Three Quarter-Centuries of Cytology

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IT IS ALWAYS DIFFICULT TO MAKE a fair comparison of the various periods that mark the development of any field of science. The pioneers who open up a virgin field are often endowed with abilities of a kind different from those characterizing the men who later develop that field. Nevertheless, most scientists will come to think of a certain period as the glorious one, of another as marking time or decline, and so on. Such a differentiation into periods is especially easy in the history of cytology, but, as elsewhere, the evaluation of these periods is definitely a matter of argument.

The history of cytology falls rather naturally into quarter-centuries. Every biologist is familiar with the first of these, which began about 1875 and terminated in 1900. This is the period of breath-taking discoveries and great expectations in which the names of Strasburger, van Beneden, the two Hertwigs, Boveri, and many others are associated with the first real recognition of the mitotic apparatus, fertilization, chromosomes, chondriosomes, gamete formation, and early embryology. It was an exciting period, pregnant with the conviction that we were on the verge of uncovering the secrets of the life processes and of life itself, and it was also a time in which there was some bitter controversy about questions of priority and ethics. There was, in any case, no doubt that cytology represented an independent field of research which was at the very spearpoint of progress of biological advance.

Though the various cytological researches into mitosis, fertilization, parthenogenesis, and embryology were continued triumphantly into the second quarter-century, the feeling that the answers to all our large questions lay almost in our grasp gradually weakened. The new and most striking development in cytology during the years from 1900 to 1925 lay in its alliance with genetics. These two disciplines started out on an almost equal footing as far as the new study of heredity was concerned, for the rediscovery of Mendel's findings at the turn of the century was followed within a year or so by Sutton's and Boveri's cytological demonstrations that Mendel's laws are closely tied to the chromosomes. The acceptance of that relationship was not an immediate one. It was only after the correlation between sex determination and sex chromosomes had been established by McClung, Wilson, and Stevens, after the convincing evidence carried by Bridges' nondisjunction experiments had been comprehended, after Carother's demonstration of random assortment had admitted of no alternative answer, and finally, after the accumulation of genetic discoveries had become really overwhelming, that the general biologist resisted no longer. These years seemed to mark the closest cooperation between cytology and genetics, as has often been stated. But toward the end of this quarter-century it appeared to the insider that the cytologist was not keeping pace with the incredibly rapid advance of his co-worker.

This dropping behind of the cytologist merits a closer examination. It must first be realized that the findings of the geneticist were usually capable of standing alone on their mathematical basis. He recognized, however, that cytology might often give him a lead to new discoveries and that his arguments could be made more convincing to biologists in general if he could say: "There can be no doubt about the correctness of my conclusions. You can actually see the mechanism at work under the microscope." Moreover, there were cases where the genetic analysis alone gave no final answer, and then the appeal to cytology was in the nature of a request for aid. Willy-nilly, the cytologist thus came to play the secondary role of the supporter, and in this role he was often expected to furnish the capstone that made the proof unassailable (again witness Bridges' cytological demonstration of nondisjunction). His prime virtue thus lay in utter reliability, and the responsibility for this began to weigh heavily upon him; he became extremely cautious, he guarded and double guarded each of his forward steps, and in his hesitation finally to commit himself he became the archdisciple of Ruskin's characterization: "In science you must not talk before you know."

It was part of this attitude of caution that also made the cytologist accept wholeheartedly Bateson's advice to "treasure your exceptions," for to him all exceptional and aberrant cases were natural experiments that constituted tests of the generalizations that he was making. When the cytologist could not get at the bottom of one of these exceptional cases, and when no soundly based explanation was available, he care-

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fully described it and filed it away in one of the scientific periodicals—somewhat in the way that valuable but useless articles are stored in the attic. Finally, the spirit of criticism was very strong within him, and any new finding or idea by one of his co-workers was immediately subjected to the most painstaking examination. It all tended to hinder progress, though, as a matter of fact, the groundwork for much future advance was being laid at this time. But it must be admitted that to the geneticist much of it was of no immediate use. Also, the cytologist gradually lost much of his value as a path-breaker and often seemed to be in the position of the doubting Thomas who did little to help the cause along.

An illustration of this position is shown in the matter of crossing over. The geneticist's evidence that some kind of interchange may occur between homologous chromosomes was quite clear, and Janssens had shown that chiasmata might well represent direct visual evidence for such a process. Rightly enough, the cytologists did not consider Janssens' evidence as conclusive, but they could not bring forward anything better on this highly important question. It was not until 20-odd years later that Stern, and Creighton and McClintock, furnished the much-needed proof. During all these years the cytologist sat rather uncomfortably and merely shook his head.

