

# THE EFFECTS OF UNEQUAL CROSSING OVER AT THE BAR LOCUS IN *DROSOPHILA*<sup>1</sup>

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## INTRODUCTION

If one thinks of mutations as being simply inherited changes, it becomes necessary to distinguish changes that involve whole chromosomes (e.g., non-disjunction or tetraploidy), changes that involve several adjacent genes (deficiencies and duplications), and what have been called “point-mutations” or “gene-mutations.” Probably this last type includes quite diverse processes. It is therefore important to collect information as to the nature of specific examples of mutation. For this purpose it will commonly be necessary to work with a frequently recurring mutation. Only one frequently mutating gene has hitherto been discovered in *Drosophila*, namely, bar. Crossing over has proved to be the key to the mutation behavior of bar, as will be shown in the present paper. The case appears not to be, strictly speaking, a point-mutation after all, but a new kind of section-mutation, in which the section concerned is so short as to include only a single known gene, and in which unequal crossing over furnishes the mechanism for bringing about the new types.

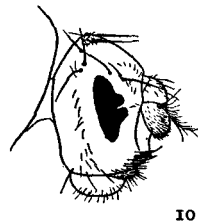
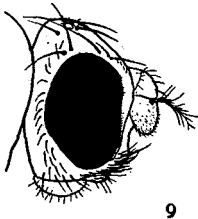
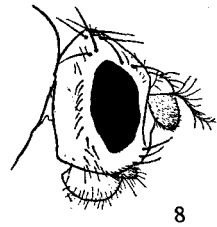
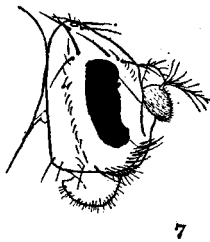
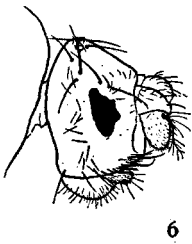
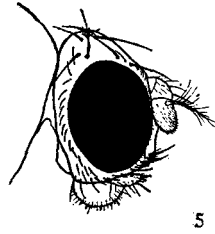
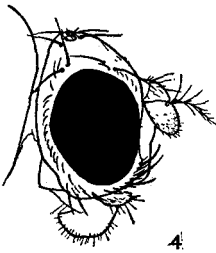
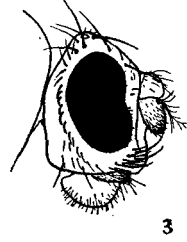
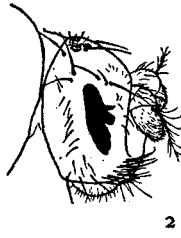
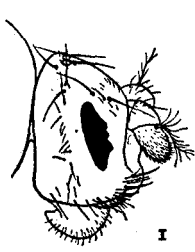
## HISTORICAL

In 1914 TICE (1914) found a single male of *Drosophila melanogaster* that had narrow eyes (see figures 1 and 2). The new type, called bar (or “barred”

<sup>1</sup> Contribution from the CARNEGIE INSTITUTION OF WASHINGTON.

## LEGEND FOR PLATE 1

- FIGURE 1.—Homozygous bar female.
- FIGURE 2.—Bar male.
- FIGURE 3.—Bar-over-round female.
- FIGURE 4.—Female homozygous for round, that was obtained by reversion.
- FIGURE 5.—Male that carries round, obtained by reversion.
- FIGURE 6.—Double-bar male.
- FIGURE 7.—Homozygous infrabar female.
- FIGURE 8.—Infrabar male.
- FIGURE 9.—Infrabar-over-round female.
- FIGURE 10.—Double-infrabar male.



in the earlier literature), was found to depend on a sex-linked gene located at 57.0 in the X chromosome. It was further found that the bar character is dominant, in the sense that females carrying one bar gene have eyes distinctly different from the wild-type or "round" eye (figure 3). Because of this dominance the type has been extensively used in linkage experiments. MAY (1917) reported that the bar gene occasionally reverts to normal (figures 4 and 5)—a process that has more recently been extensively studied by ZELENY (1919, 1920, 1921). ZELENY found that the frequency of reversion is variable, but in many stocks is such that about 1 in 1600 offspring from a pure bar stock receives a not-bar, or round, allelomorph. ZELENY also concluded that the reversion probably occurs chiefly (or perhaps exclusively) in females. His argument, based on the sex ratio found among reverted individuals, is not as convincing as the direct tests that will be described in this paper, and which verify his conclusion. ZELENY also found that homozygous bar gives rise to a new and more extreme allelomorph of bar, that he has called "ultra-bar." For reasons that will be developed in this paper, I prefer to call it "double-bar." The eyes of double-bar are distinctly smaller than those of bar (figure 6). ZELENY has shown that the type is more strongly dominant over round than is bar, and also that double-bar is largely dominant over bar.

ZELENY likewise found that homozygous double-bar stocks revert to round with a frequency not very different from that of homozygous bar stocks, and that double-bar occasionally mutates to bar; that is, it can go all the way back to round at one step, or it can give bar, which, in turn, is capable of reverting to round. ZELENY has argued that the three types, round, bar, and double-bar, have the same characteristic properties, regardless of their origin. The round eye of reverted bar is indistinguishable from wild-type; bar derived from double-bar does not differ from the original bar, etc. This point will be considered in more detail in a later section.

STURTEVANT and MORGAN (1923) showed that double-bar over bar<sup>2</sup> also gives rise to round-eyed individuals. They reported three reversions from this combination and three from homozygous bar. In all cases the mothers had been heterozygous for forked,<sup>3</sup> which lies 0.2 units to the left

<sup>2</sup> In this paper the constitution of heterozygotes will be expressed as above in order to avoid circumlocution or indefiniteness. "Double-bar over bar" is to be understood as: "carrying double-bar in one X chromosome and bar in the other X chromosome."

<sup>3</sup> Forked is a recessive bristle modification. Locus 56.8 in the X chromosome (see MORGAN and BRIDGES 1916).

of bar, and for fused<sup>4</sup> which lies 2.5 units to the right of bar. All six reversions represented crossovers between forked and fused, though the total forked fused crossovers constituted less than 3 percent of the number of individuals examined. STURTEVANT and MORGAN also reported that experiments in which bar entered only through the males had failed to give any reversions, though no numerical data were reported. The present paper is based on the results of a more detailed study of the relations first shown by STURTEVANT and MORGAN (1923).

## MUTATIONS AND CROSSING OVER

The results from homozygous bar females, that were reported by STURTEVANT and MORGAN, were from females of the constitution  $\frac{+Bf_u}{fB+}$ . A more efficient type of experiment is that in which females of the constitution  $\frac{+B+}{fBf_u}$  are mated to forked bar fused males. Table 1 (first row) shows the results obtained from an extensive series of this type. In the second row of table 1 are given the results from mating a few females of the above constitution to forked fused males.

TABLE 1  
 $\frac{B}{fBf_u}$  ♀ ×  $fBf_u$  ♂ (1st row) or  $ff_u$  ♂ (2nd row).

| TYPE OF MALE USED | BAR  |        |       |     |      |        |       |     | TOTAL | ROUND |     |   |       |     | DOUBLE-BAR |     |
|-------------------|------|--------|-------|-----|------|--------|-------|-----|-------|-------|-----|---|-------|-----|------------|-----|
|                   | ♀    |        |       |     | ♂    |        |       |     |       | ♀     |     | ♂ |       |     | ♀          | ♂   |
|                   | 0*   |        | 1     |     | 0    |        | 1     |     |       | $f_u$ | $f$ | + | $f_u$ | $f$ | $f$        | $f$ |
|                   | +    | $ff_u$ | $f_u$ | $f$ | +    | $ff_u$ | $f_u$ | $f$ |       |       |     |   |       |     |            |     |
| $fBf_u$           | 5413 | 3749   | 94    | 140 | 5218 | 4160   | 93    | 124 | 18991 | 0     | 2   | 1 | 2     | 1   | 1          | 1   |
| $ff_u$            | 374  | 359    | 6     | 6   | 352  | 324    | 10    | 7   | 1438  | 1     | 0   | 0 | 1     | 0   | 0          | 0   |
| Total . . . . .   | 5787 | 4108   | 100   | 146 | 5570 | 4484   | 103   | 131 | 20429 | 1     | 2   | 1 | 3     | 1   | 1          | 1   |

\* In this and the following tables "0" signifies non-crossover classes; "1," classes resulting from crossing over in the first region, etc.

