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# Studies on male recombination in a Southern Greek *Drosophila melanogaster* population

## (a) Effect of temperature. (b) Suppression of male recombination in reciprocal crosses

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SUMMARY

A second chromosome of *Drosophila melanogaster* (symbol *31.1*) isolated from a natural population of North-Western Peloponnesus (at a distance of 8 km from the city of Patras) was found to induce recombination in heterozygous males, both in the second and third chromosomes. The present study also revealed the following points. (1) The phenomenon is temperature-sensitive with higher male recombination at 29 °C than at 25 or 15 °C. (2) The temperature-sensitive period is during the larval stage where premeiotic divisions of germ cells take place. (3) Suppression of male recombination in both the second and third chromosomes occurred when *31.1*/CyL$^4$ females were used in the matings, and (4) the suppression of male recombination is caused by a cytoplasmic factor of the *CyL*$^4$/*Pm* stock.

## 1. INTRODUCTION

During the last few years several workers have reported male recombination among the descendants of wild-caught *Drosophila melanogaster*. Thus Hiraizumi (1971, from Harlingen, Texas), Waddle & Oster (1974*b*, North Western Ohio), Hiraizumi *et al.* (1973), Broadwater, *et al.* (1973, North Carolina), Sved (1974, Australia), Voelker (1974, Jacksonville, Florida), Mathews & Hiraizumi (1976, South Texas, U.S.A.) and Yamagushi (1976, Jugoslavia, Taiwan and Japan) have recovered, from natural populations, chromosomes which show male recombination in appropriate experimental tests. Moreover, Woodruff & Thompson (1975) discovered the same phenomenon in natural populations from England and Oklahoma City (U.S.A.) and Kidwell & Kidwell (1975), have reported male crossing over in twelve wild-type strains.

In the case of our investigation a lethal-bearing, second chromosome (symbol *31.1*), isolated from a large natural population of Southern Western Greece in autumn 1971, was discovered to induce male recombination when heterozygous for appropriate markers. The aim of the present study was to investigate (*a*) whether the properties of the *31.1* second chromosome are similar to those of

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chromosomes isolated by previous workers, (*b*) to study the effect of temperature upon the phenomenon of male recombination induced by the chromosome *31.1*, and (*c*) to assess whether or not male recombination was affected by the sex of the parent from which the 'male recombination chromosome' was inherited.

## 2. MATERIALS AND METHODS

The following strains of *Drosophila melanogaster* were used:

(1) *dp b cn bw*. A standard second chromosome line marked with four recessive mutants: *dp* (dumpy wings, 2L-13), *b* (black body colour, 2L-48.5), *cn* (cinnabar eye colour 2R-57.5) and *bw* (brown eye colour 2R-104.5).

(2) *cn bw*. A standard second chromosome line marked with two recessive eye colour mutants *cn* and *bw*.

(3) A second chromosome line isolated from a natural population of north western Peloponnesus southern peninsula of Greece (at a distance of 8 km from the city of Patras). This was the chromosome in which male recombination was first detected during the mapping of a lethal. This chromosome is inversion-free and is kept balanced with the $In(2L+2R)Cy$, $CyL^4sp^2$ chromosome.

(4) *se e*. A standard third chromosome line marked with two recessive mutants, *se* (sepia eye colour 3L-26) and *e* (ebony body colour, 3R-70.7).

(5) Ore-k. The Oregon wild type, which has been kept in our Laboratory since 1969).

A standard cornmeal food was used throughout the present experiments. Parents were 2–3 days old when the crosses were set up and progeny were scored until the 18th day after setting up matings. The cultures were maintained in vials kept at the desired temperature in incubators. The statistical method used was the $2 \times 2$ chi-square analysis.

Three different, but related, estimates of male recombination were computed.

(1) Total recombination frequency: total number of recombinants $\times 100$/total number of progeny.

(2) Minimum recombination frequency: estimated number of recombination events $\times 100$/total number of progeny.

(3) Number of males that produced recombinants: number of males with at least one recombinant progeny.