The close of this second quarter-century in 1925 was signalized by the publication of Wilson's great work on the cell. This book shows very well the conditions then obtaining in cytology. There had been many great advances, but there also had accumulated a host of pertinent questions which had no answers, as well as a great number of findings which seemed to have no application anywhere. The volume of all this was truly immense and had assumed such proportions that it has often been said that Wilson was perhaps the last man really to encompass all of it.

The beginning of the third quarter-century is marked almost as sharply as is the end of the second. It was Belling who ushered in this, our present, period, and his real claim to fame was his recognition of the experimental value of extra, or supernumerary, chromosomes in the analysis of meiotic phenomena. His skillful elucidation of chromosomal behavior (for which Blakeslee was furnishing the genetic background) opened up avenues, the vista of which no one before had fully appreciated. To be sure, both Wilson and Bridges had dealt with heteroploid conditions, but not with any intention of analyzing chromosome mechanics as such. The unique advantage of heteroploidy to the experimentalist lies in the fact that it usually does not involve detrimental effects on the life of the cell such as nearly always are the concomitants of surgical intrusions or physicochemical treatments; as Bleier has said, "probably there is no more harmless and elegant a method of intrusion into the nucleus." In Belling's recognition of the experimental value of heteroploidy and polyploidy lay the germ of most of the advances that have been made during recent years.

However, not many cytologists and very few geneticists recognized the significance of Belling's contributions at once. In his work Belling was adhering strictly to the traditional principles of cytology, which meant that his advances were made cautiously and therefore slowly. There was no immediate prospect that he would attack the growing mass of cytological data to make it available to geneticists or noncytologists in general. Given time, he might have done so, but his death in 1933 terminated his activities much too soon.

In 1929, however, two joint papers on heteroploidy in certain plants were published by Newton and Darlington. These heralded the advent of a new departure in cytology—a departure which was exactly what most geneticists had been waiting for. Since Newton died before these papers were published, it was Darlington who was mainly responsible for the generalizations and who went on to develop the "New Cytology." This had its first general expression in 1932 in his *Recent advances in cytology*.

Not often has a single man made such an impression on old established fields of science as has Darlington on cytology and genetics. Under the impact of his attack the unwieldy and undigested mass of cytological data seemed to dissolve and reprecipitate as a usable system. Viewing this change, the geneticist gained a new sense of power and once more began to look on cytology as an aid to his researches and something that he could himself employ. His change of attitude was reflected in various ways. Nearly all of the younger geneticists began to add cytological methods to those which were traditionally genetic, and even the older workers frequently did so. In short, these geneticists became cytogeneticists. Generally, to be sure, the make-up of such a cytogeneticist was still largely genetical—say, to the extent of 80 or 90%—but a portion of him was definitely cytological, and he tackled his problems with a buoyancy, a fresh courage, that had been lacking for some years. The change is shown in various ways. At the Christmas meetings of the American Genetics Society in 1929, before this revolution, 5 out of the 39 papers presented (*i.e.* 13%) involved some cytological work on the part of the author. By 1940, 11 years later, the papers which employed cytological methods in part had increased to 52% of the total. In other words, during this significant decade the percentage had quadrupled, and it is safe to say that many among the remaining papers employed some cytological reasoning even when they did not actually use a compound microscope in solving their problems. How did Darlington—for certainly his was the predominant influence in this change—bring this about?

To comprehend this, it must be realized that the change involved not merely the fashioning of new and ingenious tools of reasoning, but also rather fundamental changes of working principle and viewpoint. When stated on paper, these changes do not seem so exciting, but it must be remembered that they affected the fundamentals of a field that had a great tradition and much historic weight. Darlington, who with rare penetration had made an analysis of the needs of the geneticist and the difficulties of the cytologist, said in effect: "Let us cut the Gordian knot and reduce this mass of cytological information to what is essential, always keeping in mind the basic conclusions that have been reached through genetic procedure. We can ignore the remainder for the present, for the seeming contradictions will fall into line as time goes on. And, contrary to Ruskin, let us not be afraid of making our hypotheses public." Through this process of winnowing out the worth-while material, Darlington arrived at a very few basic generalizations, simple almost to the point of naiveté, but of immediate utility in nearly all genetic considerations.

The first and most fundamental of these generalizations is simply that, at a certain point in meiotic prophase, homologous chromosome regions attract each other in pairs, and only in pairs. If another, or third, homologous region is present in the nucleus, it will not be attracted to the region where such pairing has already occurred; in fact, it will even be repelled. But this rule applies not only to whole chromosomes but also to the longitudinal subdivisions of chromosomes. Thus, if a chromosome is already split (*i.e.* composed of two chromatids), the affinity for an association in pairs is thereby satisfied, and no other homologue is attracted at all.

It may be said that this was not new and that, for instance, Wilson and Morgan had carefully considered the rules of synapsis many years before. But they had not considered the various possibilities of numerical variations, and what would happen if a third homologue is present had not really entered their considerations. In this lay a most important key to the explanation of chromosome behavior.