The mutant females that appeared in these experiments were, of course, all heterozygous for whichever allelomorph (bar or round) they received from their fathers, but in tables 1 to 18 this paternal gene is ignored and the females classified according to the maternal gene. In all doubtful cases (including both double-bars), the classification was checked by raising offspring from the mutants, since there is sometimes difficulty in

<sup>4</sup> Fused is a recessive venation character. Its locus is at 59.5 in the X chromosome (see MORGAN and BRIDGES 1916, LYNCH 1919).

classifying single individuals, a difficulty that can be removed by examination of a series of specimens of a given constitution.

It will be observed that seven of the eight reversions, and also both of the double-bars, occurred in gametes that came from crossing over between forked and fused, though the total forked fused crossovers constituted only 2.4 percent of the population. The one exceptional case, a wild-type male, is not above suspicion of having arisen through contamination rather than reversion of bar. That he was really round-eyed was proven by tests. Only 4 other exceptions to the rule that mutation in this locus is accompanied by forked-fused crossing over have been met with in the work here reported. These will be discussed separately later.

On the basis of these results we may formulate the working hypothesis that both reversion and the production of double-bar are due to *unequal crossing over*. If we suppose that, in a female  $\frac{+B+}{fBf_u}$ , crossing over occurs in such a way that the respective points of interchange lie to the left of the bar locus in one chromosome, but to the right of it in the other one, there will result chromosomes of the constitution  $fBB+$  and  $+f_u$  (or  $f+$  and  $+BBf_u$ ). The hypothesis is that reverted round is simply *no-bar*, and that double-bar is  $BB$ ,—this being the reason for abandoning ZELENY'S name, ultra-bar.

This hypothesis makes reverted round and double-bar complementary crossovers, and they should accordingly be produced with equal frequency. Table 1 agrees with ZELENY'S more extensive data in showing that round is apparently far more frequent than double-bar; but such a result was to be expected for two reasons. Double-bar is not as viable as round, so that fewer of the double-bar mutant individuals would be expected to survive; and double-bar is not always clearly distinguishable from bar, so that some mutant individuals are probably overlooked, while it is not likely that any reversion is overlooked through difficulty of classification.

The double-bar over bar experiments reported by STURTEVANT and MORGAN (1923) can be interpreted in the same way: the reversion is here due to unequal crossing over just as in homozygous bar. In the earlier cultures of the experiments previously reported, only the reversions were classified for forked and for fused. Two of the reversions were in such incompletely classified cultures. Table 2, including all the double-bar over bar data for which complete counts are available, contains one of the previously reported reversions and one new one. This table includes only the male offspring, since the  $BB$  derived from the fathers rendered the classification of the females uncertain.

The process of unequal crossing over might be expected to give rise to triple-bar from the females that are double-bar over bar. No individual that could be so identified was obtained, though several specimens with very small eyes were tested. All those that were fertile proved to be double-bar. Apparently triple-bar is either inviable or sterile. This problem will be discussed again below.

TABLE 2  
 $\frac{B}{fBBf_u} \text{♀} \times fBBf_u \text{♂}$ .

| B OR BB ♂ |         |       |     | TOTAL | ROUND ♂ |
|-----------|---------|-------|-----|-------|---------|
| 0         |         | 1     |     |       | f       |
| +         | $f f_u$ | $f_u$ | $f$ |       |         |
| 3933      | 2741    | 62    | 73  | 6809  | 2       |

My own experiments with homozygous double-bar have not yielded any mutations, probably because I have found this type hard to breed and have therefore not obtained large numbers of offspring. It may be recalled that ZELÉNY has obtained both bar and round from such females, but not in experiments in which forked and fused were present. In table 3, showing the data I have obtained, only males are recorded, for the same reason as in table 2, and also because the females could not be classified for fused.

TABLE 3  
 $\frac{fBB}{BBf_u} \text{♀} \times fBB \text{♂}$ .

| BB ♂  |     |    |         | TOTAL |
|-------|-----|----|---------|-------|
| 0     |     | 1  |         |       |
| $f_u$ | $f$ | +  | $f f_u$ |       |
| 627   | 956 | 37 | 24      | 1644  |

In the case of double-bar over round, bar should be produced by any crossover between the two bars of the double-bar chromosome. This event might seem less unlikely to occur than the type of unequal crossing over invoked in the preceding experiments; and both of the chromosomes resulting from such crossing over should yield bar, whereas in the preceding cases, a given crossing over must always have yielded chromosomes bearing two different kinds of bar allelomorphs. It is accordingly in

agreement with the hypothesis that tables 4 and 5 show a higher percentage of mutation than do tables 1 and 2. Two non-disjunctional individuals (a  $ff_u\sigma$  and a  $\frac{+++}{fBBf_u}\varphi$ ) have been omitted from table 5.

TABLE 4  
 $\frac{BB}{ff_u}\varphi \times ff_u\sigma$ .

| SEX       | DOUBLE-BAR AND ROUND |        |       |       |         |     | TOTAL | BAR   |     |
|-----------|----------------------|--------|-------|-------|---------|-----|-------|-------|-----|
|           | 0                    |        | 1     |       | 2       |     |       | $f_u$ | $f$ |
|           | $BB$                 | $ff_u$ | $f_u$ | $fBB$ | $BBf_u$ | $f$ |       |       |     |
| $\varphi$ | 1174                 | 784    | 1     | 1     | 18      | 30  | 2018  | 0     | 1   |
| $\sigma$  | 993                  | 943    | 1     | 1     | 14      | 19  | 1971  | 1     | 1   |

No mutations are to be expected from females that are heterozygous for bar and for round ( $\frac{B}{+}$ ), since crossing over can not produce any new combination. The results of the tests are shown in tables 6 and 7, and are in agreement with this expectation. One non-disjunctional female ( $\frac{+++}{fBf_u}$ ) was also produced in this series.<sup>5</sup>

TABLE 5  
 $\frac{+}{fBBf_u}\varphi \times ff_u\sigma$ .

| SEX       | DOUBLE-BAR AND ROUND |          |         |     |       |       | TOTAL | BAR   |     |
|-----------|----------------------|----------|---------|-----|-------|-------|-------|-------|-----|
|           | 0                    |          | 1       |     | 2     |       |       | $f_u$ | $f$ |
|           | $+$                  | $fBBf_u$ | $BBf_u$ | $f$ | $f_u$ | $fBB$ |       |       |     |
| $\varphi$ | 464                  | 392      | 0       | 1   | 14    | 23    | 894   | 1     | 0   |
| $\sigma$  | 495                  | 406      | 0       | 0   | 6     | 10    | 917   | 1     | 1   |

L. V. MORGAN (1922) has described a race of *D. melanogaster* in which the two X chromosomes of the female are attached to each other, so that such a female gives 100 percent non-disjunction. This race, and others

<sup>5</sup> This series also produced one female that was wild-type in appearance. Such a female would be either a double crossover—which is not at all probable in such a short chromosome section, or a non-crossover reversion of bar. This female was mated to an unrelated bar male, and produced 124 bar-over-round daughters and 96 round-eyed sons; but none of the sons showed either forked or fused. This result must mean that the exceptional female was due to contamination.



separate in origin but having the same peculiarity of attached X's, have been used to test the mutability of bar in the male. If a round-eyed female with attached X's is crossed to a bar male, all the sons get their X chromosomes from the bar father and accordingly furnish a direct test of the

TABLE 6  
 $\frac{+}{fB f_u} \text{♀} \times f f_u \text{♂}$ .

| SEX | BAR |           |         |     |       |       | TOTAL |
|-----|-----|-----------|---------|-----|-------|-------|-------|
|     | 0   |           | 1       |     | 2     |       |       |
|     | +   | $f B f_u$ | $B f_u$ | $f$ | $f_u$ | $f B$ |       |
| ♀   | 331 | 276       | 0       | 0   | 12    | 13    | 632   |
| ♂   | 316 | 265       | 0       | 1   | 10    | 11    | 603   |

mutability of bar in males.<sup>6</sup> A total of 10,079 bar males has been observed from such matings, with no rounds or double-bars. There was, however, one other male that had an eye intermediate between bar and round. This new type, called infrabar (figures 7, 8, 9) has been shown to represent a new allelomorph of bar. Its somatic appearance will be described in more detail later in this paper.

