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SHORT PAPER

Bar reversion and hybrid dysgenesis in *Drosophila melanogaster*: reversion in males and in inversions

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SUMMARY

The effect of the P-M system of hybrid dysgenesis in *Drosophila melanogaster* on unequal crossing-over was studied using the Bar duplication. This system of dysgenesis had no demonstrable effect on the rate of Bar reversion. In the course of the study it was found that there was a greatly reduced rate of reversion in two homozygous inversion stocks. Further, one revertant was found which may result from unequal crossing over at the Bar locus in males.

1. INTRODUCTION

A transposable genetic element called the P factor has recently been identified by molecular techniques as the probable cause of the P-M system of hybrid dysgenesis in *Drosophila melanogaster* (Bingham, Rubin & Kidwell, 1982), This type of dysgenesis affects the germ line of hybrid offspring of females of M strains, which do not carry the P factor, and males of P strains, which carry the factor integrated into their chromosomes (Bingham *et al.* 1982). One interesting property of dysgenesis which is only found in association with the P-M system is the induction of male recombination. The recombination might occur at points of chromosomal breakage, at sites of P factor integration or at sites determined by an as yet unspecified process. Dysgenic recombination does not cause large deletions as a consequence of random chromatid breakage and reunion since Sved (1978) and Isackson, Johnson & Denell (1981) have shown that the recombinant chromsomes induced by dysgenesis are usually viable in homozygous condition. The possibility remains that such recombination may be unequal, although homozygous lethality does not result.

It was decided to investigate the dysgenic induction of unequal crossing over by using the Bar mutation since it has been shown that reversion of the mutation to wild type is due to unequal crossing over between chromatids carrying the 16A duplications causing the Bar phenotype (Sturtevant & Morgan, 1923; Bridges, 1936; Sutton, 1943; Peterson & Laughnan, 1963). Bar reversion has not been found in males by the technique of examining patroclinously descended *X*-chromosomes (Peterson & Laughnan, 1963). So a new experimental design using female inversion heterozygotes was utilized in this study. The changes in the pattern of female recombination induced by dysgenesis

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(Kidwell, 1977) are small when compared to the non-dysgenic levels of such recombination. However, as this might not be the case for unequal crossing over it was decided to extend the study of reversion to females.

2. MATERIALS AND METHODS

All experiments were performed using standard semolina-treacle-agar medium at 25 ± 2 °C. Cultures were contained in 190 ml milk bottles or 2·5 × 10 cm vials.

The stocks used were:

B = *B bb*$^{+}$ provided by Dr L. Sandler, Department of Genetics, University of Washington, Washington.

CS = Canton S, provided by Dr M. G. Kidwell (Kidwell, Kidwell & Sved, 1977).

dl-49 = *In*(1) dl-49, *y ct*^{ns} *v B*, provided by the Mid-America Drosophila Stock Center, Bowling Green State University.

Table 1. *Bar reversion in the progeny of crosses of tested females*

Line	Test female*	Male parent*	Males revertant	Bar males	Females revertant	Bar females
1	*B/B*	—	10†	10174	1	3492
2	*B* × (*B* × CS)	—	4	5884	—	—
3	*B* × (*B* × H)	—	3	2500	—	—
4	*B Cy Ubx*‡	—	6	4005	—	—
5	*B Cy* +‡	—	3	2306	—	—
6	*B* + *Ubx*‡	—	4	3332	—	—
7	*B* + +‡	—	2	1173	—	—
8	*dl*-49	*dl*-49	1	9207	2	8713
9	*dl*-49 × (*dl*-49 × H)	*dl*-49	0	4545	—	—
10	*dl*-49 × *B*	*dl*-49	3§	1804	2§	1436
11	*dl*-49 × (*B* × H)	*dl*-49	2‖	2202	—	—
12	*dl*-49	*f fu*	2	4594	2	4792
13	*dl*-49	*B*	1	3084	2¶	2996
14	*dl*-49	(*B* × CS)	0	5216**	2	3372
15	*dl*-49	(*B* × H)	—	—	1	2003
16	FM7	—	2	9721	0	2471
17	FM7 × (FM7 × CS)	—	0	3250	—	—
18	FM7 × (FM7 × H)	—	0	1032	—	—

* Progeny of the cross of these males and females were scored for reversion. Sibs or *B* males were used unless otherwise stated. In all crosses, the female parent is written first. Parentheses enclose the types of cross used to produce an F_1 hybrid.

