A FURTHER STUDY OF THE SO-CALLED MUTATION AT THE BAR LOCUS OF DROSOPHILA

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Received December 6, 1927

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INTRODUCTION

An earlier study (STURTEVANT 1925) of the "mutations" at the bar locus of *Drosophila melanogaster* led to the conclusion that these mutations are due to unequal crossing over at the bar locus. Reverted bar is no-bar, having no allelomorph at the bar locus; double-bar (the ultra-bar of ZELENY) has two bar genes lying next each other in the linear series of genes.

The present paper gives the results of a few experiments designed to clear up certain points left doubtful by the earlier study.

TIME OF OCCURRENCE OF BAR MUTATION

ZELENY (1921) recorded five cases in which more than one reversion appeared in a single culture, and concluded from this evidence that reversion probably occurs, sometimes at least, in oogonial cells. Since the five cultures in question were not from individual females they do not prove that several reversions may be produced by a single mother, but they are at least suggestive. If, following this suggestion, one assumed that unequal crossing over leading to bar reversion occurred at some stage earlier than the maturation divisions, it would be expected that normal crossing over between forked and fused would occur after it. The result should be that about 3 percent of the "mutations" at the bar locus would appear to be unaccompanied by forked-fused crossing over—and the data on record show a small number of such exceptional cases. A further consequence, open to experimental test, should be that bar reversion would not interfere with crossing over in neighboring regions.

Accordingly an experiment has been carried out with females homozygous for infrabar (used instead of bar in order to make the separation for eye-color easier), and heterozygous for garnet-2 forked and fused (see figures 1 and 2).
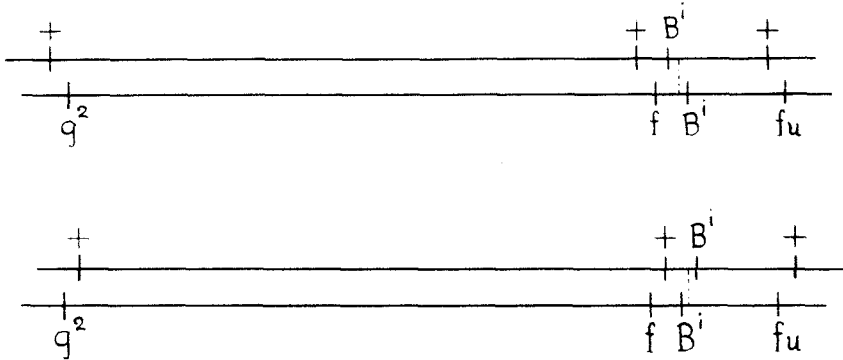

Figure 1 (above) and Figure 2 (below).

TABLE 1

$$Offspring\ from\ \frac{B^i}{g^2 f B^i f_u}\ ♀ \times g^2 f B^i f_u\ ♂.$$

	B^i									B^iB^i				+			
	0		1		2		1,2		Total	♀*		♂		♀*		♂	
¬	$g^2 f f_u$	$f f_u$	g^2	f_u	$g^2 f$	f	f	$g^2 f_u$		f_u	$g^2 f$	f_u	$g^2 f$	f_u	$g^2 f$	f_u	$g^2 f$
	19536	14939	2351	2678	574	647	4	7	40736	4	2	2	6	4	7	11	4

* The "mutant" females were, of course, all heterozygous for B^i, received from the father.

A small series (1647 individuals) from $\dfrac{f\ B^i}{g^2\ B^i f_u}$ also gave a reversion ($f f_u$) that was not a $g^2 f$ crossover. In the two series there is a total of 41 "mutant" individuals, all of them crossovers between forked and fused, but none of them garnet-2 forked crossovers. There are 5040 garnet-2 forked crossovers among 40736 unmutated offspring of the table, or 12.4 percent, in good agreement with the 12.1 percent recorded by MORGAN, BRIDGES and STURTEVANT (1925).

