compounds. It is a most suggestive one, though it is not to be taken too literally—indeed I shall employ it only as a kind of allegory or illustrative fiction. No one can fail to be struck with the really remarkable analogy, in method and in results, between the procedure of modern genetic experiment and that of modern organic chemistry. Just as the qualities of a particular protein may be definitely altered by the addition, subtraction or the substitution one for another of particular side-chains or molecular "Bausteine," so the addition, subtraction or substitution of particular "determiners" or "factors" in the zygote calls forth specific responses that lead to the production of corresponding characters. The reasoning that applies to the first of these cases seems equally applicable to the second. No one, I suppose, would hold in the first case that the particular molecular groups or "Bausteine" concerned in the change are "bearers of" (*i. e.*, are alone responsible for) the resulting new qualities. The qualities of any protein, as Kossel has recently urged, belong to the molecule as a whole, and are not to be regarded as the sum of the qualities of its constituent "Bausteine." Why should we regard in a different light the "determiners" (chemical substances?) concerned in the second case? They are, clearly, not to be regarded as "bearers" or "physical bases" of the characters which depend upon their presence or absence. They are, I repeat, only differential factors of ontogenetic reactions that belong to the germ considered as a whole or unit-system.

In all this I am but expressing what I believe to be the point of view of many recent writers on genetic problems; but what I desire to emphasize is that the problems of cytology should be regarded from the same point of view. It is our task to see whether an apparatus of ontogenetic response can be discovered in the cell that fits with such a conception of the general process of determination. Is there cytological evidence of the existence in the germ-cell of such specific factors of reaction as I have referred to—in the nucleus, in the protoplasm, or in both? I think that observation and experiment alike have produced such evidence. Such experiments as those of Boveri on multipolar mitosis, and of Herbst and of Baltzer on the relations of the chromosomes in reciprocal crosses in sea-urchins have almost conclusively shown that the chromatin does in fact play a causative rôle in determination. Observation has gradually established the existence of a complex process of segregation and distribution of the nuclear materials in karyokinesis, maturation and fertilization, that shows a most striking parallel to that of the factors of determination. That a somewhat similar apparatus of distribution may exist also in the protoplasm of the cell is indicated both by recent observations on the chondriosomes, or plastosomes, and by earlier results on experimental embryology. If in the brief discussion that follows I confine myself to certain phenomena of the nucleus it is because the history of the chromatin is more fully and accurately known.

The progress of cytological inquiry tends steadily, I think, to sustain the view first clearly formulated by Wilhelm Roux that the nucleus contains many different substances which undergo orderly groupings and distributions in the karyokinetic phenomena. These processes are in some measure made visible to us in the formation of spireme-threads, in their history in cell-division, and in the still imperfectly understood but perfectly definite events of synapsis and reduction. In his well-known paper on the significance of the karyokinetic figure, published in 1883, Roux maintained that the nucleus is the seat of many different "qualities." He committed himself to no definite view as to what these "qualities" really are; but the implication is not far to seek that they have a chemico-physical basis and may be different chemical substances. On this general assumption he based his well-known interpretation of karyokinesis, of which the essential postulate was that the "qualities" (substances?) become arranged in linear series in the spireme-thread, and by longitudinal splitting of the thread may thus be equally divided (or otherwise definitely distributed) to the daughter-nuclei. I believe that many of the later advances of cytology lend additional support to this conception.

1. In the first place, the evidence gives strong ground for the conclusion that the chromosomes, to which the spireme-thread gives rise, are not homogeneous, but compound bodies. I do not here refer to the well-known fact

that the spireme-threads often consist of linear series of granules. I have in mind the fact that the number and size-relations of the chromosomes often differ materially as between different species, even nearly related ones, and that in at least one case (that of the X-chromosome) it is an established fact that a particular chromosome which in some species is a single body may be represented in other species by two or more components that sometimes show constant and characteristic differences of size. The natural interpretation of this fact is that the chromosomes are compound bodies, consisting of different constituents which undergo different modes of segregation in different species. We may here find a rational explanation, both of sex-limited heredity, as I have elsewhere indicated, and of other kinds of coupling.

2. Recent studies on karyokinesis and maturation emphasize anew the importance of the mitotic transformation of the chromatin-substance, and add weight to Roux's original interpretation of this phenomenon. Nothing in recent cytological research is more interesting than the discovery by Bonnevie, Pinney, Davis, and others that new chromosomes may arise within the old ones in the form of tightly coiled or convoluted threads, which uncoil or unravel to form separate spireme-threads. In karyokinetic division these threads may be formed already inside the telophase-chromosomes of the preceding division, as was discovered by Bonnevie. In other cases, an example of which is given by certain Orthoptera first reported upon by Miss Pinney, they are first visible in the early prophases, when they are seen uncoiling from massive bodies formed from the old chromosomes and equal in number to them. The same remarkable process occurs in the early auxocytes, as the chromosomes are preparing for conjugation in synapsis, as has been shown particularly by Davis, whose observations, like those of Pinney, I have recently been able to confirm and extend. In many insects the presynaptic spireme-threads do not arise, as has often been described in other

forms, directly from a chromatin-network. They arise from massive bodies, each of which resolves itself into a closely convoluted thread which then uncoils before conjugation takes place. Why should chromosomes that are already formed as massive bodies delay their division or conjugation until so remarkable a redistribution of their substance has taken place? It is not a necessary condition of conjugation, as is proved by the case of both the sex-chromosomes and the *m*-chromosomes, which do in fact conjugate in the massive condition. All the facts become intelligible in the light of Roux's hypothesis that the formation of the spireme-threads effects a linear alignment of different constituents in preparation either for division or for a definite type of association in pairs in synapsis.

