# CHAPTER 8

# DEVELOPMENT OF DROSOPHILA WORK

One of the striking things about the early Drosophila results is that the ratios obtained were, by the standards of the time, very poor. With other material it was expected that deviations from the theoretical Mendelian ratios would be small, but with Drosophila such ratios as 3 : 1 or 1 : 1 were rarely closely approximated. This was recognized as being due to considerable differences in the relative mortalities of the various classes in larval and pupal stages, before counts were made. In fact the culture methods were poor, so that these viability differences were very marked, and they still are a source of difficulty.

There has, however, been a steady improvement in the technique of handling the material—most of the early work here being due to Bridges, who brought improved optical equipment and temperature control devices into use, and who had the largest share in the development of improved and standardized culture media.

Bridges was also responsible for the finding and analyzing of a great many new mutants. This undertaking, which was the source of usable material for the studies, was participated in by everyone in the laboratory, but Bridges had the best "eye" for new types and contributed many more of them than did the rest of us. He also had the skill and patience required to build up useful combinations of mutant genes, and many of his multiple strains are still among the most useful, twenty-five years after his death.

Aside from these matters of routine technique, a series of new genetic relations was worked out, and new genetic techniques were developed which made it possible to attack problems that were not recognized or were not approachable before. But one of the first needs was a convenient symbolism; the development of such a system was, in fact, connected with a theoretical question.

Mendel used arbitrary letters as gene symbols—*A* and *a*, *B* and *b*, and so on. He started the custom of using a capital letter for the dominant and the corresponding small letter for its recessive allele, a custom that soon became widespread. It was so widespread, in fact, that Cuénot's failure to follow it led to his not being understood.

Bateson introduced the use of mnemonic symbols, the pair being named for the striking characteristic of the dominant allele, for example, *Y* and *y* for yellow and green seeds in peas. Along with this went Bateson's "presence and absence" hypothesis, according to which the recessive was merely the absence of the dominant, that is, the symbol *y* for green seeds indicated merely that the gene *Y* was absent. This hypothesis dominated the field for a good many years, at least until multiple alleles were recognized. There were exceptions, however, such as Johannsen, who was aware that the hypothesis was unnecessary and might be misleading.

Cuénot in 1904 understood the relations between the mouse colors yellow, agouti, and black, which he treated as alleles. However, he gave the genes symbols (*J, jaune; G, gris; N, noir)* which did not suggest this relation, and he seems to have felt that there was nothing unusual or unexpected about the relation. Rather, he felt that the unexpected relation was *lack* of allelism, as in the cases of chocolate, and of pink eyes. By 1907 he listed five "determinants" (= systems of alleles) for colors:

1. *C*, for color in general, and its mutant *A*, for albinism.
2. *M*, for dark eyes and intense coat color and its mutant *E*, for pink eyes and paler coat.
3. "*G* est une déterminant spécial de la teinte du pelage en présence de *C*; il présente un grand nombre de mutations: *G'*, *N*, et *J*."
4. *F* and its mutation *D*, affecting the black pigment (the recessive is now known as chocolate).
5. *U*, for uniform color of whatever hue, and its mutation *P* (panachure) "with a series of variants, $p^1$, $p^2$, $p^3$, . . . , $p^n$, which correspond to varying degrees of spotting."

The last series has not been borne out by later results; but in the case of *G* he had correctly included what are the now-recognized alleles for yellow, white-bellied agouti, agouti, and black, with the order of dominance as now known.

Morgan showed in 1911 that the relation of agouti, black, and yellow was unusual, and the case was interpreted by Sturtevant (1912) as being due to complete linkage between two gene pairs, one for yellow vs. nonyellow, and the other for agouti vs. black. This conclusion was dis-

puted by Little (1912). Then, in 1914, I developed the idea of multiple alleles—basing it largely on the relation between Himalayan and albino rabbits and that between white and eosin eyes in Drosophila, but without realizing that the same interpretation would fit the mouse case. Finally, in 1915, Little pointed out that Cuénot had already given the multiple allele interpretation in papers that were cited and discussed by both Morgan in 1911 and Sturtevant in 1912. Our failure to realize that Cuénot had understood and explained the situation can only be excused by his use of unorthodox symbols and by the fact that he apparently felt there was nothing about the relation that called for elaboration or emphasis.*

With the development of the multiple allele concept, the presence-and-absence hypothesis was abandoned and, at about the same time, the system of gene symbols associated with it broke down for Drosophila. According to that scheme, each pair of genes was named for the somatic effect of the dominant allele, but with the accumulation of many recessive mutant genes, including, for example, a dozen or so for eye-color, it became necessary to name each one for the mutant allele, usually the recessive. The wild type was considered as a standard of reference, usually symbolized as "+." This system, as gradually developed, is now universally used in the Drosophila literature, and essentially the same scheme is applied to microorganisms. The older scheme, or some compromise between it and that used for Drosophila, is still usual for most other higher organisms—a difference in language that sometimes makes for confusion and misunderstanding. It may be added that the Drosophila system itself is now under a good deal of strain, as a result of the phenomenon of pseudoallelism (see Chapter 14).