If mutation and garnet-2 forked crossing over are unrelated, the chance that none of the 41 mutant individuals should be a garnet-2 forked crossover is therefore $(1-.124)^{41} = .004$, or 1 in 250. One may safely conclude that the unequal crossing over that gives rise to mutation at the bar locus

interferes with crossing over in the garnet-2 forked region, just as does ordinary crossing over between forked and fused, and hence that the unequal crossing over occurs at the same time as ordinary crossing over.

There must, therefore, be other explanations of the two relations that led to the experiment. The occurrence of mutations not accompanied by forked fused crossing over is open to two interpretations, as previously pointed out (STURTEVANT 1925). Crossing over between sister strands is a possible explanation, but if it occurred at all would be expected to give many more apparent non-crossover mutations than have been observed. The most probable view seems to be that all such records are due to experimental errors. It is to be noted that none occurred in the present series of experiments.

As pointed out above, ZELENY's data do not prove that more than one mutation was produced by a single mother in any case. However, in the present series there were four separate cultures in which a single mother produced two mutant offspring, as follows:

Culture	Mutant offspring
21794	$B^i B^i f_u$, $g^2 f B^i B^i$
22254	f_u, $g^2 f$
23611	f_u, f_u
23898	$g^2 f B^i B^i$, $g^2 f$

Examination of these data shows that in three of the four cases the two individuals cannot have been produced by one crossing over.[1] In the first case, for example, the crossing over that produced a $B^i B^i f_u$ chromosome would also give a $g^2 f$ (figure 1)—but could not give $g^2 f B^i B^i$, the combination actually recovered. It follows that the production of more than one mutant offspring by a single mother must be due to the occurrence of more than one unequal crossing over in that mother. Whether or not such coincidences are more frequent than would be expected with a random distribution can hardly be determined without the collection of a vast amount of accurate data.

RELATIVE NUMBERS OF REVERSIONS AND DOUBLE-BARS

On the hypothesis of unequal crossing over a homozygous bar stock should produce equal numbers of double-bar and wild-types "mutants," but all observers have found distinctly more wild-type than double-bars.

[1] Unless by chance one of the rare "normal" forked fused crossovers followed the mutation in one egg and not in the other in each case. The chance that this should have happened in all three instances is negligible.

I have suggested (STURTEVANT 1925) that this is due to two factors—lower viability of double-bar and greater likelihood of overlooking mutant individuals. The viability factor can be directly measured. Using the inbred stocks prepared for the facet counts recorded in my earlier paper, I have made counts from double-bar over round mated to round and to bar. A small series gave 60 double-bar over bar females to 82 bar over round, and 126 double-bar males to 161 round males. Equality is expected in each case, and the deviations are due to differential viability. It follows that, in the combinations met with as mutant individuals in a homozygous bar stock, double-bar has a viability about 75 percent that of round.

ZELENY (1921) records the production of 52 reversions and 3 double-bars among 85008 specimens examined. In addition there were at least 5 double-bars that were not tested, so that we may take $52:8 = 6.5:1$ as the observed ratio. ZELENY argues that there were probably very few mutant individuals overlooked—probably only a few of the double-bar females. I obtained (STURTEVANT 1925) 8 reversions and 2 double type from homozygous bar, 18 reversions and 3 double types from homozygous infrabar, and 2 reversions and 3 double types from bar over infrabar—a total of $28:8 = 3.5:1$. This is somewhat closer to the expected equality than ZELENY's $6.5:1$, but the experiments here reported for homozygous infrabar gave $27:14 = 1.9:1$. This is still a definite excess of reversions, over what can be accounted for by differential viability, especially as the viability difference is less for double-infrabar than for double-bar, but more carefully conducted experiments and more experience having lowered the ratio from $3.5:1$ to $1.9:1$, I feel confident that there is still room for technical improvement sufficient to remove the remaining discrepancy.

<center>POSITION EFFECT</center>

It was shown (STURTEVANT 1925) that two bar genes lying in the same chromosome are more effective in reducing facet number than are the same two genes when they lie in different chromosomes. It seemed possible that such a relation might also hold for non-allelomorphic genes, but the one test, now to be described, has given a negative result.