## 3. RESULTS

### (i) *The effect of temperature on male recombination*

In order to determine whether temperature affects the frequency of male recombination, virgin *dp b cn bw* females were mated to $31.1/CyL^4$ males and, after allowing egg-laying to occur for 20 h at 25 °C, the cultures were kept at 15, 25 or 29 °C. The *31.1*/*dp b cn bw* sons of these crosses were then mated ($2\ ♀ \times 1\ ♂$) to *dp b cn bw* virgin females and the $F_2$ progenies (cultured at 25 °C) were scored for recombination. The data presented in Table 1 show clearly that the

frequency of recombinant progeny increases with the culture temperature of the heterozygous males. In control crosses, in which Ore-K/*dp b cn bw* males were kept at either 25 or 29 °C, not only is the frequency of male recombination much lower but also there is no apparent temperature effect.

Since larval tests contain only premeiotic cells (Cooper, 1950), two temperature-shift experiments were made to see whether the effect of the high (29 °C) temperature occurs only in larval, or only in pupal or in both developmental stages. Again *31.1.CyL*$^4$ males were crossed to *dp b cn bw* virgins and the females allowed to lay eggs at 25 °C for 4 h. After 20 h one set of vials was kept at 29 °C until the first puparia formed. These puparia were discarded and the vials returned to 25 °C. The other set of vials was kept at 25 °C until puparia formed and white prepupae were picked from them and subsequently maintained at 29 °C. The *31.1/dp b cn bw* $F_1$ males that emerged from these vials were mated as in the previous experiment and their progeny scored for recombinants. The data are also shown in Table 1. It is clear that 29 °C during the pupal period has little if any effect on recombination frequency, whilst the frequency in males kept at 29 °C only as larvae is as high as in those kept at 29 °C throughout their development.

The fact that male recombination events are clustered (these data and Hiraizumi *et al.* 1973), taken together with the finding that temperature affects the frequency of male recombination in the larval, but not the pupal, period suggests that male recombination is a premeiotic event.

As previous studies have shown (Hiraizumi *et al.* 1973; Voelker, 1974; Waddle & Oster, 1973; Woodruff & Thompson, 1975), the distribution of male recombinant events is dissimilar to that of female recombinant events. For example region 1 (*dp-b*) is 35 map units long from standard meiotic data whilst region 2 (*b-cn*) is only 9 map units long. Yet male recombinant events are about twice as frequent in region 2 as in region 1. Frequencies of male recombination in region 3, very similar to those reported in Table 1 for *31.1/dp b cn bw*, were found when *31.1/cn bw* males (from *31.1/CyL*$^4$ fathers) were backcrossed to *cn bw* virgins. In this experiment (at 25 °C) 25 of 67 tested males gave recombinant progenies and 35/4721 recombinants (0·74 %) were found.

As noted by Hiraizumi (1971), the *k* value (i.e. the proportion of wild type amongst total progeny (excluding crossovers)) of chromosomes inducing male recombination is low in the present experiments. Indeed the data of Table 1 show a positive correlation between the frequency of male recombination and the *k* value as the temperature increases.

### (ii) *The reciprocal cross effect*

The previous data had all been derived from *31.1/dp b cn bw* males who inherited their *31.1* chromosome from their father. It was found that when these males were derived from the reciprocal cross the results were very different (Table 2). When the *31.1* chromosome had been inherited maternally the frequency of male recombination was very low (in fact not greater than that seen in Ore-k/*dp b cn bw* males, Table 1) and was not increased by an increase in culture temperature.

Table 1. *Second chromosome male recombination in* 31.1/dp b cn bw *males* (*from* 31.1/CyL$^4$ *fathers*) *and Or-K*/dp b cn bw *males* (*from Oregon-K fathers*) *grown at different temperatures: cross over regions: 1* dp-b, *2* b-cn, *3* cn-bw

| Male parent | Temperature (°C) | No. of males tested | No. of progeny | No. of recombinants by region | | | | | | recombination frequency | Minimum recombination frequency | No. of males that produced recombinants | Average *k* |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | 1 | 2 | 3 | 1, 2 | 1, 3 | 2, 3 | | | | |
| *31.1/CyL*$^4$ | 15 | 56 | 3071 | 0 | 2 | 3 | 0 | 0 | 0 | 0·16% | (5) 0·16% | 5 (8·9%) | 0·52 |
| *31.1/CyL*$^4$ | 25 | 90 | 4902 | 10 | 20 | 55 | 0 | 0 | 1 | 1·75% | (63) 1·28% | 50 (55·56%) | 0·40 |
| *31.1/CyL*$^4$ | 29 | 92 | 3099 | 16 | 36 | 44 | 1 | 0 | 2 | 3·19% | (63) 2·03% | 52 (56·52%) | 0·36 |
| Or-K | 25 | 117 | 8014 | 1 | 5 | 1 | 0 | 0 | 0 | 0·09% | (4) 0·05% | 3 (2·56%) | 0·54 |
| Or-K | 29 | 94 | 6485 | 0 | 1 | 4 | 0 | 0 | 0 | 0·08% | (3) 0·05% | 4 (4·25%) | 0·52 |
| *31.1/CyL*$^4$ | 25 → 29* | 93 | 5962 | 19 | 23 | 45 | 0 | 1 | 0 | 1·48% | (70) 1·18% | 54 (52·06%) | 0·39 |
| *31.1/CxL*$^4$ | 29 → 25* | 126 | 6021 | 35 | 55 | 164 | 0 | 1 | 2 | 4·25% | (120) 1·99% | 87 (69·05%) | 0·37 |

