CHAPTER 12

CYTOLOGICAL MAPS AND THE CYTOLOGY OF CROSSING OVER

Rearrangements of the chromosomal material were first detected in Drosophila by genetic methods. Deficiencies were reported by Bridges (1917) and by Mohr (1919), duplication by Bridges (1919), translocation by Bridges (1923), and inversion by Sturtevant (1926). These were all of spontaneous occurrence, and none of them were cytologically identifiable by the methods then available.

The first successful cytological attempt to analyze the chromosomes from a genetic point of view, in terms of units less than a whole chromosome, was made by Belling. By 1924, Belling and Blakeslee reported a series of extra-chromosome types in Datura. In this paper they developed the idea that the meiotic pairing of chromosomes, even at a late stage (diakinesis), could be used to determine the homologies of separate arms. They described "secondary trisomics," in which the extra chromosome was made up of two like arms of a normal chromosome, presumably having arisen by a somatic division that was normal except that the centromere had divided transversely to the long axis of the chromosome instead of parallel to that axis. These types gave evidence as to the phenotypic effects of the separate arms. Unfortunately, however, there were few mutant genes available in the plant, and the more critical earlier stages (pachytene) of meiosis were not favorable for study in Datura.

A different attempt to get cytological information on the genetic composition of individual chromosomes was developed for maize by McClintock, Randolph, Longley, and others. Here it was possible to study the pairing of homologous chromosomes at pachytene, when they were longer and showed more recognizable detail than at the later stages

chiefly studied by Belling. A large series of mutant genes was known, with linkage maps well understood, and a series of chromosome rearrangements was collected. This was the most hopeful material for a detailed correlation between cytologically visible structures and linkage maps—until the development of the salivary gland chromosome technique for Drosophila in 1933 (see p. 75).

In Drosophila, the first evidence relating a cytologically visible structure to a linkage map was Anderson's proof (1925) that the centromere end of the X is the right end of the linkage map—as pointed out in Chapter 8.

Muller's original report on the mutagenic effects of X rays (1927) stated that he had also recovered "a high proportion of changes in the linear order of the genes." The presence of rearrangements was confirmed by cytological studies (Painter and Muller, 1929). They showed, especially by a study of long deletions, that the genetical map of the X, based on crossing-over frequencies, does not correspond with the intervals measured on the metaphase chromosomes—although the sequential order is mutually consistent. By 1931 they showed that a large section of the right (centromere) end of the X is "inert" (contains very few genes) and in 1930 produced a "cytological map" of the X.

Dobzhansky (1929, 1930) studied X-ray-induced translocations involving the second and the third chromosomes with the small fourth. The positions of the break-points were determined cytologically, and also genetically, by determining the apparent locus of a fourth-chromosome gene (eyeless) on the maps of the longer chromosomes. There resulted cytological maps of these chromosomes, which were consistent with the sequences of loci on the genetical maps but showed that, as Muller and Painter found for the X, the intervals were not proportional. That is, there were relatively long sections with relatively little crossing over, and relatively short ones with much crossing over.

In 1931 two papers that appeared independently demonstrated that recombinants arising from genetic crossing over are accompanied by exchange of cytologically visible markers. The first of these, by Creighton and McClintock, utilized a translocation and a "knob" (heterochromatic end) in maize; the second, by Stem, utilized an X of Drosophila with an arm of Y attached to its right end, and an X-IV translocation. In both cases, two marker genes between the cytologically identifiable regions were available, and it was demonstrated that recombination between the marker genes was regularly accompanied by recombination between the cytological markers. These papers gave the final cytological proof that

genetic crossing over is accompanied by an exchange of parts between chromosomes.

The metaphase chromosomes of Drosophila are very small and show little structural detail. The use of brain cells, introduced by Frolowa (1926), gave somewhat larger figures than the previously studied oögonial cells, but the breakpoints were still only approximately identifiable. No cytological analysis was possible for short deficiencies or duplications, for inversions within a single chromosome arm, or for translocations involving exchanges of nearly equal parts.