TABLE 7  
 $\frac{B}{f f_u} \text{♀} \times f f_u \text{♂}$ .

| SEX | BAR  |         |       |       |         |     | TOTAL |
|-----|------|---------|-------|-------|---------|-----|-------|
|     | 0    |         | 1     |       | 2       |     |       |
|     | $B$  | $f f_u$ | $f_u$ | $f B$ | $B f_u$ | $f$ |       |
| ♀   | 1792 | 1356    | 3     | 7     | 36      | 41  | 3235  |
| ♂   | 1523 | 1334    | 4     | 3     | 26      | 36  | 2926  |

Tests soon showed that infrabar behaved as a single unit in inheritance. Bar can not be recovered from it, and it shows the same linkage relations as bar. The convincing proof that it represents a modification of the bar gene will appear below.

Homozygous infrabar behaves like bar in that it reverts to normal, and also produces a new and more extreme type, double-infrabar (figure 10),

<sup>6</sup> Occasionally the attached X's separate, and a regular son carrying a maternal X is produced. In the present series of experiments this source of error was eliminated by having the two maternal X's differ from the paternal one in at least one other mutant gene besides bar.

analogous to double-bar. As shown in table 8, both these events are again associated with forked-fused crossing over.

Of the three double-infrabar individuals, one was sterile, and one was accidentally lost, but the constitution of the other was established by breeding tests. The type was also obtained in another experiment; and its appearance and mutation behavior will be described later. This series shows the same excess of rounds over double forms as did the bar series, and the explanation is doubtless the same. The difficulty of separation is even greater here, owing to a greater variability in eye size (see below).

TABLE 8  
 $\frac{B^i}{fB^if_u} \text{ } \varphi \times f B^i f_u \text{ } \sigma^7$ .

| SEX | INFRABAR |        |       |     | Total | ROUND |     | $B^iB^i$ |     |
|-----|----------|--------|-------|-----|-------|-------|-----|----------|-----|
|     | 0        |        | 1     |     |       | $f_u$ | $f$ | $f_u$    | $f$ |
|     | +        | $ff_u$ | $f_u$ | $f$ |       |       |     |          |     |
| ♀   | 4607     | 3606   | 94    | 142 | 8449  | 0     | 5   | 0        | 1   |
| ♂   | 4341     | 3905   | 97    | 106 | 8449  | 2     | 11  | 2        | 0   |

Bar-over-infrabar females of three different constitutions have been tested. They have produced rounds, and also a new double type that had eyes only very slightly larger than those of double-bar. These must evidently have bar and infrabar in the same chromosome. Tables 9, 10 and 11 show these results. In most cases the fathers of these cultures did not

TABLE 9  
 $\frac{f B^i}{B f_u} \text{ } \varphi \times \textit{various } \sigma^7 \sigma^7$ .

| BAR AND INFRABAR MALES |     |    |        |       | DOUBLE TYPE |        |
|------------------------|-----|----|--------|-------|-------------|--------|
| 0                      |     | 1  |        | Total | ♂           |        |
| $f_u$                  | $f$ | +  | $ff_u$ |       | +           | $ff_u$ |
| 623                    | 682 | 26 | 18     | 1349  | 1           | 1      |

carry fused; for this reason the females, among which no mutants were detected, are not listed. The forked fused mutant male was sterile, but the constitution of the other was tested. All the experiments here described, on "bar-infrabar" (figure 10), concern this mutant gene. The stability of this new type in the male has also been tested. Matings of bar-infrabar males to attached-X females (also differing in at least one other sex-linked

gene) have produced 9042 non-disjunctional sons,—all of them bar-infrabar.

TABLE 10  
 $\frac{B^i}{f B f_u} \text{♀} \times f B f_u \text{♂}$ .

| SEX | BAR AND INFRABAR |         |       |     |       | MUTANTS |
|-----|------------------|---------|-------|-----|-------|---------|
|     | 0                |         | 1     |     | Total |         |
|     | +                | $f f_u$ | $f_u$ | $f$ |       |         |
| ♀   | 497              | 415     | 10    | 18  | 940   | 0       |
| ♂   | 505              | 476     | 15    | 16  | 1012  | 0       |

The double-type male of table 11, which resembled those from table 9, was tested. His descendants are discussed below under the name "infrabar-bar."

TABLE 11  
 $\frac{B}{f B^i f_u} \text{♀} \times f B^i f_u \text{♂}$ .

| SEX | BAR AND INFRABAR |         |       |     | Total | ROUND |     | DOUBLE TYPE |
|-----|------------------|---------|-------|-----|-------|-------|-----|-------------|
|     | 0                |         | 1     |     |       | $f_u$ | $f$ | $f$         |
|     | +                | $f f_u$ | $f_u$ | $f$ |       |       |     |             |
| ♀   | 2445             | 2203    | 59    | 74  | 4781  | 0     | 1   | 0           |
| ♂   | 2365             | 2347    | 64    | 71  | 4847  | 1     | 0   | 1           |

These new double types have made it possible to devise crucial tests of the theory of unequal crossing over, which may now be described. The first test made was that of bar-infrabar over round. Tables 12 and 13 show the results obtained. In addition one culture of this series gave two wild-type females, one in the first count and another in the last count,

TABLE 12  
 $\frac{f B B^i}{f_u} \text{♀} \times f f_u \text{♂}$ .

| SEX | BAR-INFRABAR AND ROUND |           |         |         |    |               | Total | BAR     | INFRABAR |
|-----|------------------------|-----------|---------|---------|----|---------------|-------|---------|----------|
|     | 0                      |           | 1       |         | 2  |               |       | $f f_u$ | +        |
|     | $f_u$                  | $f B B^i$ | $B B^i$ | $f f_u$ | +  | $f B B^i f_u$ |       |         |          |
| ♀   | 303                    | 394       | 4       | 0       | 14 | 8             | 723   | 0       | 1        |
| ♂   | 321                    | 308       | 0       | 2       | 7  | 3             | 641   | 1       | 1        |

ten days later. These females give rise to the same difficulties as did the wild-type obtained in the series reported in table 7. One of them was tested, and gave unexpected results. The mother had carried vermilion in her forked fused X, and the father had also been vermilion forked fused. The tested exceptional female was found to carry one wild-type X and one forked fused X, but did not carry vermilion at all. Under the circumstances it is open to question whether these two exceptional individuals were not due to contamination. They will be discussed again later.

TABLE 13

$$\frac{BB^i}{f f_u} \text{♀} \times f f_u \text{♂}.$$

| SEX | BAR-INFRABAR AND ROUND |                        |                      |                         |                                     |          | Total | BAR                  | INFRABAR |
|-----|------------------------|------------------------|----------------------|-------------------------|-------------------------------------|----------|-------|----------------------|----------|
|     | 0                      |                        | 1                    |                         | 2                                   |          |       | <i>f<sub>u</sub></i> | <i>f</i> |
|     | <i>BB<sup>i</sup></i>  | <i>f f<sub>u</sub></i> | <i>f<sub>u</sub></i> | <i>f BB<sup>i</sup></i> | <i>BB<sup>i</sup> f<sub>u</sub></i> | <i>f</i> |       |                      |          |
| ♀   | 2549                   | 1684                   | 3                    | 1                       | 43                                  | 74       | 4354  | 0                    | 0        |
| ♂   | 2322                   | 1857                   | 5                    | 8                       | 48                                  | 63       | 4303  | 2                    | 4        |

Tables 12 and 13 show that bar and infrabar can both be recovered from bar-infrabar. It appears then that in the double form the individual elements maintain their identity. Even more important, however, is the indication that they maintain their sequence in the chromosome. As shown in table 9, the bar-infrabar first came from the combination  $\frac{f B^i}{B f_u}$ , as a not-forked not-fused male. If the two elements of double forms are arranged in the same linear series as the rest of the genes, this result must mean that the bar now lies to the left of the infrabar. This supposition is entirely borne out by tables 12 and 13, which are experiments of the usual type used to establish sequence of genes. All the 9 single types recovered agree with the supposed sequence.