One of the most interesting applications of this view to genetic phenomena is that suggested by Janssens in his theory of the "chiasmatype," which has recently been applied by Morgan to the explanation of coupling and repulsion. In the twisting together of the spireme threads, either in synapsis or at a succeeding stage, and the subsequent secondary splitting of the thread in one plane is provided a very simple mechanical basis, both for the free interchange of factors between the homologous chromosomes and for the phenomena of coupling or repulsion, which are otherwise so difficult to comprehend. I do not maintain that this particular interpretation, or the more general one of Roux, is demonstrably true, or that no other explanation can be found. I only hold that they are legitimate conceptions which may be tested by observation and experiment, and which must be fully reckoned with as intelligible interpretations of the facts before we can set these facts aside as utterly mysterious or as a meaningless by-play.

3. I would lastly recall the experimental proof by Boveri that the chromosomes differ among themselves in their physiological relation to development, and the corresponding cytological fact that they differ among them-

selves also in respect to size, behavior, or both. In one case only has it thus far been possible to demonstrate a constant relation between particular chromosomes and particular characters, namely in the case of sex and sex-limited characters. It is true that in this case we are not able to assert that the sex-chromosomes are the primary determining cause of sex—indeed, there seems to be good evidence to the contrary. Unless, however, we are prepared to defend the proposition that the sex-chromosomes are absolutely functionless we shall not, I think, escape the conclusion that they form one of the factors in sex-heredity.

I will not enter upon the analytical subtleties of the problem whether the chromosomes, or the substances that they contain, are permanent and self-propagating elements or are merely temporary products of an unseen underlying activity—whether they are causes, effects or mere accompaniments of the specific reactions with which they are somehow connected. These are fundamental questions; and some of them can not yet be answered. But we should not hesitate to adopt what seems likely to be for the time the most reasonable and fruitful working view. "Hypotheses," said Pasteur, "come into our laboratories by armfuls; they fill our registers with projected experiments, they stimulate us to research—and that is all." In my view studies in this field are at the present time most likely to be advanced by adopting the comparatively simple hypothesis that the nuclear substances are actual factors of reaction by virtue of their specific chemical properties; and I think that it has already helped us to gain a clearer view of some of the most puzzling problems of genetics. But even if we adopt the opposite view that the formation, segregation and distribution of these substances are only signs or indices of what lies behind them, we still have in this direction one of the most promising paths of approach to a study of the activities of the germ-cells in heredity.

It will perhaps be said that such conceptions of the nuclear organization as have here been indicated are both vague and artificial. Vague and crude they undoubtedly are; and so they will remain until we have far more thoroughly explored a field of inquiry in which we must for the present make a shift with crude ideas unless we are content to work with no ideas at all. They may be artificial too; but it appears to me that in this respect they differ only in degree from the graphic formulas of structural chemistry. The chemist does not hesitate to picture definite topographical or spatial relations in the complex organic molecule—symmetrical and asymmetrical forms, cyclic or ring-formations, linear series, side-chains and other such graphic constructions. It is by their use that the whole science of organic chemistry has been built up, and that such men as Emil Fischer and Kossel have made nearly all of their advances in our knowledge of those most complex of known organic compounds the proteins. And these constructions are regarded by eminent investigators as something more than mere figurative expressions or symbols. They are taken more literally as representations or models—rude, no doubt, but as far as they go real—of the actual arrangements in space of the various molecular groups or protein "Bausteine." If therefore observation and experiment lead the cytologist to postulate definite topographical relations among the nuclear substances, and if such a conception help him to explain the results of genetic studies, he finds himself in good company, even though his present clumsy notions regarding the nuclear organization can as yet make no approach to the exact and elegant constructions of the chemist.

The essential conclusion that is indicated by cytological study of the nuclear substance is that it is an aggregate of many different chemical components, which do not constitute a mere mechanical mixture, but a complex organic system, and which undergo perfectly ordered processes of segregation and distribution in the cycle of cell-life. That these substances play some definite rôle in determination is not a mere assumption, but a conclusion based upon direct cytological experiment, and one that finds support in the results of modern chemical research. Professor Kossel has recently said that every peculiarity of the species and every occurrence affecting the individual may be indicated by special combinations of protein "Bausteine." The point of view that has here been indicated is entirely in accordance with such a conception. The results of cytological inquiry fit with the view that there are many such combinations in both nucleus and protoplasm; and the interest of cytological study lies in the fact that we can in some degree follow out their modes of segregation and distribution with the microscope. We are still utterly ignorant as to how these processes are determined; and the more one studies them the more one's wonder grows. I would certainly be one of the last to disparage the brilliant results that have been attained through the prolonged and patient labors of cytological observation and experiment. They stand, I believe, among the most interesting and valuable achievements of modern biology. But these studies have as yet made no approach to their limit, and a vast unexplored field still lies before us. We may as well recognize the fact that our present rude notions of cell-organization have not yet progressed very far beyond the paleolithic stage of culture; but they are of use in so far as they help to open new points of view or to discover new facts, whether in cytologic or in genetic inquiry. It seems to me that in both regards they have already proved worth while.