This was radically changed with the advent of the salivary gland chromosome analysis. The existence of large, banded strands in the salivary gland nuclei of Chironomus larvae was recorded by Balbiani in 1881, and this condition in the salivary glands, Malpighian tubes, and in some cells of the gut of several groups of Diptera was studied by several authors after that date. The condition was observed in living, intact larvae and was also studied in fixed and stained sections. The usual interpretation was that these strands formed a continuous spireme, with only two free ends. Only in 1933 (January) was this shown to be incorrect, when Heitz and Bauer studied the Malpighian tube cells of Bibio by the squash technique instead of sections. Pressure spread the threads, and they were able to show that there was a definite number of distinct worm-like bodies, tangled in an unanalyzable mass in the living cells or in sectioned material, but separate and countable in their squash preparations. They further found that the number of bodies was the haploid one, and that the relative sizes were like those of the metaphase chromosomes. They concluded that each of the worm-like bodies was a closely conjugated pair of homologous chromosomes,* and they also pointed out that each of them had a characteristic banding pattern and characteristic ends, recognizable from cell to cell and from one larva to another one.

Heitz had previously (1928) shown that in the liverwort Pellia there are heterochromatic regions in the chromosomes at somatic divisions, and that these tend to aggregate into a common chromocenter in resting stages. In 1933 (December) he showed that similar relations are to be found in Drosophila—specifically, that much of the basal region of the X is heterochromatic (a result which he correlated with the "inert" region of Muller and Painter), and that there is a common chromocenter to which the salivary gland chromosomes are attached. However, he found the salivaries difficult to study and did not carry his analysis very far.

* It had already been shown, by Stevens and by Metz, that homologous chromosomes of Diptera usually show "somatic pairing" at ordinary somatic divisions.

In the same month (December, 1933) there appeared Painter's account of the salivary gland chromosomes of *Drosophila melanogaster*, which he showed were quite workable, if one studied old larvae nearly ready to pupate. In this paper he presented a drawing of the euchromatic part of the X, with over 150 bands, and with 13 corresponding points shown, that had been determined both cytologically and genetically from a long deletion, seven translocations, and two inversions (one of the latter being the familiar ClB). Here at last was a detailed correspondence in sequence between the crossover map and cytologically visible landmarks, and a technique that was clearly capable of refinement to give the precise loci of genes in terms of recognizable bands. Instead of two or three landmarks per chromosome (the ends and centromeres), there were now hundreds, and there soon came to be thousands for the whole complex.

There followed a series of studies in several laboratories, which rapidly gave more and more detailed cytological maps of all the chromosomes, both of melanogaster and of other species. In the case of melanogaster, where the available genetic data were much more extensive, the detailed studies of Bridges were especially useful, and his drawings of the salivary gland chromosomes of that species (1935, 1938, 1939) are still the standards.

In 1935 Bridges recognized 725 bands for the X chromosome, 1320 for the second, 1450 for the third, and 45 for the fourth. In 1938 the number for the X was raised to 1024, and in 1939, the number for the right limb of the second was raised from 660 to 1136. He recognized that even these numbers did not exhaust the potential resolving power of the method. Bridges also developed a convenient system for designating each band—a system that is still in use.

An early result of the salivary gland studies was the discovery of *repeats* by Bridges (1935) in Drosophila and by Metz (1938) in Sciara. These repeats were shown to be either "direct" or "reverse" in their orientation with respect to each other, and to be either adjacent or separated by other regions. Their origin is not altogether clear, but their frequent occurrence is, as Bridges pointed out, of considerable evolutionary interest, since they furnish extra genes that are presumably not needed by the organism, and that may be of importance in making possible the origin of new genes with new functions.

Another result of these studies, recently found to be of great interest, is that of the "puffing" of certain regions. It was shown by Metz (1938) that certain regions of the salivary gland chromosomes undergo a reversible process in which the bands swell and show a much looser struc-

ture. Pavan (1952) found that in Rhynchosciara this is a regular phenomenon, particular bands undergoing puffing at specific developmental stages.

This has been fully confirmed by Rudkin, Beermann, and others, and the subject is currently being actively studied—especially by Beermann and his co-workers—because of its bearing on questions relating to the timing of gene action in development.

The original interpretation of chiasmata by Janssens (1909) rested on the assumption that the initial separation of the four strands involved in a tetrad was always, at every level, such that two sister chromatids remained together in each of the separating areas. On this basis there is a one-to-one correspondence between a visible chiasma and a genetic exchange; at such a visible chiasma two of the four chromatids have undergone a crossing over, and these two are nonsister chromatids.