The first major undertaking after 1913 was the mapping of the new genes as they became available. Here again, while we all took part, it was Bridges who did most of the spadework, and who gradually accumulated and organized the data to produce the maps, which still remain essentially the same in form. With these maps, and with the carefully planned multiple mutant stocks with conveniently located markers, it gradually

* It should be added that the view that multiple allelism was to be expected was in accord with the thinking of the time. In 1902 Bateson criticized Mendel's hypothesis of two independently segregating recessives for white flower color in Phaseolus, on the ground that these two postulated recessives should be allelic to each other, and in 1903 he published a note titled "On Mendelian Heredity of Three Characters Allelomorphic to Each Other," the three characters being rose, pea, and single combs in fowl, which are due to two independent pairs of genes. Cuénot's achievement was the recognition of such a case when he found it, and the understanding of its difference from the more usual case of independent pairs of genes.

became possible to work with a precision that was heretofore impossible with any other material.

One of the early discoveries was that of lethal genes. The history of lethals goes back to the work of Cuénot with mice. He reported in 1905 that he had been unable to produce homozygous yellows. On mating yellow × yellow, he obtained 263 yellow : 100 nonyellow, and on testing 81 of these yellows, he found that all were heterozygous. This result led to much discussion by several authors, but in 1910 Castle and Little showed that the ratio of yellow to nonyellow is 2 : 1 (they got 800 : 435, or, adding Cuénot's data, 1063 : 535). There could be no doubt that homozygous yellows were formed, but died before birth. That is, the yellow gene was dominant for coat color and also had a recessive lethal effect.

Baur had already analyzed a similar situation in snapdragons. In 1907 he reported that the form *aurea*, with yellowish-green leaves, when selfed, gives a ratio of 2 yellowish : 1 green, and that the greens breed true, while yellowish × green gives 1 : 1. He postulated that the homozygous yellowish embryos die and, in 1908, showed that most of them germinate but produce seedlings that are almost white. However, they die (evidently from lack of photosynthesis) before counts are ordinarily made. This was the first clear demonstration of a lethal gene.

In 1912, Morgan reported the first sex-linked lethal in Drosophila, which was also the first lethal in which the heterozygote had no detectable phenotypic effect. This gene had no dominant effect, but males carrying it invariably died, giving a 2 : 1 sex ratio; the introduction of marker genes made it possible to locate the lethal on the map of the X. It soon became evident that such recessive lethals constitute the largest single class of mutants in Drosophila; as will appear in Chapter 11, they have been very useful in studies of mutations, and they have also been useful in special techniques for making up and maintaining some complex types of stocks.

The process of crossing over was at once recognized as presenting a mechanical problem capable of experimental study. It was already apparent when the first maps were constructed that one crossing over tends to prevent the occurrence of another one near it. This question of interference was subjected to detailed study by Muller, by Bridges, and by Weinstein. Much accurate information was collected, so that the phenomenon can be described in detail, but no wholly satisfactory interpretation has emerged. It is now known that "negative interference," where a crossover *increases* the probability of another one near it, may occur in the small fourth chromosome of Drosophila, and in certain microorganisms. This matter of interference has again become one of the more

actively investigated problems in genetics.

It was soon shown by Bridges that in some regions the frequency of crossing over changes with the age of the female, and by Plough that, in these same regions, the frequency can be influenced by temperature; here again the facts are clear, but their interpretation is not understood.

The chief advance in the understanding of the geometrical relations in crossing over came as a result of the discovery of attached X's. Mrs. Morgan found a very unusual mosaic individual in one of her cultures. As she was examining it, it recovered from anesthetization and flipped off the microscope stage onto the floor. She searched the floor thoroughly, but was unable to find it. Then she reasoned that flies go toward the light when disturbed, so perhaps the mosaic was on the window; there she found and captured it, and was able to recognize it with certainty because of its unusual appearance. The offspring she then obtained from this specimen showed that its ovaries had two X's that always segregated together, and cytological study showed that they were attached at one end, forming a **V** instead of the usual two rods (L. V. Morgan, 1922). This attached-X strain immediately came into very general use as a convenient tool for maintaining sex-linked mutants or combinations in which the females are weak or sterile, and for the rapid multiplication of new sex-linked mutants, or combinations of sex-linked genes.