TABLE 14

$$\frac{BB^i}{f B^i f_u} \text{♀} \times f B^i f_u \text{♂}.$$

| SEX | <i>BB<sup>i</sup></i> AND <i>B<sup>i</sup></i> |                                      |                                    |                         |                                     |                        | Total | <i>B<sup>i</sup>B<sup>i</sup></i> |
|-----|--|--------------------------------------|------------------------------------|-------------------------|-------------------------------------|------------------------|-------|-----------------------------------|
|     | 0  |                                      | 1                                  |                         | 2                                   |                        |       | <i>f</i>                          |
|     | <i>BB<sup>i</sup></i>                          | <i>f B<sup>i</sup> f<sub>u</sub></i> | <i>B<sup>i</sup> f<sub>u</sub></i> | <i>f BB<sup>i</sup></i> | <i>BB<sup>i</sup> f<sub>u</sub></i> | <i>f B<sup>i</sup></i> |       |                                   |
| ♀   | 850  | 702                                  | 3                                  | 3                       | 17                                  | 28                     | 1603  | 0                                 |
| ♂   | 867  | 860                                  | 4                                  | 2                       | 22                                  | 23                     | 1778  | 1                                 |

Bar-infrabar has also been tested against infrabar (table 14).

Here again there is an opportunity for the production of a triple form,—but since the corresponding round did not appear, its absence is not significant. The one double-infrabar confirms the sequence of the component parts of bar-infrabar. It was tested, and all the results from double-infrabar reported below were obtained from flies descended from it.

Examination of the data in table 11 shows that the double form obtained there must have had infrabar to the left of bar; that is, it was infrabar-bar instead of bar-infrabar. The tests made with it appear in table 15.

TABLE 15  
 $\frac{f B^i B}{f_u} \text{♀} \times f f_u \text{♂}.$

| SEX | INFRABAR-BAR AND ROUND |           |         |         |    |               | Total | BAR | INFRABAR |
|-----|------------------------|-----------|---------|---------|----|---------------|-------|-----|----------|
|     | 0                      |           | 1       |         | 2  |               |       | +   | $f f_u$  |
|     | $f_u$                  | $f B^i B$ | $B^i B$ | $f f_u$ | +  | $f B^i B f_u$ |       |     |          |
| ♀   | 483                    | 469       | 1       | 1       | 15 | 15            | 984   | 2   | 0        |
| ♂   | 478                    | 408       | 1       | 9       | 17 | 15            | 928   | 0   | 1        |

These results show, in fact, that the sequence is infrabar-bar as supposed. Except for this difference the mating is the same as in table 12. It will be observed that the two not-forked not-fused single types in that table were both infrabar, while the two obtained here were bar; the one forked fused there was bar, here it was infrabar. There is a total of 13 single-type mutants in tables 12 to 15, all of them agreeing in indicating that the two elements of double types maintain not only their individuality but their sequence.

The double-infrabar obtained in table 14 has been tested against round (tables 16 and 17).

TABLE 16  
 $\frac{f B^i B^i}{f_u} \text{♀} \times f f_u \text{♂}.$

| SEX | $B^i B^i$ AND ROUND |             |           |         |    |                 | Total | $B^i$ |
|-----|---------------------|-------------|-----------|---------|----|-----------------|-------|-------|
|     | 0                   |             | 1         |         | 2  |                 |       | +     |
|     | $f_u$               | $f B^i B^i$ | $B^i B^i$ | $f f_u$ | +  | $f B^i B^i f_u$ |       |       |
| ♀   | 538                 | 629         | 1         | 1       | 12 | 17              | 1198  | 0     |
| ♂   | 574                 | 505         | 1         | 1       | 13 | 7               | 1101  | 2     |

The results in these tables show that double-infrabar was correctly identified, and that it behaves as was to be expected, giving infrabar by both kinds of crossing over,—just as double-bar gives bar in both cases.

TABLE 17

$$\frac{B^i B^i}{f f_u} \text{♀} \times f f_u \text{♂}.$$

| SEX | DOUBLE INFRABAR AND ROUND         |                        |                      |                                     |   |          | INFRABAR |                      |          |
|-----|-----------------------------------|------------------------|----------------------|-------------------------------------|---|----------|----------|----------------------|----------|
|     | 0                                 |                        | 1                    |                                     | 2   |          | Total    | <i>f<sub>u</sub></i> | <i>f</i> |
|     | <i>B<sup>i</sup>B<sup>i</sup></i> | <i>f f<sub>u</sub></i> | <i>f<sub>u</sub></i> | <i>f B<sup>i</sup>B<sup>i</sup></i> | <i>B<sup>i</sup>B<sup>i</sup> f<sub>u</sub></i> | <i>f</i> |          |                      |          |
| ♀   | 1664                              | 1404                   | 3                    | 3                                   | 39  | 37       | 3150     | 1                    | 1        |
| ♂   | 1449                              | 1193                   | 3                    | 3                                   | 29  | 42       | 2719     | 1                    | 2        |

Double-infrabar over infrabar has also been tested, in the hope of obtaining triple-infrabar (table 18).

No triple-infrabar was detected; but its absence is not surprising, since the corresponding round occurred only once, and since it is not at all sure that the triple form could be distinguished from the double one.

TABLE 18

$$\frac{B^i B^i}{f B^i f_u} \text{♀} \times f B^i f_u \text{♂}.$$

| SEX | DOUBLE INFRABAR AND INFRABAR* |                        |                      |          |       | ROUND    |
|-----|-------------------------------|------------------------|----------------------|----------|-------|----------|
|     | 0                             |                        | 1                    |          | Total | <i>f</i> |
|     | +                             | <i>f f<sub>u</sub></i> | <i>f<sub>u</sub></i> | <i>f</i> |       |          |
| ♀   | 851                           | 777                    | 25                   | 31       | 1684  | 1        |
| ♂   | 665                           | 750                    | 26                   | 29       | 1470  | 0        |

\* The double-infrabar and infrabar flies are not entered separately. In the original counts they were separated; but the classification is uncertain at times.

One further type of female was tested, namely, bar-infrabar over infrabar-bar (table 19).

The one mutant infrabar obtained in the series is in agreement with the sequence in which bar and infrabar were supposed to lie in the two X chromosomes of the mother; and this mutant is also the only one yet obtained from a mother carrying a double-type allelomorph in each X, where forked and fused were heterozygous. It therefore serves to complete the demonstration of the relation of crossing over (between forked and fused) to mutation in the bar locus.

The experiment of table 19 was, however, planned for another purpose. It will be seen that in the mother, which was  $\frac{B B^i}{B^i B}$ , equal crossing over might give rise to new types, namely, double-bar and double-infrabar.

TABLE 19  
 $\frac{BB^i}{fB^iB f_u}$  ♀ ×  $f B^iB f_u$  ♂ (1st row), or  $f f_u$  ♂ (2nd row).

|    | BB <sup>i</sup> AND B <sup>i</sup> B |                  |                |    |     |                  |                |    | B <sup>i</sup> |   |
|----|--------------------------------------|------------------|----------------|----|-----|------------------|----------------|----|----------------|---|
|    | ♀                                    |                  |                |    | ♂   |                  |                |    | ♂              |   |
|    | 0                                    |                  | 1              |    | 0   |                  | 1              |    | Total          | f |
|    | +                                    | f f <sub>u</sub> | f <sub>u</sub> | f  | +   | f f <sub>u</sub> | f <sub>u</sub> | f  |                |   |
| 1* | ...                                  | ...              | .              | .. | 95  | 74               | 3              | 4  | 176            | 0 |
| 2  | 428                                  | 335              | 8              | 15 | 341 | 319              | 7              | 16 | 1469           | 1 |

\* Females not counted in the mating to B<sup>i</sup>B male.

The first could not be distinguished, in somatic appearance, from the unmutated double types (BB<sup>i</sup> and B<sup>i</sup>B); but the double-infrabar should be readily detected. Such an individual would be forked. It may accordingly be concluded that none of the 35 forked (not-fused) offspring represented equal crossing over between the halves of the two double-type bar allelomorphs present. It therefore seems probable that crossing over of this kind is not much, if any, more frequent than is that between the two elements of a double-type allelomorph when the other chromosome carries round (tables 4, 5, 12, 13, 15, 16).

Several of the above tables agree with a small series of infrabar over round, heterozygous for forked and for fused, in showing that infrabar lies between forked and fused. It must clearly be either an allelomorph of bar, or bar plus a modifier that lies near bar.