$\chi^2$ 15 °C *v.* 25 °C = 42·4, D.F. = 1, $P < 0{\cdot}001$.
$\chi^2$ 25 °C *v.* 29 °C = 17·4, D.F. = 1, $P < 0{\cdot}001$.
$\chi^2$ 25 → 29 *v.* 29 → 25 = 87·7, D.F. = 1, $P > 0{\cdot}001$.
* Until puparium formation at first temperature and until eclosion at second.

Despite the fact that *31.1/dp b cn bw* males derived from *31.1/CyL*$^4$ mothers show low male recombination their sons show high frequencies of recombination, in fact as high as *31.1/dp b cn bw* sons derived from a *31.1/CyL*$^4$ father (Table 2, line 5).

It has been suggested (Kidwell & Kidwell, 1975) that this type of reciprocal cross effect on male recombination is due to cytoplasm–chromosome interactions. Since the *31.1* chromosome was isolated from a wild male by crossing to *CyL*$^4$*/Pm* females and has been kept by mass mating in the laboratory for many years, the cytoplasm as well as the *X* chromosomes of the *31.1/CyL*$^4$ balanced stock derived almost entirely from *CyL*$^4$*/Pm* stock.

When *31.1/CyL*$^4$ males were mated to $F_1$ females from the cross between *dp b cn bw* males and virgin *CyL*$^4$*/Pm* females their sons showed a very low rate of male recombination (Table 2, line 6). This suggests that it is the cytoplasm of the *CyL*$^4$*/Pm* stock which suppresses male recombination.

### *Male recombination on chromosome 3*

Hiraizumi *et al.* (1974) and Waddle & Oster (1974*a*, *b*) have reported that their second chromosome male-recombination-inducing chromosomes also induce male recombination in chromosome 3. In addition Kidwell & Kidwell (1975) reported strains that produce high recombination frequencies in chromosome 3. This male recombination also showed the reciprocal cross effect. To see whether the *31.1* chromosome had similar properties, *se e* flies were crossed reciprocally to *31.1/CyL*$^4$ males or virgin females and the *31.1/+*; *se e/+ +* sons backcrossed to *se e* in order to measure recombination. The data (Table 3) confirm that recombination is induced on chromosome 3 and that the frequency is about 10-fold higher when the *31.1* chromosome is inherited from a father than a mother.

No appreciable male sterility had been observed in any of the crosses of the present work (see Sved, 1976; Kidwell & Kidwell, 1975).

## 4. DISCUSSION

Investigations up to now on male recombination factors isolated from natural populations (Hiraizumi, 1971; Hiraizumi *et al.* 1973; Waddle & Oster, 1974*b*; Volker, 1974; Sved, 1974; Woodruff & Thompson, 1975; Mathews & Hiraizumi, 1976; Yamagushi, 1976) seem to suggest that their properties are more or less the same in every population studied. However, we do not yet know whether the factors studied from different populations are or are not identical. Some differences have been reported between these factors, but they may result from either differences in the genetic background (e.g. suppressors), or from experimental conditions or differences in the structure of those specific factors responsible for male recombination.