The second rule is almost but not quite as basic. According to Darlington, after the homologous, single chromosomes have joined in pairs during meiosis, each one splits. At about this time there may occur an interchange between two of the four resulting chromatids. The tendency of the four chromatids is to repel one pair from the other, but if the interchange has taken place between chromatids of two different chromosomes, a bridge or chiasma will prevent this separation. A chiasma is therefore visual evidence of one of the most important processes in evolutionary and meiotic mechanics, and the presence of crosses, rings, and similar configurations involving four chromatids is sufficient evidence that this important process—that is, crossing over—has occurred.

Darlington's elaboration of these two basic rules and their employment in the solution of many cytological questions of long standing were at all times resourceful and occasionally brilliant. Thus, the factors that bring about meiosis, the mechanics of mitosis, the structure and function of the kinetochore, and many more problems were explained on the basis of these fundamental generalizations. Indeed, no question came up that did not receive an explanation of some sort, and these answers were given with superb confidence and authority.

The geneticists accepted the help given them by the "new cytology" with appropriate thankfulness. This is reflected very nicely in the textbooks that have been written since 1932. Without exception, all of these books utilizing cytogenetics regard Darlington's rules as a constituent part of the science, and it is very rare indeed that even a hesitation is expressed over accepting his conclusions. This represents very fairly the attitude of the cytogeneticists and geneticists in general, and there can be no doubt, as I have already said, that the effect was an inspiring one.

However, that is not to say that there were not some exceptions among the cytogeneticists who did not use these tempting tools quite so uncritically and who retained a certain concern over the underlying verities; after all, there is a McClintock. There was also still a group of cytologists who, true to their basic and, if you wish, old-fashioned principles, did not accept these generalizations of the "new cytology" so wholeheartedly. As might be expected, they objected, first, that in selecting the data that supported each generalization, much pertinent information had been ignored because it was contrary to the hypothesis arrived at. The very last publication of Belling, in 1933, has some 25 pages of objections to various statements and conclusions reached in Darlington's book, and the reviews written by some other cytologists expressed similar dissatisfaction even while voicing admiration for the boldness and ingeniousness of the reasoning.

These objections have not diminished in the years since 1932, at least as far as the cytologists are concerned. Whenever they are specific, they can certainly not be ignored, and I will take time to outline two of them because they affect the very basis of the whole structure:

(1) I have already emphasized the most basic of these rules, the one that in some form or fashion underlies all of the others, to wit: it is the split and unsplit conditions of the chromosome that determine its behavior with respect to homologous chromosomes also present in the nucleus. Although such a generalization does not explain what the underlying forces may be, merely to establish the existence of such a numerical rule of behavior is immensely important. Aside from its immediate applicability to genetic reasoning, it would seem to point out the direction which the final attack on this all-important question in cellular mechanics must take.

The trouble is that almost all cytologists, except for Darlington and his followers, are now convinced that the factual findings are erroneous. Each chromosome is already split in telophase—that is, it is then composed of two chromatids, and the evidence is mounting that there is even a subdivision into half chromatids. In short, unless there is then some mysterious healing just at the time of synapsis, we are dealing with two or four parted chromosomes instead of single, unsplit ones. It has been suggested that the situation can be saved by considering such chromosomes to be "physiologically single." Although this may be a legitimate restatement of the actual conditions, it cannot, however, hide the fact that the numerical generalization which seemed to open the way to the final solution has irretrievably been lost. It is not the singleness of a chromosome which underlies its attraction to another single homologue, and, as Huskins states it, "the general problem now appears to be not to what degree the chromosome is subdivided, but how it comes to behave as a bipartite unit in inheritance."

(2) The second generalization, only less important than the first, that the chiasma represents an interchange between two chromosomes and that it serves to hold such chromosomes together even when they are split, still is valid as far as it goes. But, to the cytologist, it is a matter of importance that there are other forces as well which must be considered in the formation of tetrads, ranging from terminal and somatic attraction to heterochromatin, collochores, and other, yet unexplained, mechanisms. Indeed, in some cases such alternative mechanisms seem to have entirely replaced the chiasma, which can therefore no longer be considered the sine qua non in tetrad formation. Darlington himself now recognizes the existence of several of these additional factors, though his co-workers have not always done so, and the identification of chiasmata by cytogeneticists in general has not always been above cavil.