The two dominat genes delta and hairless lie 3 units apart in the third chromosome (BRIDGES and MORGAN 1923). Hairless causes a reduction in bristle number, and delta partially counteracts this effect. These genes were selected for study because they lie close together, and because bristle number is convenient for quantitative work. An inbred strain was established, in which repeated brother-sister matings and repeated use of crossovers between delta and hairless made it probable that modifiers were

uniform. Matings were made in parallel, delta×hairless and delta hair-less×wild-type. All offspring from these matings were reared at 26°C. The results are shown in table 2.

TABLE 2

Number of dorsocentrals.

Mating	3	4	5	Total	Mean
Δ X H	5	95	0	100	3.95
Δ H X +	10	167	1	178	3.95

While the numbers here are small, the exact agreement in mean number of dorsocentrals makes it seem certain that there is no position effect.

As pointed out in my earlier paper, the pairing of homologous chromosomes that occurs in somatic divisions in Drosophila suggests that there may be a position effect normally present of such a nature that allelomorphs reinforce each other's effects. Such an effect was offered as an explanation of the different dominance relations observed in triploids and in cases of translocation (MORGAN, BRIDGES and STURTEVANT 1925). A new set of facts makes this interpretation now seem improbable. I have shown (STURTEVANT 1926) that the C_{III} present in ebony and other stocks is due to the inversion of a large section of the third chromosome, and there is now evidence that the $C_{II\ L}$ of the curly stock and two other $C_{III\ R}$'s are due to similar inversions. In flies heterozygous for such inversions the position relations of many genes are greatly changed, but no difference in somatic appearance has yet been detected in such flies. It follows that changes in position of this order are not usually of developmental significance. One may conclude that the association of the two elements in double-bar is much more intimate than the association between identical loci in homologous chromosomes, and that the "position effect" perhaps rests on something more than mere closeness together.

FACET COUNTS OF DERIVED TYPES

Facet counts recorded (STURTEVANT 1925) for a reversion from bar and one from infrabar suggested that these derived rounds might be different from the wild-type, since both strains gave lower facet numbers with bar-infrabar (one of them also with double-bar and in males) than did wild-type. The inference that there is a wild-type allelomorph of bar, absent in reversions, and having an effect on facet-number opposite to that of bar, was further tested by studying an infrabar from bar-infrabar over round, and a bar from double-bar over round. Both of these derived types when tested against double-bar, gave somewhat larger facet-numbers than the controls (original infrabar and original bar, respectively). It was suggested

that perhaps the derived types carried wild-type allelomorphs, which partially counteracted the effects of the bar and infrabar genes. However, the differences were small in all these experiments, and it was emphasized that more tests were needed.

Several new derived types have now been studied, and have not borne out the assumption of a wild-type allelomorph.[2]

Two new infrabars, both from double-infrabar over reverted infrabar, would have been expected to give the same results as the old infrabar, even if there is an effective wild-type allelomorph—since such an allelomorph should not be present in either the old or the derived types. The result of a test against double-bar was: control (old infrabar) 35.7 facets, new infrabar 33.6 and 35.4 facets, respectively (individuals counted, 51, 30, and 30). The expectation is clearly correct; there are no significant differences.

A new reverted double-infrabar was tested, and at the same time the reverted infrabar of the earlier experiments was used. These and wild-type were tested against double-bar. Forty females of each type were counted, with these results: wild-type, 43.8; reverted infrabar, 44.4; reverted double-infrabar, 43.2. The reverted infrabar differs from wild-type in the opposite direction from the earlier test, and none of the differences here seem significant.

Two new bars, both from double-bar over round, were tested in three ways, as shown in table 3.

TABLE 3

Facet counts.

	OVER B^iB^i		HOMOZYGOUS ♀		MALE	
	n	M	n	M	n	M
Bar (control)	45	37.4	25	61.8	30	69.8
First derived bar	45	38.2	25	54.4	30	67.6
Second derived bar	45	35.4	25	49.6	60	61.0

Both of the derived types give lower facet numbers than the control, and in the case of the second derived type this difference is probably signi-

[2] The reverted double-infrabar and the first derived bar arose in the inbred forked stocks that were used in the earlier study. The other derived types were crossed at least five times to these stocks before counts were made. All the other stocks used in the present experiments were the same as those described in the earlier paper. The arguments for supposing modifiers to be alike are the same as in the earlier experiments. The temperature was $25° \pm 1°$.

ficant—but the difference is in the opposite direction from that found in the earlier experiments.