Our data show that male recombination is temperature-sensitive, with higher values at 29 °C than at 25 or 15 °C; moreover, the *k*-values are negatively correlated with the temperature. On the contrary, Kidwell & Kidwell (1975) reported that

Table 2. *Male recombination in the reciprocal crosses*

| Male parent | Temperature (°C) | No. of males tested | No. of progeny | No. of recombinants by region: 1 | 2 | 3 | 1, 2 | 1, 3 | 2, 3 | Total recombination frequency | Minimum recombination frequency | No. of males that produced recombinants | *k* |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1. *31.1/CyL*$^4$ ♂ | 25 | 90 | 4902 | 10 | 20 | 55 | 0 | 0 | 1 | 1·75% | (63) 1·28% | 50 (55·56%) | 0·40 |
| 2. *31.1/CyL*$^4$ ♀ | 25 | 77 | 6049 | 0 | 1 | 3 | 0 | 0 | 0 | 0·07% | (3) 0·05% | 4 (5·19%) | 0·53 |
| 3. *31.1/CyL*$^4$ ♂ | 29 | 92 | 3099 | 16 | 36 | 44 | 1 | 0 | 2 | 3·19% | (63) 2·03% | 52 (56·52%) | 0·36 |
| 4. *31.1/CyL*$^4$ ♀ | 29 | 113 | 6566 | 0 | 2 | 0 | 0 | 0 | 0 | 0·03% | (2) 0·03% | 2 (1·77%) | 0·51 |
| 5. *31.1*/dp b cn bw from cross 2 ♂ | 25 | 52 | 3000 | 11 | 9 | 24 | 0 | 0 | 1 | 1·50% | (32) 1·07% | 25 (48·08%) | 0·43 |
| 6. *31.1/CyL*$^4$ × *CyL*$^4$/dp b cn bw ♀* | 25 | 54 | 3741 | 0 | 0 | 2 | 0 | 0 | 0 | 0·05% | (2) 0·05% | 2 (3·70%) | 0·53 |

* See text.

Table 3. *Third chromosome male recombination in* 31.1/+ ; se e/+ + *males*

| Male parent | Temperature (°C) | No. of males tested | No. of progeny | Total recombination frequency | No. of males that produced recombinants | *k* |
|---|---|---|---|---|---|---|
| 1. *31.1/CyL*$^4$ ♂ | 25 | 74 | 3805 | (40) 1·05% | 29 (39·19%) | 0·49 |
| 2. *31.1/CyL*$^4$ ♀ | 25 | 60 | 5273 | (5) 0·11% | 4 (6·67%) | 0·55 |

male recombination was considerably reduced at both high (28 °C) and low (18 °C) temperatures. No explanation can be provided for the difference between our findings and those reported by the previously mentioned workers. Since the crossover-inducer causes distorted segregation by decreasing considerably the number of chromosomes which bear it, we may assume that temperature affects, at least partially, the frequency of the crossover-inducer in natural populations.

Hiraizumi *et al.* (1973) concluded that a considerable fraction of male recombination is premeiotic in origin. His conclusion is based on the clustering occurrences of recombinants, the unequal recovery of the complementary recombinant classes for each cluster case, and on a much higher frequency of recombination around the centromeric region. Our data, which are very similar to Hiraizumi's when correlated with the fact that the temperature sensitive period is during the larval stage, where only mitotic divisions occur, favour the assumption that male recombination is premeiotic in origin.

Another observation is that the activity of the crossover-inducer was suppressed when *31.1*/$CyL^4$ females were mated to males homozygous for recessive markers. Moreover, suppression of male crossing over had the effect of increasing the *k*-values to the control level. Kidwell & Kidwell (1975) suggested that the differences between male recombination frequencies in reciprocal crosses in some wild-type strains are due to cytoplasm–chromosome interactions. Table 2 shows that the cytoplasm of the $CyL^4$/*Pm* stock is very probably responsible for the suppression phenomenon in males.

There is also evidence that the *31.1* induces recessive lethals both in the second and *X* chromosomes (experiments in progress). This finding is in agreement with what other investigators have reported (Slatko & Hiraizumi, 1973; Kidwell, 1975; Woodruff & Thompson, 1975; Cardellino & Mukai, 1975). Despite the distorted segregation, the lethal mutations and the chromosomal aberrations (Voelker, 1974; Cardellino & Mukai, 1975) induced by the male-recombination inducer factor(s), they have been found in high frequencies in natural populations. (Hiraizumi, 1971; Broadwater *et al.* 1973; Waddle & Oster, 1974*b*). These high frequencies may, at least partially, be attributed to both the existence of suppressor factor(s) (either chromosomal or cytoplasmic) in these natural populations and to the fact that the effects of the male recombination factors may be decreased by, for example, low temperature.

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