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ETHYL METHANESULPHONATE-INDUCED MALE RECOMBINATION AND CHROMOSOMAL ABERRATIONS IN *DROSOPHILA MELANOGASTER*

G. S. MIGLANI AND MINI SINGH

Department of Genetics, Punjab Agricultural University
Ludhiana 141004

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ABSTRACT

Ethyl methanesulphonate (EMS)-induced stable chromosomal aberrations did not show a direct association with male recombination in *Drosophila* although one out of four chromosomal aberrations observed in the TC_1 larvae overlapped the *dp-b* region. Highest frequency of male recombination was observed in the *dp-b* region. No aberrations was observed in TC_1 larvae decendents and F_1 larvae larvae of the cross involving female from a homozygous male recombinant line and a male from the complementary stock, or its reciprocal. Thus, involvement of chromosomal aberrations in male recombination in *D. melanogaster* still remains an open question and warrants further investigation.

Key words: Ethyl methanesulphonate, male recombination, chromosomal aberrations, *Drosophila melanogaster*.

Male crossing over and the occurrence of "unique" chromosomal aberrations, mostly inversions, in *D. melanogaster* are thought to be under common genetic control [1, 2]. Several chromosomal rearrangements in the *dp–b* and *b–cn* regions have been observed in the TC_2 larval progeny of ethyl methanesulphonate (EMS)- and hydroxylammonium sulphate (HAS)-induced TC_1 recombinant males of *D. melanogaster* [3, 4]. An inversion was observed *dp-b* region on chromosome 2L induced by 0.25% formaldehyde (FA) and this region also showed very high frequency of male recombination [5]. On this basis it was suspected that chromosomal aberrations may be associated with male recombination.

The present investigation was designed to examine the role of chromosomal aberrations in male recombination using a potent chromosome breaking agent and recombinogen, ethyl methanesulphonate (EMS).

MATERIALS AND METHODS

A wild type laboratory stock, Oregon-K, and a mutant stock homozygous for four second-chromosome recessive markers, *aristales* (*al*: 2—0.4), *dumpy* (*dp*: 2—13.0), *black* (*b*: 2—48.5) and *cinnabar* (*cn*: 2—57.5), were used. Gene symbols and map distances are as given in the standard compilation [6]. Ethyl methanesulphonate (Sigma, batch No. 890-0439) at LD_{50} dose of 0.75% was fed to 54–86 h old larvae at 25°C [7] by a method published earlier [8]. Control experiments were also done simultaneously. The protocol of the experiment has been described previously [9] and is given here in Fig. 1.

All the TC_1 male recombinants recovered were verified genotypically/phenotypically by test crossing each of them with *al dp b cn* females. The recombinant status of a TC_1 male was verified when it produced recombinant and quadruple recessive type flies in TC_2. Procedure used for construction of homozygous male recombinant lines (HMRLs) is given in Fig. 1. Temporary smears of salivary gland cells were prepared [9] at different stages of the experiment as shown in Fig. 1. Chromosomal aberrations were detected at 1,000 x magnification and breakage–union points located by comparing the aberrations with the standard salivary chromosome map [10].

RESULTS

Twenty-two untreated F_1 (+/ *al db b cn*) males on test crossing produced a pooled TC_1 progeny of 2303 flies comprising 1182 males and 1121 females but no recombinant was detected. Sixty EMS-treated F_1 males test crossed yielded a pooled TC_1 progeny of 5181 flies comprising 2539 males and 2642 females. The number of progeny types expected from a four-point test cross are 2^4 for 16. However, only 7 (2 parental + 5 recombinant) types were recovered in this experiment. The recombinants included 34 males (4 *al dp cn*, 19 *al dp*, 10 *b cn* and 1 $\overline{dp}$) and 35 females (1 *al b cn*, 1 *al dp cn*, 23 *al dp* and 10 *b cn*). The *al dp* was the most and *b cn* the next most frequent recombinant phenotypes observed (Table 1). The recombination frequency recorded separately or together in the three regions, *al–dp*, *dp–b* and *b–cn*, was significantly higher over the control ($p < 0.001$). The recombination frequency was highest in the region *db-b* (1.3318%), followed by *b-cn* (0.0956%) and *al-dp*) (0.0386%) regions (Table 2).

