CHAPTER 14

POSITION EFFECT

In the absence of radiation, one mutation appeared in Drosophila with sufficient frequency to be worth detailed study: namely the case of Bar eye. Although the analysis was successful, the situation turned out to be too special to serve as a basis for any general picture of mutation, but it did lead to the discovery of the "position effect," which has played a large part in later developments.

Bar is a sex-linked dominant that reduces the size of the eye. It was studied intensively by Zeleny and his students, especially with respect to the effects of temperature on the size of the eye, as measured by counting the numbers of facets. In the course of these experiments it was noticed by May (1917) that Bar stocks occasionally revert to wild type. This phenomenon was studied by Zeleny (1919, 1920, 1921), who found that about 1 in 1600 offspring from a Bar stock carries a wild-type allele (B^+). He concluded that the event occurs in females, late in the development of their eggs. He also found that there is a more extreme type produced, a type that he called ultra-Bar, which I later gave the name double-Bar. He showed that double-Bar stocks also revert to wild type and also may give rise to Bar.

Zeleny's evidence indicated that these mutations occurred in females near the time of meiosis, and consequently Morgan and I were led to an investigation of whether the mutations had any relation to crossing over. Our result was clear: 6 reversions were obtained, and all were crossovers between marker genes (forked and fused) lying on opposite sides of the locus of Bar and less than 3 units from each other. That is to say, all 6 reversions were in a class (the crossovers) that included less than 3 percent of the population (Sturtevant and Morgan, 1923).

I then carried out more extensive tests (Sturtevant, 1925) that confirmed this result, not only for reversion of Bar but for the production of double-Bar and for other changes, such as the production of Bar from double-Bar/wild-type heterozygotes.

The interpretation developed was that of "unequal crossing over," according to which it occasionally happens that one chromosome breaks just to the left of Bar, the other one just to the right of it, yielding two crossovers—one carries no Bar (reversion to wild type) and the other carries two Bars (double-Bar, which is the reason for renaming it). This interpretation was later substantially confirmed and extended by study of the salivary gland chromosomes by Muller, Prokofieva-Belgovskaya, and Kossikov (1936), and by Bridges (1936). It appears from these studies that Bar itself is due to a "repeat" of the salivary section including the seven bands of section 16*A*. In double-Bar this section is present in triplicate. In a homozygous Bar female the pairing evidently sometimes occurs thus:

$$\frac{15 \cdot 16A \cdot 16A \cdot 16B}{15 \cdot 16A \cdot 16A \cdot 16B}$$

Crossing over within the apposed 16*A* sections then gives rise to $15 \cdot 16A \cdot 16B$ (wild type) and $15 \cdot 16A \cdot 16A \cdot 16A \cdot 16B$ (double Bar).

The two types, double-Bar/wild type and Bar/Bar, each have the 16*A* section represented four times, but facet counts (Sturtevant, 1925) showed that the former regularly has about 30 percent fewer facets than the latter. That is to say, three 16*A* sections in the same chromosome, and one in its mate, are more effective in reducing facet number than are two in each chromosome. This was at the time a wholly unexpected result, since all previous data had indicated that the position of a gene in the chromosome had no effect on its activity. The position effect here demonstrated has since been found to be rather widely distributed, and it is still being actively studied for its bearing on questions about gene action.

Later results (Dobzhansky, 1932; Bridges, 1936; Griffen, 1941; E. Sutton, 1943, and others) indicate that the original Bar phenotype is itself a position effect, due to the presence of a 16*A* section that is removed from its normal 15 neighbor, rather than directly to the dosage effects of the duplication. It may be surmised that the originally discovered position effect is due to a greater effect on the rightmost 16*A* section of double-Bar, since it is now still further removed from 15.

It has gradually become evident that there are two essentially different types of position effects, designated by Lewis (1950) as the S-type (stable) and the V-type (variegated). Bar represents the S-type, and this will accordingly be discussed first.

A position effect not dependent on a chromosome rearrangement was

discovered by Lewis (1945). The two mutants Star (dominant) and asteroid (recessive) have very similar phenotypes and lie adjacent to each other. Lewis studied the heterozygote (Star/asteroid) and recovered from it both the wild type and the double mutant, Star asteroid. If we compare the two kinds of double heterozygotes—the *cis* type (Star asteroid/wild type) and the *trans* type (Star/asteroid)—it is clear that the *trans* type differs more decidedly from the wild-type phenotype than does the *cis* type. This case is complicated by the dominance of Star, but more recent examples have shown that the principle illustrated here is a general one in such cases: the *cis* heterozygote (carrying a normal unmutated chromosome) is more nearly wild type in phenotype than is the *trans* (Bar is an exception to this rule).