The two X's in the original attached-X strain were alike, but soon afterward Anderson got a new attached-X line from X-ray experiments, in which the two X's differed in several pairs of genes. Finding that these X's underwent crossing over with each other, he analyzed the results (Anderson, 1925)—an analysis greatly extended with more favorable material later by Beadle and S. Emerson (1933, 1935).

Anderson's study showed that the two X's were attached by their centromere ends, and that genetically these were what had been arbitrarily called their "right" ends. More important, however, was his demonstration that the results could only be accounted for if each chromosome was split lengthwise at the time of crossing over, and if crossing over occurred only between two of the four resulting strands at any one level.

It had been recognized from the beginning of the crossing-over studies that the phenomenon was perhaps one that involved four strands rather than two, that is, perhaps it occurred after the equational split of conjugated chromosomes. This was Janssens' interpretation of the cytological picture, and it was also suggested by Bridges' results on "primary nondisjunction" in 1916. However, there was an alternative explanation possible for Bridges' results, and it seemed simpler to keep to the 2-

strand diagrams as long as they were adequate to explain the data. With these results of Anderson's, and the confirmatory results from triploid females, published at the same time by Bridges and Anderson, the 4-strand interpretation was firmly established.

Crossing over may occasionally occur at mitotic divisions, as was shown by Stern (1936) for Drosophila. Here again it occurs after the chromosomes have undergone a split, and the crossing over is between two (nonsister) strands of the four that are present. The result may be the production of sister cells homozygous for genes that were heterozygous in the original cell.* If these cells then undergo further division and differentiation, there arise adjacent "twin spots" which may show the phenotypes of recessive genes that were originally heterozygous, one in each homologous chromosome.

The production of twin spots was used by Demerec to study the effects of recessive lethal genes that had become homozygous in small areas of a heterozygous individual. He found that some of these are "cell lethal" under these conditions, while others are viable in at least some tissues—either because their normal alleles are not essential for the reactions going on in these tissues or because the necessary substances can somehow be supplied from the rest of the animal.

More recently, it has been found that somatic crossing over occurs regularly in some fungi; this has proved useful for linkage studies, especially in Aspergillus (Pontecorvo and co-workers) and in yeast (Roman and co-workers). Somewhat similar phenomena also occur in bacteria in connection with transformation and transduction and are now under intensive study for their bearing on the mechanism of crossing over.

Bridges followed his genetic and cytological studies of nondisjunction of X with a similar account of nondisjunction of the small IV-chromosomes (1921), and with genetic demonstrations of sectional duplications, deficiencies, and translocations involving the longer chromosomes; these had to wait for the discovery of the properties of the salivary gland chromosomes before they could be fully analyzed (see Chapter 12). Also in 1921 came his studies on triploidy, which will be discussed later (Chapter 13).

Another type of chromosome modification that was worked out step by step may be described here, since it furnished part of the working methods for the analysis of mutation and other phenomena. This is the

* That is, somatic segregation occurs. The process is much too rare and too erratic in its incidence to furnish support for Bateson's hypothesis of somatic segregation (Chapter 6), though it sometimes occurs in the germ line and leads to the production of crossover gametes.

inversion of a section of chromosome.

Both Muller and I had earlier found what we considered to be genes that greatly decreased crossing over in the chromosomes in which they were located. We also both found the surprising result that this reduction occurred only when these "genes" were heterozygous; when they were homozygous the reduction disappeared (Sturtevant, 1917).

In 1921 I suggested that there was probably an inversion in the third chromosome of *Drosophila simulans* as compared to that of *D. melanogaster*, and that perhaps this was the nature of the "crossover reducers" in melanogaster. In 1926 Plunkett and I succeeded in demonstrating the existence of the simulans inversion by the locating of more parallel mutants, and, later in the same year, I obtained definite genetic evidence that this was truly the nature of the crossover reducers.

Inversions were soon found to be not rare and were made use of in various ways, especially in keeping sterile or semisterile genes, or combinations of genes. Later they were studied by Sturtevant and Beadle (1936) for the light they throw on the mechanics of crossing over and segregation, and by Sturtevant and Dobzhansky (1936, 1938) in helping to unravel problems in phylogeny. The most important immediate use, however, was by Muller in his studies on mutation (Chapter 11). The full analysis of inversions was not possible until the advent of the salivary gland chromosome technique.