The number of flies recovered in different complementary classes (Table 1) were compared to see whether EMS-induced male recombination was reciprocal or not. When two complementary classes appeared in 1:1 ratio, male recombination was regarded as reciprocal, but when two complementary classes failed to appear in 1:1 ratio, male recombination was termed as nonreciprocal [4]. The wild type phenotype phenotype was predominant over quadruple mutant (*al dp b cn*) in the control experiment ($p < 0.001$) while

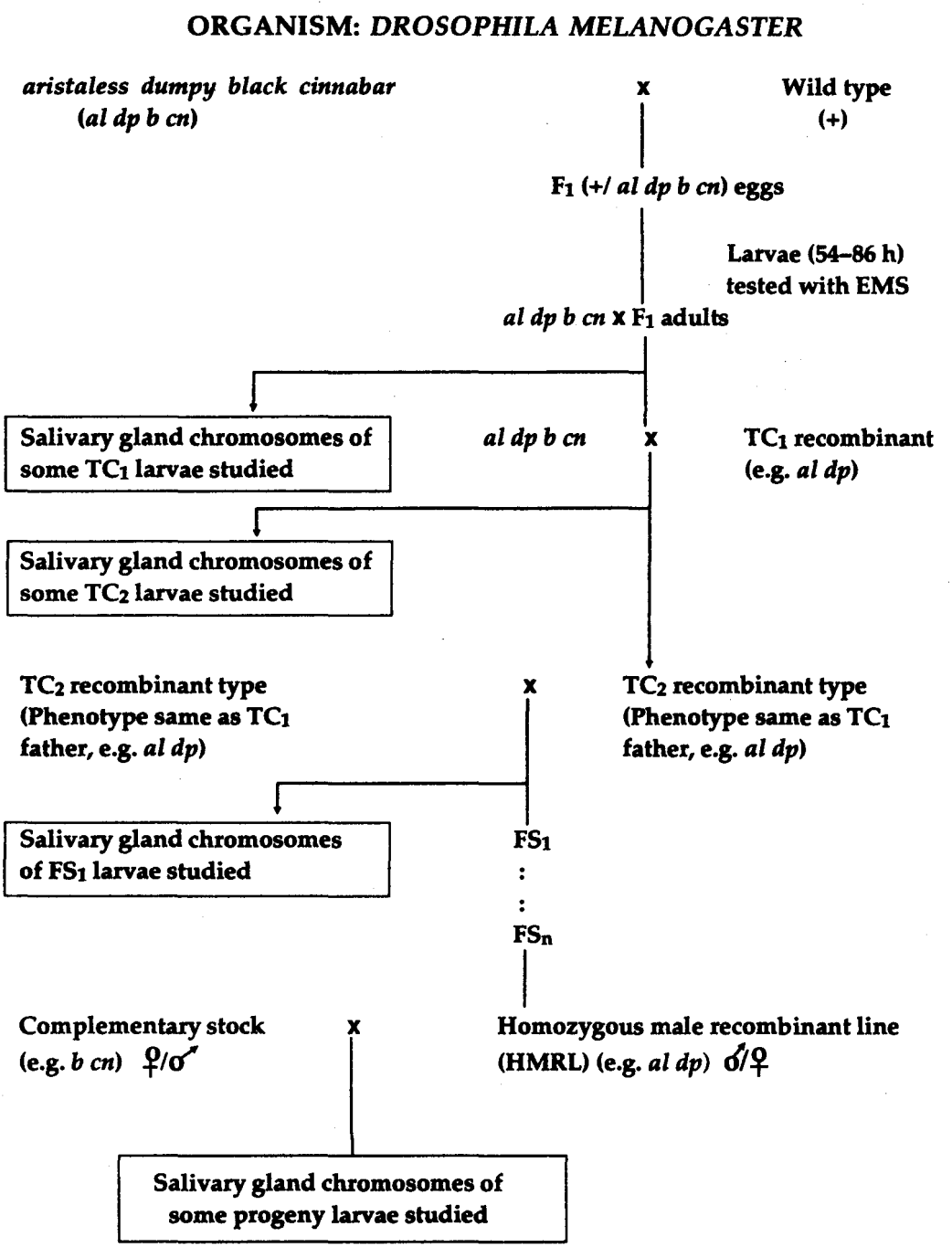

Fig. 1. Protocol of the experiment to study male recombination in *Drosophila*.

Table 1. Pooled TC_1 progeny produced by untreated and 0.75% EMS- treated F_1 (+/ *al dp b cn*) males of *D. melanogaster*

Phenotype	TC_1 progeny					
	control (22)			0.75% EMS (60)		
	males	females	total	males	females	total
+ + + +	693	672	1365	1117	1149	2266
al dp b cn	489	449	938	1388	1458	2846
al dp + *cn*	0	0	0	4	1	5
al + *b cn*	0	0	0	0	1	1
al dp + +	0	0	0	19	23	42
+ + *b cn*	0	0	0	10	10	20
+ *dp* + +	0	0	0	1	0	1
Total recombinants	0	0	0	34	35	69
Total progeny	1182	1121	2303	2539	2642	5181

Values in parentheses indicate number of F_1 males test-crossed.

the opposite was observed with 0.75% EMS ($p < 0.001$). Two pairs of complementary phenotypes, *al b cn: dp* and *al dp cn: b* were observed in equal frequency while the pair of complementary classes *al dp: b cn* ($p < 0.001$) had the number of flies of the former phenotype of majority over the latter.