Situations like this were soon found in which the dominance complication was absent. The first of these was reported by Green and Green in 1949, for the mutant lozenge in Drosophila. Oliver (1940) had shown that females heterozygous for two independently arisen lozenge mutants, called glossy and spectacled (that is, glossy/spectacled), had the typical phenotype of the lozenge series but gave some wild-type chromosomes that were always crossovers for outside markers. Oliver failed to detect the contrary crossover and was therefore in doubt as to the significance of the result. Green and Green used glossy (lz^{g}) and two new independently arisen types (lz^{BS} and lz^{46}). They were able to recover from each of the double heterozygotes of the *trans* type, both the wild type and the double mutant; all these events were again associated with crossing over between outside markers. These results showed the sequence in the chromosome map to be lz^{BS} lz^{46}, lz^{g}. When the six possible heterozygotes for two mutants were made up, it was found that all three *cis* types were wild type in phenotype, whereas all three *trans* types (lz^{BS}/lz^{46}, lz^{BS}/lz^{g}, lz^{46}/lz^{g}) were lozenge in appearance.

The same kind of result was soon demonstrated for several other series of independently arisen alleles—for vermilion and for beadex by Green, for white and for bithorax by Lewis, and for several other series by other investigators. The case of white was particularly unexpected, for this had long been the type case of multiple allelism, and it became clear that the then-current hypothesis must be revised.

Multiple alleles had been supposed to represent changes in a single original gene, and there were two criteria for their recognition: they occupied the same locus in the chromosome and were not separable by crossing over; and their heterozygote *(trans* type) was mutant with respect to their common recessive phenotype, since neither carried the wild-type allele of the other. With the discoveries noted above, these two criteria were shown

not to agree. In such cases, which came to be called "pseudoallelic" (Lewis), the *trans* heterozygote is mutant in phenotype (the mutants do not complement each other), but both the wild type and the double mutant can nevertheless be reconstituted by crossing over. Evidently, each mutant carries the wild-type composition that the other has lost, but the section of chromosome that includes them is a functional unit that must be intact in at least one chromosome to produce the wild-type phenotype.

This conclusion, which has been shown to apply to many (most ?) loci in many (all ?) organisms, has had a very wide influence. A minor consideration is that it has complicated the terminology of the subject in several ways. The symbolism for genes had grown up on the basis of the older view, and it is still not clear what will be the most effective compromise. The older terms *gene, allele,* and *locus* are now in a fluid state so far as current usage is concerned, and several newer terms are in general use: *cistron* (Benzer) to denote an area that must be intact (that is, in the *cis* form) to produce the wild-type phenotype, and *site* (or *recon* of Benzer) to denote the smallest unit separable by recombination. It is still not clear what will be the most convenient system of terminology; probably it will depend on developments in the study of the genetic coding system (Chapter 16).

There has also been much discussion of the implications of the position effect for the basic theory of genes and their effects on development. The most extreme view is that of Goldschmidt (1946), who suggested that the whole idea of genes be given up—the chromosome being a single developmental unit and all mutant effects being due to rearrangements (usually minute) of its parts, with resulting position effects. This view has few adherents, but at one time did figure largely in the literature of genetics.

In general, the frequency of recombination within a cistron is very low, and this is the reason why the phenomenon was overlooked in early work. It is still one of the limiting factors in the study of higher organisms. But in microorganisms, and especially in bacteriophage, it is possible to develop methods for studying recombinations that occur only with very low frequencies. It is largely for this reason that current studies in the field, leading to what is called "fine-structure analysis," are often carried out with such material. These studies (for example, see Benzer, 1961) are outside the scope of this book.