These criticisms of the two fundamental generalizations, of course, also affect the more subsidiary ones. Suffice it to say here that much of the foundation on which modern cytogenetics has been erected is in need of reconstruction—although this may be possible without destroying all of the superstructure that the cytogeneticist has erected on it. It should here be pointed out that the cytologist's task of examining this foundation was at times a thankless one, for he played the role of the man who pokes sticks into the wheels of a nicely rolling vehicle. Furthermore, he was free to admit that his own accumulation of unexplained facts had begun to hinder progress, and that the formulation of generalizations at least set up targets at which to shoot. His complaint was that the cytogeneticists as a group never shot at them, and possibly they can not be blamed too much for that. They were busy enough as it was, and one can do just so much in one's working day. However, it seems not too much to ask that they recognize the necessity for such a testing of the rules that have been set up, and that the cytologist for whom the analysis of cell structure and function constitutes the primary interest is duty bound to make such tests. I fear that the cytogeneticist has not always recognized that, when the cytologist is remiss in this respect, both disciplines will suffer in the end, by either a slowing of progress or waste of effort. Let me illustrate:

The salivary chromosomes represent somatically paired homologues of huge size, which are strikingly marked by transverse bands, individually recognizable. These bands are correlated in position with the loci of genes. The recognition of these general facts by Painter, Heitz, and Bauer has given us one of the most useful tools imaginable, and its employment by the student of evolution has opened up paths that have resulted in really great advances in that old field of endeavor. But, oddly enough, today—14 years after the great utility of the salivary chromosomes was first recognized—we still do not know their exact nature, and we are still puzzled about the origin and structure of the transverse bands. There is no denying that the answers to these problems are not there for the mere picking up; nevertheless, it is remarkable that scores, or even hundreds, of workers have used the salivary chromosomes as a tool in their work, whereas a scant half dozen or so have seriously concerned themselves with their more basic aspects. Our ignorance is now beginning to prove a hindrance, and the question arises of why more work has not been done to remove it. I think the answer lies in the fact that the question involved is primarily cytological and that the ranks of the cytologists have become so thinned out that there are not enough to do all the work.

Here is a second illustration: The male of *Drosophila* has no crossing over, and in conformity to the correlation between crossing over and chiasmata, the autosomal bivalents show no chiasmata. However, the x and y chromosomes enter into a combination that results in a normal, cross-shaped tetrad. Since on the Darlington hypothesis this must mean that a chiasma is involved, why is there no genetic evidence of crossing over? Darlington's explanation is characteristically ingenious. Crossing over does indeed occur in the sex chromosomes, but it is always restricted to a single region which is devoid of active genes. Further, since a single cross over would interchange an arm of the x with an arm of the y—which certainly does not occur—he postulated that there is always a reciprocal cross over, which means that in the immediate vicinity of the first there is another one which brings both arms of the x (and y) together again. An apparent exception to his basic rule is thus shown to constitute a striking confirmation. But this explanation, as Cooper has recently shown, involves three major assumptions as well as eight steps in the cytological argument which admit of alternative explanations. Undeniably, the hypothesis is clever, but the cytologist of 25 years ago would never have accepted it as it stands. The "new cytology" presents a striking contrast to that older attitude, for in the 10 years prior to Cooper's analysis no one seriously questioned Darlington's explanation, and at least half a dozen cytogeneticists undertook extensive investigations which were based on a full acceptance of all of its points. If the moral is not obvious, it must at least be recognized that the waste of effort that results from such a state of affairs may well be very considerable.

I have said enough to portray the status of cytology during the years since 1925. It is a period dominated by bold generalizations which have induced a vast amount of research with some splendid results, chiefly by the cytogeneticists. But work on the fundamental questions to which we must all return sooner or later has not progressed so satisfactorily, and the relatively small group of cytologists that have kept it in mind have not been rewarded with much appreciation. As I have already noted, that is largely due to the mild contempt with which other workers have come to regard the painstaking principles of the cytology of former years. But, in view of the weaknesses that I have pointed out, I would venture the opinion that a judicious return to some of those principles would exert a decidedly wholesome influence in the present day. If the free and untrammeled making of speculations and hypotheses continues, these will gradually encumber our movements as much as did the piling up of unsolved cytological puzzles in former years. Destructive criticism, if constructive cannot be had, had better not be removed from the cytological arsenal; the restless questioning of a Belling and the coldly analytical mind of a Belar have been sorely missed during the past 15 years. Nor can we afford to hold our noses quite so high as we encounter the aberrant cases that trouble our beautiful generalizations; after all, in the cytology of *Drosophila* itself there is much that does not conform to what we have set up as the standard course of events.

It is taking no great risk to predict that 1950 will mark the end of a period, just as sharply as did 1925 and 1900. Whether he is pleased or not, the cytologist of the next quarter-century will find his co-workers in the laboratories of the biochemist and biophysicist—if the handwriting that workers like Caspersson and Mirsky have put on the wall means anything at all. Moreover, it is already obvious that in this newer alliance the cytologist will not play the subsidiary role that he has had in the recent past, for it is on the foundations constructed by him that this building of the future will arise.