† Including one cluster of 13 revertants treated as one event.

‡ See text for derivation of these female types.

§ Revertants were wild type in eye colour except for one male.

‖ Both revertants were vermilion in eye colour.

¶ Both revertant events were clusters of two individuals.

** Lumped with *dl*-49 × (*B* × H) males.

FM7 = *In*(1) FM7, y^{31d} sc^{8} w^{a} sn^{x2} v^{of} g^{4} *B*, provided by Dr R. Frankham, School of Biological Sciences, Macquarie University N.S.W.

f fu = *f fu* (balanced over *ClB*) provided by the Mid-America Drosophila Stock Center.

H = Harwich, provided by Dr Kidwell (Kidwell *et al.* 1977).

H-41 = *In*(1) sc^{11} sc^{2sR+s} w^{a} *B*; *In*(2LR) SM1, al^{2} *Cy* cn^{2} sp^{2}/*In*(2LR) bw^{v1}, ds^{33k} dp^{ov} bw^{v1}; *In*(3LR) Ubx^{130}, Ubx^{130} e^{s}/*In*(3LR) *C*, *Sb*; spa^{pol} provided by Dr Frankham.

All of these stocks are M except for Harwich which is P.

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Females with two Bar chromosomes were crossed with males (Bar-eyed unless otherwise specified) which enabled the distinction to be made between patroclinous descent and reversion. Progeny of these crosses were scored for reversion, but not for Ultrabar since the overlap of the ranges of Bar and Ultrabar phenotypes makes scoring uncertain. Males were considered revertant if they were wild type in appearance. The genotype of apparently heterozygous (revertant) female progeny was confirmed by

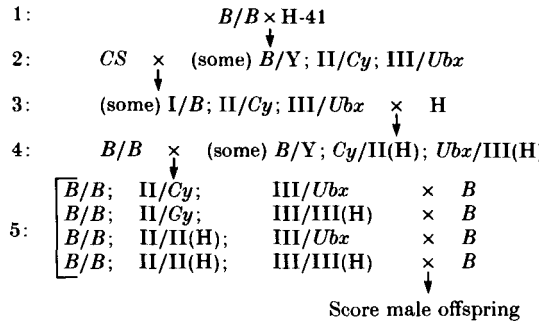

Fig. 1. Mating scheme used to provide Bar females of known autosomal constitution. Harwich chromosomes are identified by (H).

backcrossing to Bar males. This backcross also enables identification by linkage relations of the parental chromosome which has undergone reversion when the F_1 female is heterozygous for a locus other than Bar. Cytological examinations of revertants were made on polytene chromosomes prepared by the method of Lefevre (1976). Tests of the fertility of females followed Colgan & Sved (1982).

Reversion was scored among the progeny of pure strain flies, of hybrids of two Bar-carrying strains and of dysgenic and non-dysgenic backcross flies. Details of the crosses are given in Table 1. The effect of individual chromosomes from the Harwich P stock was tested using the design shown in Fig. 1. This scheme produces a male in generation 4 that carries the Bar chromosome of the *B* stock and which is heterozygous for the major Harwich autosomes and dominantly marked balancer chromosomes. The male was mated to a *B*/*B* female and the genotypes of the female progeny were scored. These females were also scored for fertility and Bar reversion was scored in their male progeny.

3. RESULTS AND DISCUSSION

The results for the various crosses are given in Table 1. The female parent is written first in each cross. Parentheses enclose a cross used to produce an F_1 individual. The rate of reversion in the progeny of $B \times (B \times H)$ females (line 3) is not significantly higher than the rates in the non-dysgenic flies of lines 1 and 2. However, the level of hybrid dysgenesis in these females would be reduced by chromosomal segregation in the F_1 male, as some of them would have few or no Harwich chromosomes. This complication is avoided by the tests of the effects of individual Harwich chromosomes. The results of this experiment are given in lines 4–7. In fertility tests of these females six of 37 *B Cy Ubx* females were sterile, as were eight of 40 *B Cy*+ (carrying the Harwich third chromosome), 23 of 57 *B*+ *Ubx* (carrying the Harwich second chromosome) and 32 of 46 *B*+ +. The fact that there is no commensurate increase of reversion rate with increasing intensity of dysgenic effects suggests that P-M dysgenesis does not markedly increase unequal crossing over at the Bar locus in females.