The second or "V-type" position effect probably is different in kind from the S-type. Most examples are associated with chromosome rearrangements induced by X rays. Muller reported in 1930 on certain "eversporting" types in which dominant genes were lost or inactivated in some

cells of individuals carrying rearrangements, producing irregularly spotted patterns for eye color, body color, or other mutant types. It was evident that these spots were due to failure of action of genes near the break-points of the rearrangements. When more cases accumulated it became clear, as pointed out by Schultz (1936), that the inactivation usually occurs when genes in euchromatin are brought near heterochromatin or, less often, when genes in or near heterochromatin are brought nearer to euchromatin.

Dubinin and Sidorov (1935) described a translocation between the third and fourth chromosomes of Drosophila, with the breakpoint in III near the locus of hairy; this locus was brought near the heterochromatin of IV. They showed that the h^+ gene in this translocation exhibited reduced dominance over the *h* mutant allele. They were able to get crossing over between the translocation point and the locus of hairy and thus to recover the h^+ allele in a normal chromosome; its usual complete dominance was at once restored. A different h^+ allele was also introduced into the translocation chromosome by crossing over, and at once acquired the reduced dominance. In the same year, Panshin obtained similar results from another translocation that affected the dominance of the cu^+ gene.

These results furnished proof that these position effects did not depend on any transmissible changes in the h^+ or cu^+ genes but on an interference with their developmental effects.

There was one uncertainty about the phenomenon: Were the genes in question lost or only inactivated in the "mutant" areas? There is no increase in frequency of germinal losses, that is, the next generation from variegated individuals is variegated, not pure for the recessive alleles. Evidently this means either that the process does not occur in the germ line, or that it is reversed in the formation of the gametes. With the discovery of the same phenomenon associated with translocation in Oenothera (Catcheside, 1939, 1947, and later in mice by L. B. Russell and others) it became probable that the inactivation is reversed at meiosis, since there can scarcely be anything other than meiosis common to the history of the germ line in Drosophila and in Oenothera. That is to say, the V-type variegation is due to a suppression of the phenotypic effects of genes that are still reproducing in the usual manner at each cell division, so that reversal of the effect is always possible, though it usually does not occur except at meiosis. One may surmise that reversion is somehow associated with the uncoiling and lengthening of the chromosomes in the meiotic prophases.

Muller found in 1930 that this type of variegation might affect the action of several genes newly brought near heterochromatin. This phenomenon was studied by Gowen and Gay, by Patterson, and by Schultz;

the most detailed and illuminating studies were by Demerec (1940, 1941) and by Demerec and Slizynska (1937). These studies showed that there is a "spreading effect." If heterochromatin is represented by the symbol *H* and if a series of wild-type alleles *A, B, C,* and so forth be brought next to it in the sequence *HABC,* then the suppression of gene activity proceeds from *H; A* is inactivated first, then *B,* then *C,* and so on. Tissue with inactivation of *A* alone or of both *A* and *B* may occur but not with *A* active and *B* inactive. It appears that there is no skipping of genes, that is, there seem to be no genes immune to the effect. (A supposed exception to this rule reported for the fourth chromosome has been found to be based on an incorrect map of the fourth chromosome.)

The most likely interpretation is that there is a progressive inhibition of the production of gene products but not of gene replication; that is, in modern terms, RNA is not produced, but DNA replication does occur. One possible interpretation is that the timing of the DNA replication is retarded as it seems to be in heterochromatin; these matters are, however, beyond the scope of this book.

It is, in fact, premature to formulate any definitive scheme for the V-type position effects, since several facts remain to be further analyzed: the effect of removal from heterochromatin upon genes normally in or near it (possibly an inhibition of suppressors normally present ?);* the striking effects of temperature and of the number of Y chromosomes present (both reported in 1933 by Gowen and Gay); the occurrence of dominant V-type effects, and numerous other unexplained relations. These are now under active study in several laboratories, and there can be no doubt that the V-type effects will contribute largely to future ideas about the nature of gene action in development and differentiation.

* The most studied example of a gene normally located in or near heterochromatin that shows variegation when removed from most of this heterochromatin is that of cubitus interruptus (*ci*). It was shown by Dubinin and Sidorov (1934) that approximately half of the translocations that involve the fourth chromosome lead to a weakening of the dominance of ci^+ over the mutant *ci.* This case has been studied in great detail, especially by Dubinin and Stern and their co-workers. There are many interesting observations, some of which are rather puzzling, but it does not seem (to me, at least) that they have led to any close insight into the nature of such cases—in part because the nature of the *ci* phenotype makes it difficult to study.