The experiments with bar-infrabar and with infrabar-bar show that these two types both contain infrabar as a unit distinct from bar.

Since bar-infrabar was produced by an unequal crossover that occurred very close to the left of infrabar, it becomes unlikely that a modifier can lie on that side of the bar locus; infrabar-bar furnishes similar evidence that there is no modifier to the right. All the evidence thus indicates clearly that the infrabar gene is really a modification of the bar gene itself.

#### FREQUENCY OF BAR MUTATIONS

The data presented in tables 1 to 19 have been examined in an attempt to formulate some general statements as to the relative frequency of the

various types of mutation in the bar locus. It is probable that homozygous double types, and double over single show the lowest frequencies of mutation, and that double type over round shows the highest. Both these results might have been expected. There is, however, so much variability among crosses of the same general nature that these conclusions must be accepted with caution. For example, the two largest series are those from homozygous bar (20,438 offspring) and from homozygous infrabar (16,918 offspring). The mechanical conditions should be alike in the two cases, since both represent homozygous single types. Yet from the first there appeared 0.03 percent of reversions, or 1 in each 2920 offspring; from the second there were 0.11 percent, or 1 in 940 offspring. In view of such unexplained differences as this, and in view of the statistical difficulty of determining probable errors for such small percentages, it does not seem profitable to discuss further this aspect of the data, except to note that mutation frequency does not appear to be correlated with frequency of forked-fused crossing over.

#### THE CROSSOVER VALUES FOR FORKED, BAR AND FUSED

The experiments recorded in tables 1 to 19 include by far the largest series of data yet accumulated for the crossover values of the three loci, forked, bar and fused. These are summarized in tables 20 and 21. In these tables all the mutant individuals have been omitted. Their inclusion would not have affected any of the values appreciably. In table 22 the

TABLE 20  
*f B f<sub>u</sub> crossing over.*

| TYPE OF FEMALE TESTED   | NON-CROSSOVERS | CROSSOVERS |          | TOTAL  |
|-------------------------|----------------|------------|----------|--------|
|                         |                | Region 1   | Region 2 |        |
| Single type over round. | 7,193          | 18         | 185      | 7,396  |
| Double type over round  | 22,937         | 52         | 603      | 23,592 |
| Double type over single | 3,279          | 12         | 90       | 3,381  |
| Total.....              | 33,409         | 82         | 878      | 34,369 |
| Percentage.....         | 97.21          | 0.24       | 2.55     |        |

data already published on these loci are included with the above, in order to arrive at final "map values" for the three loci.

On the basis of these data, it seems best to map forked 0.2 unit to the left of bar, that is, at 56.8; and fused 2.5 units to the right of bar, at 59.5, thus making the forked-fused interval 2.7 units.



## FACET NUMBER

It has been shown by ZELENY and MATTOON (1915), MAY (1917) and ZELENY (1922) that selection for number of facets is effective in isolating lines of bar flies with high facet numbers or with low. Though no detailed genetic analysis has yet been reported, there is abundant evidence in

TABLE 21  
*ff<sub>u</sub> crossing over.*

| TYPE OF FEMALE TESTED          | NON-CROSSOVERS | CROSSOVERS | TOTAL  |
|--------------------------------|----------------|------------|--------|
| Single type over single. . . . | 48,966         | 1290       | 50,256 |
| Double type over single. . . . | 9,717          | 246        | 9,963  |
| Double type over double. . . . | 3,175          | 114        | 3,289  |
| Total. . . . .                 | 61,858         | 1650       | 63,508 |
| Percentage. . . . .            | 97.40          | 2.60       |        |

these papers that ordinary bar stocks are heterogeneous for modifiers (not in the bar locus) that affect facet number. This is also the impression I have gained from extensive but less exact studies, with numerous crosses involving bar.

TABLE 22  
*Total linkage data.*

| LOCI                  | SOURCE OF DATA          | CROSSOVERS | TOTAL  | PERCENT |
|-----------------------|-------------------------|------------|--------|---------|
| <i>fB</i>             | MORGAN and BRIDGES 1916 | 8          | 1,706  | 0.5     |
|                       | BRIDGES 1917            | 5          | 980    | 0.5     |
|                       | Table 20                | 82         | 34,369 | 0.2     |
|                       | Total                   | 95         | 37,055 | 0.26    |
| <i>ff<sub>u</sub></i> | WEINSTEIN 1918          | 200        | 8,298  | 2.4     |
|                       | Table 21                | 1650       | 63,508 | 2.6     |
|                       | Total "primary" data    | 1850       | 71,806 | 2.58    |
|                       | MORGAN and BRIDGES 1916 | 37         | 1,201  | 3.1     |
|                       | Table 20                | 960        | 34,369 | 2.8     |
| Grand total           | 2847                    | 107,376    | 2.65   |         |
| <i>Bf<sub>u</sub></i> | MORGAN and BRIDGES 1916 | 222        | 8,768  | 2.5     |
|                       | BRIDGES 1917            | 46         | 1,401  | 3.3     |
|                       | Table 20                | 878        | 34,369 | 2.5     |
|                       | Total                   | 1146       | 44,538 | 2.57    |

Another source of variability in facet number is temperature. It was shown by SEYSTER (1919) that high temperature decreases the facet number of bar, and this relation has been studied in great detail by KRAFKA (1920), ZELENY (1923), A. H. HERSH (1924) and R. K. HERSH (1924). These observers have shown that the effect is present, though in varying degree, in double-bar, round, and in various heterozygotes, as well as in bar. Although these studies furnish essential data for any complete analysis of the mode of action of the bar series of allelomorphs upon development, they need not be further discussed here.

The evidence just reviewed indicated that it would be necessary to get stocks as nearly uniform for modifiers as possible, and also to control the temperature, if any reliable data were to be collected as to the relative effectiveness on facet-number of the various combinations of bar and infrabar. Accordingly, a female that was  $\frac{fBf_u}{fBB}$ ,<sup>7</sup> was mated to a round-eyed vermilion female from vermilion stock. The descendants from this mating were inbred (brother-sister pair matings) for seven generations. In each generation a female heterozygous for two of the three bar allelomorphs concerned was mated to a male carrying the third allelomorph. The line was made homozygous for forked; but both vermilion and fused were eliminated. No other selection was practiced. A female of the third inbred generation was mated to an infrabar male, and a daughter that was infrabar over round was mated to a male from the fourth generation. For three more generations the infrabar series was crossed to the inbred line. After the eighth generation the pedigrees are somewhat more complex, but as close inbreeding as was compatible with maintaining four allelomorphous sex-linked genes was continued for six more generations before the facet counts were begun. The other types studied (bar-infrabar, reverted bar, etc.) were all crossed to the inbred stock just described, at least five times (mostly using females to allow crossing over and get as much of the X's uniform as possible) before being used in the counts. This procedure should have resulted in making the various stocks practically alike with respect to modifying genes, and the results obtained are sufficiently consistent to indicate that there was no heterogeneity in major modifiers, though it is still possible to interpret some of the minor differences observed as being due to uneliminated diversity in modifiers.

<sup>7</sup> Derived from the experiments of table 1, so that the *BB* of the following discussion is known to have come from the *BB* male of that table, and to have been derived from homozygous bar by forked-fused crossing over.

The temperature control used was not very exact, but maximum-minimum daily records show that 25°C was maintained to within about  $\pm 1^\circ\text{C}$ , and even these deviations were probably of short duration. For the main body of the experiments it has not proved possible to detect any systematic effect of the fluctuations in temperature that did occur. The few experiments in which such an effect is perhaps present will be specified when described.

The facets of the smaller eyes,—up to and including homozygous bar,—were counted directly under the binocular microscope, usually on etherized flies, but in some cases on alcoholic specimens. The eyes larger than this were not found to be workable by this method. Such specimens were killed and cleared in KOH. The surface of each eye was then removed and mounted on a slide. By the aid of a camera lucida a drawing was made, representing each facet by a dot, and these dots were then counted, each dot being marked by a check as counted. In all cases the right eye alone was used.

The main series of data is shown in table 23.