There is also no apparent effect of dysgenesis on the rate of reversion in male progeny of females homozygous for the *dl*-49 or FM7 inversions. Interpretation of this observation is complicated by a significantly low rate of reversion in the control crosses. The absence

of reversion in the progeny of dysgenic flies does, however, imply that no mechanism, such as breakage of chromosomes, which is peculiar to dysgenic flies is operating to increase the level of reversion above the base rate of the particular stock.

Reversion in female progeny of inversion homozygotes is not reduced to the same extent as reversion in males. There were, in total, nine revertants in 21876 female progeny of *dl*-49 homozygotes. This can be compared with the 32 revertants in the 29374 male progeny of the *B*/*B* homozygotes. It is hard to assess the significance of the comparison since some of the female revertants may derive from the male parent (see after) and since the rate of reversion is underestimated by scoring females because of the overlap of the homozygous Bar and heterozygous phenotypes.

The low rate of reversion in the progeny of inversion homozygotes deserves further comment since it contrasts with the rate found in the B stock and with that found in other Bar-eyed stocks (Zeleny, 1921; Sturtevant, 1923). Zeleny (1921) did find, however, an 'emarginate' stock which showed an unexplained, reduced level of reversion. There is no obvious reason why revertants from the inversions should have a low relative viability. One of the two (*dl*-49 × *B*) revertants in females was semi-lethal in males. But none of the other 11 revertant chromosomes tested were reduced in viability. It is unlikely that the reduction in reversion is due to an effect of the inversion *per se* since the rate of reversion is not greatly affected by other rearrangements like ring chromosomes (Green, 1968) and inverted attached-*X* chromosomes (Gabay & Laughnan, 1973). Nor, in general, are large reductions in ordinary crossing-over found in inversion homozygotes (Roberts, 1976). In the present case, the distance between *y* and *f* in *dl*-49 homozygous females was scored in male progeny of *In*(1) *dl*-49, *y ct v f fu*/*In*(*l*) *dl*-49, *ct v B* genetic heterozygotes. The observed recombination frequency of 40% is comparable with the frequency of 57% found in chromosomes of wild-type sequence when the probable occurrence of multiple crossovers between *y* and *f* is taken into account. A number of types of genes which affect recombination along short regions of the chromosome are known in *Neurospora* and other organisms (Catcheside, 1981). Similar genic effects may be operative here. Whatever the cause of the reduction, the restitution of apparently normal levels of reversion in *dl*-49/*B* heterozygotes shows that the effect is not dominant.

Linkage relations suggest that all except one of the revertants in female progeny of the *dl*-49 × *B*, *dl*-49 × (*B* × CS) and *dl*-49 × (*B* × H) crosses occurred in the *dl*-49 chromosome. The exception was a cluster of two revertants in the *dl*-49 × *B* cross. When crossed to *dl*-49 males these two revertants produced 57 *v B* males, 61 + + males and 1 *v*+ male. (Vermilion is inside the *dl*-49 inversion.) These results imply that the reversion occurred in the wild-type sequence Bar-chromosome. The results also show that, since the revertant females carry the *dl*-49 chromosome, they were not contaminants. Cytological examination of the chromosomes showed that both had only one copy of the 16A region. It seems that the event causing the reversion was a pre-meiotic unequal exchange in the male. This example is the first instance of such an event at the Bar locus. Indeed such an event has been reported only once before – for reversion of a duplication of the white locus in patroclinously descended males (Green & Lefevre, 1979). Reversion to normal eye phenotype and normal chromosomal sequence may be due to an excision event mediated by a transposing element. It is significant, however, that no revertants derived from dysgenic males, particularly as the P factor has the ability to mobilize other transposable elements in *Drosophila* (Rubin, Kidwell & Bingham, 1982).

The results of the present study show that P-M hybrid dysgenesis has no significant effect on Bar reversion and hence, that such dysgenesis does not lead to a general increase in unequal crossing-over. The experiments are not, however, sensitive enough to detect the localized effects of P-M dysgenesis on unequal crossing over which would be expected under the hypothesis that crossing over occurs at or near the sites of chromosomal integration of the P factor. Under this hypothesis, dysgenesis would cause few revertants in these experiments since low frequencies of transposition render it unlikely that the

P factor would have moved into a site near the Bar locus in many of the flies scored. To test this hypothesis it would be necessary to obtain a strain with a P factor inserted in the 16 A duplication. The labour of establishing such a strain would be reduced by accumulating P factors in an *X* chromosome by crossing P males to attached-X M females and backcrossing the male progeny to such females for a number of generations (Bingham *et al.* 1982).

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