The conclusion seems to be justified that the observed differences are due to uneliminated modifiers or to chance environmental differences. In any case the new results do not at all support the hypothesis of a wild-type allelomorph affecting facet-number.

The three homozygous bar types recorded above averaged 55.3 facets; a double-bar over-round series of 40 females reared at the same time gave a mean of 43.8. This gives a position effect of the same kind as the data in the earlier paper, but rather less in extent.

REVERTED INFRABAR AND CROSSING OVER

Reverted bar or infrabar may be considered as a deficiency which is so short that it does not have the lethal effect of most deficiencies. It has been shown by BRIDGES (1917, 1919) and MOHR (1923) that females hetero- zygous for the usual type of deficiency give no crossing over in the section covered by the deficiency. This fact suggested another test of the relation between reverted bar and wild-type. It has been shown (STURTEVANT 1925) that any double-type over wild-type gives about 0.1 percent of single-type, the mutant individuals always being forked-fused crossovers. That is, in such heterozygous females there is crossing over between the two elements of the double-type. A test has now shown that reverted infrabar gives the same result as normal with double infrabar (table 4).

TABLE 4

$$\text{Offspring from } \frac{B^iB^i}{fr_{ev}f_u} \, ♀ \times fr_{ev}f_u \, ♂.$$

0		1		2		TOTAL	MUT.
B^iB^i	ff_u	f_u	fB^iB^i	$B^iB^if_u$	f		fB^i
1474	1415	1	3	35	42	2968	2

IS A WILD-TYPE ALLELOMORPH OF BAR NORMALLY PRESENT?

The results reported in this paper indicate that reverted bar and re- verted infrabar do not differ from wild-type in their effect on facet-number or in their crossing over relations in heterozygotes with double-types. No other kind of test appears to be available, so the simplest conclusion is that ordinary stocks carry no allelomorph of bar. This is consistent with the fact that the change from round to bar has been detected only once in D. melanogaster, and no similar mutation has occurred in any other species.

One may surmise that the stock that gave rise to the original bar mutation had some sort of allelomorph already present—though other assumptions may also be made. It should be noted that, if a normal allelomorph were present in a stock and underwent unequal crossing over, as bar and infra-bar do, the only stable condition would be that in which no bar allelomorph was present. While unequal crossing over does not alter the number of bar allelomorphs present in a stock, any strain without such allelomorphs will breed true, while any strain in which they are present will still give occasional individuals lacking them. If it be granted that Drosophila populations show great fluctuations in numbers, and often are reduced to very few individuals—as is very probable from their habits—then it would, in fact, be expected that unequal crossing over would have reduced most strains to the "no-bar" condition.

SUMMARY

1. "Mutation" at the bar locus interferes with crossing over in the neighboring garnet-forked interval, indicating that unequal crossing over at the bar locus occurs at the same time and by the same mechanism as normal crossing over.

2. Homozygous bar or infrabar stocks probably give equal numbers of reversions and of double-types; but the latter are somewhat less viable and much less easily detected, so that reversions are found more often. Improved technique has, however, decreased the observed difference.

3. The dominant mutant types Delta and Hairless show no "position effect" on bristle number—that is, $\Delta H/+$ and Δ/H do not differ in bristle number. This and other evidence suggests that relative position of genes is not usually significant in development—though new data agree with old in showing that it is significant in the case of bar.

4. New data indicate that reverted bar (or reverted infrabar) is not different in facet-number from wild-type, and that derived types in general show no effects of their past history.

5. Double-infrabar over reverted infrabar gives infrabar.

6. The last two points indicate that reverted bar (or reverted infrabar) is not different from wild-types, and the conclusion is drawn that ordinary wild-stocks carry no bar allelomorph.

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