The table shows that homozygous infrabar is about like bar over round in facet number, but the two types can be separated by a peculiarity common to all the larger infrabar and double-infrabar types, namely, a roughened appearance of the eye, due to irregularities in the rows of facets. This peculiarity is not present in bar eyes, and is almost completely recessive in bar over infrabar. In infrabar over round (which is not far from round in facet number) the roughness is variable in extent, and may be not at all evident,—in which case the type can not be distinguished with certainty from homozygous round. In other stocks, where the modifiers are different, it often happens that infrabar over round is regularly conspicuously roughened and is easily distinguishable from round. This roughness of the eyes may be taken as evidence that the infrabar gene is qualitatively different from bar, rather than being merely a fraction of bar.

The table shows that in general when bar and infrabar are both present in an individual, the infrabar produces almost as great an effect in reducing facet number as would another bar, though in the absence of bar the infrabar is far less effective. For example,  $\frac{B}{B} = 68$ ,  $\frac{B^i}{B^i} = 348$ ; but  $\frac{B}{B^i} = 74$ . And in general,  $BB^i$  is practically as effective as  $BB$  throughout the table. In two cases the observed differences, though surely not significant, indicate that  $BB^i$  is more effective than  $BB$  (that is, the combinations

with  $BB$  and with  $B^i$ ). Similar relations are shown in other parts of the table.

TABLE 23  
Facet numbers of flies carrying various combinations of bar allelomorphs.

|            |          | $BB$            | $BB^i$          | $B^iB^i$         | $B$              | $B^i$            | $+$             |
|------------|----------|-----------------|-----------------|------------------|------------------|------------------|-----------------|
| $BB$       | $n$      | 47              |                 |                  |                  |                  |                 |
|            | $M$      | $24.96 \pm .30$ |                 |                  |                  |                  |                 |
|            | $\sigma$ | $3.03 \pm .21$  |                 |                  |                  |                  |                 |
| $BB^i$     | $n$      | 20              | 51              |                  |                  |                  |                 |
|            | $M$      | $24.10 \pm .35$ | $26.69 \pm .29$ |                  |                  |                  |                 |
|            | $\sigma$ | $2.36 \pm .25$  | $3.07 \pm .21$  |                  |                  |                  |                 |
| $B^iB^i$   | $n$      | 25              | 32              | 27               |                  |                  |                 |
|            | $M$      | $26.68 \pm .29$ | $27.91 \pm .36$ | $38.19 \pm .71$  |                  |                  |                 |
|            | $\sigma$ | $2.13 \pm .20$  | $2.98 \pm .25$  | $5.48 \pm .50$   |                  |                  |                 |
| $B$        | $n$      | 51              | 86              | 28               | 50               |                  |                 |
|            | $M$      | $36.43 \pm .30$ | $37.01 \pm .25$ | $38.29 \pm .59$  | $68.12 \pm 1.09$ |                  |                 |
|            | $\sigma$ | $3.18 \pm .21$  | $3.49 \pm .18$  | $4.64 \pm .42$   | $11.15 \pm .77$  |                  |                 |
| $B^i$      | $n$      | 30              | 53              | 3                | 30               | 18               |                 |
|            | $M$      | $41.77 \pm .41$ | $37.79 \pm .52$ | 138.2            | $73.53 \pm 1.29$ | $348.4 \pm 12.4$ |                 |
|            | $\sigma$ | $3.34 \pm .29$  | $5.62 \pm .37$  | —                | $10.52 \pm .92$  | $78.0 \pm 8.8$   |                 |
| $+$        | $n$      | 161             | 70              | 27               | 25               | 25               | 25              |
|            | $M$      | $45.42 \pm .24$ | $50.46 \pm .40$ | $200.2 \pm 8.6$  | $358.4 \pm 7.9$  | $716.4 \pm 3.4$  | $779.4 \pm 4.1$ |
|            | $\sigma$ | $4.57 \pm .17$  | $4.93 \pm .28$  | $66.7 \pm 6.1$   | $58.2 \pm 5.6$   | $24.9 \pm 2.4$   | $30.6 \pm 2.9$  |
| $\sigma^8$ | $n$      | 261             | 247             | 40               | 40               | 25               | 25              |
|            | $M$      | $29.02 \pm .17$ | $29.68 \pm .15$ | $45.98 \pm 1.19$ | $91.03 \pm 1.76$ | $478.1 \pm 13.7$ | $738.8 \pm 6.5$ |
|            | $\sigma$ | $4.02 \pm .12$  | $3.94 \pm .10$  | $11.13 \pm .84$  | $16.61 \pm 1.25$ | $101.8 \pm 9.7$  | $48.1 \pm 4.6$  |

The most striking relation shown by table 23 is that the relative position of identical genes affects their action on facet number. There are three similar comparisons to be made:

$$\frac{B}{B} = 68.1 \text{ versus } \frac{BB}{+} = 45.4$$

$$\frac{B}{B^i} = 73.5 \text{ versus } \frac{BB^i}{+} = 50.5$$

$$\frac{B^i}{B^i} = 292.6^8 \text{ versus } \frac{B^iB^i}{+} = 200.2$$

<sup>8</sup> This value for  $\frac{B^i}{B^i}$  is different from the one of table 23. It is based on a series reared at the same time as the  $\frac{B^iB^i}{+}$  with which it is here compared. The difference between this value and that of the table is probably due to temperature.

Since the bar allelomorphs are to be thought of as inhibitors of facet development, it will be seen that this comparison indicates that *two bar allelomorphs lying in the same chromosome are more effective than are the same two allelomorphs when they lie in opposite chromosomes.*

Such an unexpected result must of course be checked up carefully. Only two possibilities of avoiding the above conclusion seem open. The results are due to (1) accidental differences in temperature or modifiers; or (2) the round allelomorph of bar brings about a reduction in facet number just as does bar. Both of these possibilities can be eliminated, as the following paragraphs will show.

There is no temperature effect, since in each case the cultures were reared side by side; and in the case of  $\frac{B}{B}$  versus  $\frac{BB}{+}$ , several different tests all gave the same type of result.

If the results are due to accidental modifiers it is scarcely conceivable that these should lie anywhere but near the bar locus, because of the inbreeding to which the stocks have been subjected. As will be shown below, another bar chromosome (derived by mutation from the inbred  $BB$  of this strain) has been found to give results sufficiently close to the bar used here so that the conclusions as to the effect of position must apply also to the new bar. And two other not-bar ("round") chromosomes have been found to give substantially the same result as the one employed here (see below). These facts eliminate the possibility of explaining the result as due to accidental genetic or environmental differences.

The second possible escape from the conclusion as to the effect of position lies in the assumption of an effect produced by the round allelomorph. This has been tested by determining the effect on facet number of reverted bar and reverted infrabar. Round obtained by reversion from homozygous bar or infrabar stocks cannot carry a normal allelomorph on the view advanced in this paper, unless such an allelomorph is already present in the parent stocks. But such a gene is, almost by definition, not an allelomorph of bar; and in any case cannot be supposed to produce the effects here under discussion, since the  $\frac{B}{B}$  and the  $\frac{BB}{+}$  would both carry it.

Two different rounds by reversion have been introduced into the inbred stocks, by the same method as used for  $BB^i$  and  $B^iB^i$ . Care was taken never to use flies carrying one of these reverted rounds in the same culture with the old round of the inbred stock, so that it is certain that these new rounds are really due to reversion,—if not from the supposed source, then from some of the allelomorphs within the inbred stock, since a new

reversion may have occurred during the process of getting the desired modifiers into the reverted round stocks. One of the reverted rounds, called "rev. *B*," came from the homozygous bar experiments of table 1; the other, called "rev. *B*<sup>*i*</sup>," came from the homozygous infrabar experiments of table 8. The results of these tests are shown in table 24.

TABLE 24  
*Facet counts to test nature of reversions.*

|   | CHROMOSOME TESTED AGAINST |                               |             |
|---|---------------------------|-------------------------------|-------------|
|   | <i>BB</i>                 | <i>BB</i> <sup><i>i</i></sup> | Y (i.e., ♂) |
| Wild-type (control)                     | 45.42 ± .24               | 50.46 ± .40                   | 738.8 ± 6.5 |
| Rev. <i>B</i> .....                     | 45.63 ± .45               | 45.48 ± .47                   | .....       |
| Rev. <i>B</i> <sup><i>i</i></sup> ..... | 43.92 ± .26               | 46.13 ± .36                   | 704.4 ± 8.0 |

These data suggest that the reversions,—especially the more thoroughly tested one from infrabar,—may give slightly smaller eyes than does the wild-type allelomorph. Certainly they do not give larger eyes. And still more certainly, with *BB* or *BB*<sup>*i*</sup> they do not give as large eyes as do the corresponding types  $\frac{B}{B}$  and  $\frac{B}{B^i}$ . These data eliminate the second possible method of explaining away the effect of the position of the bar allelomorphs on facet number. The conclusion must stand as stated on p. 137.