This assumption was not proven then, as was soon pointed out by Robertson (1916) and others—and it has still not been proven. It may be that, at some levels in a tetrad, the initial separation does not separate one pair of sisters from the other (reductional separation), as Janssens supposed, but is equational, separating two nonsisters from the other two (also nonsister) chromatids. Otherwise stated, it may be that if one visualizes the four chromatids as straight untwisted rods, the initial two-by-two separation may occur in either of the two geometrically possible planes. If this assumption is accepted, then there is no necessary relation between visible chiasmata and genetic crossing over. As Wilson put it in 1925:

> To the author, all seems to point to the conclusion that the mechanism of crossing-over must be sought in the pachytene stage during the period following synapsis. . . . The genetic evidence . . . leads almost inevitably to the conclusion that crossing-over must involve some process of torsion and subsequent splitting apart . . . but we must admit that on its cytological side the problem still remains unsolved.

The cytological study of the meiotic process was actively prosecuted at about this period, in an attempt to see what really happened at crossing over. Among the numerous workers then, perhaps the most important was Belling, who studied more especially plants of the lily and related families. From 1926 to 1931 he published several "working hypotheses," based on the assumption of random breaking of the thin, paired chromosome strands, with reunion of the broken ends, which could lead to interchanges between homologues if two breaks happened to occur at the

same level. In the later forms of these models, he related the phenomenon to the production of new daughter chromatids—an idea that has been involved in many of the more recent interpretations.

The most ambitious attempt at a general scheme is that of Darlington, embodied in a long series of papers and first developed in detail in his book of 1932. This scheme was very generally accepted, and for a time came to be considered the very backbone of cytogenetics. It depends on Darlington's "precocity theory," which he sums up as follows: "Meiosis differs from mitosis in the nucleus entering prophase before the chromosomes divide instead of after they divide."

According to this scheme there is a tendency for chromosomes and their constituent parts to form pairs of like elements at the beginning of prophase. If chromosome division has already occurred (as at mitosis), this affinity is satisfied by the fact that daughter chromatids are still closely apposed; at meiosis it leads to a conjugation between homologues. In the latter case, when the conjugated chromosomes divide there are four apposed strands, and the attractive force is supposed to be satisfied when two elements are apposed. Therefore there occurs a separation (reductional) into two double bodies, each made up of a pair of sister elements. If, now, there has been an exchange (that is, a crossover), there will be a chiasma corresponding to it, since only in this way can each part undergo a reductional separation. These chiasmata hold the structure together and ensure that the orientation at the metaphase of the first meiotic division will lead to the passage of two chromatids to each pole.

This scheme was elaborated in great detail, and gave a satisfying geometrical picture, which was correlated with the genetic results by many workers. To many of us, it came to be accepted as basic (see, for example, Sturtevant and Beadle, 1939). But there were skeptics from the beginning. Belling was very critical of much of the scheme, as were Sax and others. It was soon apparent that, in some forms, the chromosomes are visibly double at the time they first conjugate; the view that the initial separation is always reductional at each level was questioned as being an unsupported hypothesis. It was pointed out that quite regular first meiotic segregation occurs without any accompanying crossing over in the Drosophila male and, sometimes, also in the female. Some of the supporting observations themselves were questioned—notably the quantitative agreement between observed frequencies of crossing over and counted numbers of chiasmata. Here the fact is that the counting of chiasmata can be carried out in a really convincing and unambiguous manner in only a few very favorable objects, and these unfortunately do not

include any forms in which there is a considerable body of evidence on the total frequency of crossing over.

Much of the critical discussion in this field is too recent for inclusion here; it is based in part on the suggestion that crossing over may occur much earlier than the detailed side-by-side pairing at synapsis, through chance overlapping of the very thin (and mostly unpaired) threads—in which case the genetically important event occurs before cytologists normally begin looking (Taylor, Grell, and others). It is also probable that a final scheme will depend in large part on the results obtained with bacteria and with bacteriophage, which cannot yet be fully evaluated in comparison with the chromosomes of higher forms.*

* I should like here to enter a protest against the current use, especially by students of bacteria and bacteriophage, of the word *chromosome* as synonymous with *linkage group.* A chromosome is a body that is visible under the light microscope, contains both DNA and other material, and has a whole series of reasonably well-understood properties. The bodies so designated in bacteria and in bacteriophage are very much smaller, seem to be wholly DNA, and lack many of the properties of true chromosomes. They do agree in containing the genes and in being subject to recombination. No one can question the importance of the studies being made about them—but it seems essential to avoid confusion by using a different term; *genophore,* suggested by Ris, seems appropriate and desirable.