It seems probable that such an influence of the relative position of genes on their effectiveness in development may be interpreted in terms of diffusion and localized regions of activity in the cell. This idea is, however, scarcely worth elaborating until more evidence is obtained. It may, however, be pointed out that there is another possible application of the hypothesis of a position effect.

It has been shown by BRIDGES (1921) that in triploid individuals the recessive genes brown, plexus and speck do not become dominant to their normal allelomorphs even when two recessive allelomorphs and one dominant are present. Unpublished data collected by BRIDGES (in part verified in an independently arisen series of triploids in my own experiments) show that this relation is a general one for allelomorphs that do not produce an obviously intermediate diploid heterozygote. But BRIDGES (BRIDGES and MORGAN 1923) has shown that a different relation may occur, even for some of the same genes that show the former relation in triploids. In the example referred to, a portion of the second chromosome, carrying the normal allelomorph of plexus (among other genes) has become attached to a chromosome III. It is possible, therefore, to obtain individ-

uals with two complete second chromosomes, each of these carrying the plexus gene, while a normal allelomorph of plexus is present in the section attached to a chromosome III. Such individuals are plexus in appearance, —not as extreme as those in a pure plexus stock, but far more like such a stock than like the ordinary triploids carrying two plexus and one not-plexus allelomorphs. While it is possible that the difference here is due to a different “balance” of modifying genes in the extra section of chromosome II, it seems likely that the effective agent is a difference in position. It is to be remembered that in *Drosophila* the homologous chromosomes lie closely apposed in somatic divisions (see METZ 1916), so that there is probably a real difference in relative positions in the two cases.

Besides the comparison of round (ultimately from vermilion stock) with round obtained by reversion from bar and from infrabar, two other derived types have been compared (as to facet number) with the corresponding original types.

An infrabar from bar-infrabar over round (table 13) was introduced into the inbred strain by six successive backcrosses, and was then compared with the old inbred infrabar, both types being made heterozygous for double-bar. The control (old infrabar) value is rather lower than the value given in table 23, probably because the temperature ran slightly higher.

Since the difference between the two means is about 4.5 times its own probable error, it is probably significant, but more extensive data will be required to establish this point.

A female of the inbred series that was double-bar over round, mated to a round male, gave rise to one bar male by mutation. This male was mated to double-bar-over-round females, and the resulting double-bar-over-bar daughters were compared with double-bar over the old inbred bar, derived from cultures made up at the same time and put side by side. Here again there is a slight difference from the value of table 23, perhaps due to a temperature difference.

The difference between the means is slightly over 3 times its probable error, and may be considered as doubtfully significant. Here again, more data are needed.

It may be pointed out that in tables 25 and 26 the derived type is presumably the larger in both cases. It is possible that this result is to be correlated with that recorded in table 24, namely, that round by reversion is perhaps smaller than wild-type. Both relations are consistent with the view that there exists a normal allelomorph of bar that has an effect on facet number opposite to, but much weaker than, that of bar; for both

of the derived single types tested were from double type over round, so that these derived forms may really be single type plus normal allelomorph. This possibility requires further experimental investigation.

TABLE 25  
Facet counts from  $\frac{BB}{B^1}$ .

|                 | NUMBER | MEAN        | $\sigma$   | DIFFERENCE OF MEANS |
|-----------------|--------|-------------|------------|---------------------|
| Old $B^1$ ..... | 40     | 34.73 ± .35 | 3.24 ± .24 | 2.05 ± .46          |
| New $B^1$ ..... | 36     | 36.79 ± .31 | 2.76 ± .22 |                     |

TABLE 26  
Facet counts from  $\frac{BB}{B}$ .

|               | NUMBER | MEAN        | $\sigma$   | DIFFERENCE OF MEANS |
|---------------|--------|-------------|------------|---------------------|
| Old $B$       | 31     | 33.10 ± .33 | 2.76 ± .24 | 1.77 ± .56          |
| New $B$ ..... | 30     | 34.87 ± .45 | 3.65 ± .32 |                     |

#### ARE MUTATIONS IN GENERAL DUE TO UNEQUAL CROSSING OVER?

One of the first problems raised by the discovery of the nature of bar reversion is as to how widespread may be the phenomenon of unequal crossing over. One direct test has been attempted, making use of MULLER'S method of testing for the frequency of occurrence of new lethal mutations. Females were made up that carried one wild-type X chromosome and one X with the mutant genes scute (locus 0.0), echinus (5.5), crossveinless (13.7), cut (20.0), vermilion (33.0), garnet (44.4), and forked (56.8). Such females were mated to males carrying all the mutant genes named. In such matings it is possible to detect practically all the crossing over that occurs in the X chromosome, except that to the right of forked (about 13 units). Counts were made from individual females, in order to make sure that they carried no lethals. Forty-one wild-type daughters (non-crossovers) were tested from such matings, to see if the non-crossover X chromosomes carried new lethals. The only lethal that occurred was in a paternal (i.e., scute echinus crossveinless cut vermilion garnet forked) chromosome. Thirty-eight double-crossover daughters and one triple-crossover (i.e., a total of 79 crossings over) were also tested; and again no lethals occurred in any of the maternally derived chromosomes, though there were two doubtful cases of new lethals in the chromosomes derived from the multiple-recessive fathers. While this experiment was done on a



small scale, it furnishes no indication that crossover chromosomes are more likely to contain new lethals than are non-crossovers.

There is, however, another kind of evidence that argues against any general applicability of the unequal-crossing-over explanation of mutation, namely, the cases in which mutations can be shown to have occurred in the X chromosomes of males, since it may be taken as established that crossing over does not occur between the X and the Y of a male.

We have seen earlier in this paper that infrabar arose from bar in a male, and that its later behavior was not in agreement with the view that it represented a quantitative change in the bar gene, as it should if due to unequal crossing over. I have also obtained yellow, a fused allelomorph, and a lozenge allelomorph under the same circumstances, namely, from mothers with attached X's and in experiments where known sex-linked genes were present, so that breaking apart of the attached X's was known not to have occurred.

Unpublished data are available for 5 other cases of the same sort, either from attached-X or from "high-non-disjunction" mothers, as follows: rudimentary (C. B. BRIDGES), a dusky allelomorph (C. B. BRIDGES), a sable allelomorph (E. M. WALLACE), white (L. V. MORGAN), and a new lozenge allelomorph from lozenge (C. B. BRIDGES). In all of these cases, as in that of infrabar, the mutant type first appeared as a single male.

MULLER (1920) reported the occurrence of white as a "somatic" mutation in a male. From a stock in which white was not present he obtained a male with one wild-type eye and one white eye. This male also transmitted white to some (all that were tested) of his daughters. In the same paper MULLER described briefly a mosaic male that was partly yellow, and transmitted the new character to his offspring. Doctor BRIDGES informs me that he has a similar (unpublished) record for yellow. MOHR (1923 a) reports a similar case for a singed allelomorph, though here some of the X-bearing sperms carried singed, while others did not. I have observed two other such cases,—both in *D. simulans*. The mutant types dusky and fused (both corresponding to the types of the same names in *D. melanogaster*) each appeared first in an individual that showed the new character in only one wing; and in each case tests showed that some of the X-bearing sperms carried the new gene, while others did not. In all cases discussed in the last two paragraphs, genetic tests have established the allelomorphism of the new mutant genes to the old ones whose names they bear.

There is thus clear evidence that mutations have arisen in the following sex-linked loci in the germ-cells of males: yellow (3 times), white (twice), lozenge (twice), dusky (twice), fused (twice), singed (once), bar (once). It should be noted that all these loci are among the more mutable ones of *Drosophila*.

Another class of cases to which the unequal-crossing-over hypothesis is probably not applicable is that in which mutation can be shown to have occurred at some stage other than maturation. A number of such instances are on record for *Drosophila*. The mosaic males described above are examples, and a longer list of cases for autosomal mutant types and for sex-linked mutations in females could easily be compiled. But since there is evidence that crossing over does very rarely occur at somatic divisions, this evidence can hardly be considered decisive. In the case of certain types of frequently recurring somatic mutations in plants, however, the mutation occurs far too often to make an appeal to somatic crossing over seem plausible. The clearest example of this sort is the variegated pericarp of maize studied by EMERSON (1917) and others, in which a given gene mutates many separate times in a single individual plant.

Mutations are known in which there appeared to be no crossing over in the region concerned,—both in females and in males where crossing over does not normally occur at all. The previously cited cases of mutations in the X chromosomes of males are examples. These may seem to furnish conclusive evidence that mutation need not be accompanied by crossing over. There is, however, one possibility that needs to be considered in this connection.

Recent results (not yet published, but soon to appear) obtained with triploid females (BRIDGES and ANDERSON) and with females having unlike attached X's (ANDERSON, L. V. MORGAN, and STURTEVANT) have shown that crossing over must normally occur when the homologous chromosomes are doubled: that is, in a "four-strand stage" (in diploid females). These results show also that crossing over may occur between only two of the strands at a given level. Now, if it be supposed that *sister* strands may cross over with each other, there will result chromosomes in which no rearrangement of mutant genes has occurred, since sister strands come from the division of one chromosome and will be identical in the genes that they carry. Yet it is conceivable that such crossing over might be unequal, and in such a case might lead to the production of a new mutation that did not appear to be due to crossing over.

The data presented in this paper show that such an event must be extremely rare in the case of bar, since no clear case was found of bar mutation (in a female) unaccompanied by evident crossing over between forked and fused. The few exceptional cases may be accounted for in this way; but, as pointed out when they were described, it seems at least equally probable that all of them are due to experimental errors. We must conclude that sister strands do not cross over with each other; or, if they do, that the crossing over is rarely, if ever, unequal.

It is therefore unlikely that apparent non-crossover mutations in other loci are to be referred to crossing over between sister strands.

#### “PRESENCE AND ABSENCE” AND QUANTITATIVE VIEW OF MUTATION

It will be observed that the hypothesis advocated in this paper makes bar, double-bar and round by reversion (or infrabar, double-infrabar and round by reversion) represent quantitative variations of the same substance. In the case of bar and round, the hypothesis is the same as the original and most special type of quantitative view, the “presence and absence” hypothesis. But the present scheme differs from the earlier ones in that it is based on definite evidence for the occurrence of unequal crossing over. That is, the mechanism whereby the quantitative differences are brought about is an essential part of the hypothesis. In the preceding section we have seen that there is definite evidence to show that unequal crossing over is not usual in the production of new mutant types. It is especially noteworthy that this evidence was derived in part from the white locus of *Drosophila* and the variegated locus of maize,—two of the best-known examples of loci that have produced large series of multiple allelomorphs. It is clear, therefore, that the bar case does not furnish support to the idea that mutations in general are quantitative in nature. Even with respect to multiple allelomorphs, where the quantitative view has often been urged, it is obvious that, at least in the cases of white and variegated, the bar evidence does not in any way support that view.

#### ARE DEFICIENCIES DUE TO UNEQUAL CROSSING OVER?

The “section-deficiencies” described by BRIDGES (1917, 1919) and by MOHR (1919, 1923 b) are probably to be interpreted as due to losses of definite sections of chromosomes. It will be observed that bar reversion has here been treated as due to the loss of a very short section; it may accordingly be described as a deficiency that is too short to show the lethal effect and other properties of the previously described deficiencies.

When the case is stated in this way, the question at once arises: is it probable that notch and other deficiencies have also arisen through unequal crossing over? If so, the contrary crossover should be a chromosome that was double for a region corresponding to the deficient section. Such a chromosome has never been identified, but it may be doubted if it would be detected even if present. Furthermore, it might well be lethal even in heterozygous females, in which case it would not be capable of detection.

There is evidence that deficiencies may arise in other ways than by unequal crossing over. In at least one case (BRIDGES and MORGAN 1923) the section missing from a second chromosome was found to be present, but attached to a third chromosome. In this case, then, the deficiency can not have been due to unequal crossing over. The first deficiency described, that for forked and bar (BRIDGES 1917), occurred first as a single female that had obtained the deficient X from her father. Here the deficiency arose (either in a male or very early in the cleavage of a female zygote) at a time when crossing over (and bar reversion) does not normally occur. In the case of notch, also, there is evidence that the deficiency may originate at stages other than maturation. LANCEFIELD (1922) records the occurrence of a notch (probably corresponding to that of *D. melanogaster*) in *Drosophila obscura*; the mutation was first detected as two females from a pair mating that gave numerous offspring. In this case the deficiency must have originated in the gonial cells of one parent, unless the two notch females received their notch chromosomes from the father, in which case it is just possible that they came from two sperms derived from a single spermatocyte. But in this case the hypothesis of unequal crossing over remains as improbable as before. I have observed two cases in *D. melanogaster* that represent "somatic" (i.e., not occurring at the maturation divisions) occurrences of notch. In one case three notch females were produced from a single mother. The X's of the mother were attached, and the notch daughters, like all their sisters, did not carry a paternal X. These three females were all sterile, so here it was not possible to demonstrate that the new type was actually notch; but the numerous characters of notch make the identification very probable. The other case also occurred in a line in which the females all had attached X's. A female, from a line with no notch ancestry, was notch in the left wing but not in the right. The offspring showed that this female was, like her mother, heterozygous for several sex-linked genes. These included scute, 3 units to the left of notch, and crossveinless, 10 units to the right of it. Some of the eggs of the mosaic carried notch, but many of them did not.

Furthermore, tests showed that scute and crossveinless were in opposite chromosomes in both types of eggs; that is, the mutation to notch occurred at a cleavage division, and was not accompanied by crossing over between scute and crossveinless. From these three instances we may conclude that the notch deficiency may arise at stages in the life cycle at which crossing over and bar reversion do not normally occur, and, in the third case, there is definite evidence that crossing over did not occur. While it may still be supposed that unequal crossing over will sometimes give rise to section deficiencies, the evidence indicates that the three best-known examples of section deficiencies in *Drosophila* have not arisen in that way.

#### UNEQUAL CROSSING OVER AND THE EXACT NATURE OF SYNAPSIS

The data on crossing over have all indicated consistently that when two chromosomes cross over they do so at exactly corresponding levels. The case of bar is the first one in which any inequality of crossing-over levels has been detected; and we have seen in the preceding sections that an analysis of other possible instances of such an occurrence makes it probable that they must be explained in some other way. The case of bar is clearly quite exceptional. But it does serve to suggest that the exact correspondence of crossover levels, that is so constant, is not to be referred to a property common to all the genes. For unequal crossing over occurs in females that are homozygous for bar or for infrabar, and in such females these loci are alike in the two X chromosomes that cross over unequally. It is difficult to imagine how the chromosomes can pair so extremely exactly as they must do, unless in some way like genes come to lie side by side. But the present case indicates that this interpretation will have to be applied with some caution.

#### SUMMARY

1. Sixteen different kinds of changes at the bar locus are shown to occur exclusively, or nearly so, in eggs that undergo crossing over at or near the bar locus.

2. This result can be explained if it is supposed that such crossovers are unequal, so that one daughter chromosome gets two representatives of the bar locus while the other receives none.

3. Only one mutation in this locus has been shown to have occurred in the germ track of a male. This one gave rise (from bar) to a new and less extreme allelomorph called infrabar.

4. Infrabar does not appear to represent a quantitative change in the bar gene.

5. When, by unequal crossing over, bar and infrabar come to lie in the same chromosome, they maintain their separate identities, and may be recovered again.

6. In such double forms the two elements also maintain their sequence in the same linear series as the rest of the genes. It is thus possible to obtain bar-infrabar and also infrabar-bar. These two types look alike, but can be distinguished by their origin and by the usual tests for determining the sequence of genes.

7. Facet counts are given for all the possible combinations of the following members of the bar series: round, infrabar, bar, double-infrabar, bar-infrabar, double-bar.

8. Analysis of these data shows that two genes lying in the same chromosome are more effective on development than are the same two genes when they lie in different chromosomes.

9. A general survey makes it seem improbable that many mutations in other loci are to be explained as due to unequal crossing over.

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