Through several full-sib matings (shown as FS_1 ....FS_n in Fig. 1) of TC_2 males and females having same phenotype as their TC_1 recombinant male (father), a total of 34 (1 *dp*, 4 *al dp cn*, 10 *b cn*, 19 *al dp*) homozygous male recombinant lines (HMRLs) were constructed.

Table 2. Frequency of male recombination (%) induced with EMS in the *al–dp*, *dp–b* and *b–cn* regions of *D. melanogaster*

Region	Control	EMS
al–dp	0.0	0.0386
dp–b	0.0	1.3318
b–cn	0.0	0.0965

The number of larvae sacrificed, cells examined, aberrations detected in control and EMS experiments from TC_1, TC_2, FS_1 and F_1 (HMRL x complementary stock, or its reciprocal) generation are presented in Table 3. The TC_1 larvae of the control experiment revealed no aberrations. Among the TC_1 larvae of EMS experiment, two deletions, 2R (91F–93C) and 3L (74F–76A), and two inversions, 2L (32A–35C) and 3R (96F–98F) were detected. The number of aberrations per larva was 0.0145 and the number of aberrations per 100 cells examined was 0.013. No chromosomal aberration was detected in the TC_2 and FS_1 larvae. The F_1 larvae of the crosses HMRL x complementary phenotype, or their reciprocals, also did not reveal any chromosomal aberration (Table 3).

Table 3. Studies of salivary gland chromosomes from the larvae sampled from different generations of *D. melanogaster*

Parameter	Control	EMS			
		TC_1	TC_2	FS_1	F_1*
No. of larvae sacrificed	96	276	154	154	613
No. of cells examined	10906	30738	17435	17086	67484
No. of aberr. detected	0	4	0	0	0
No. of aberr./larva	0	0.0145	0	0	0
No. of aberr./100 cells	0	0.0130	0	0	0

*Progeny of the crosses HMRLs x Complementary stock or their reciprocal.

DISCUSSION

In the present study, 0.75% EMS dose was found to be superior over 20 µg/ml bleomycin [11], 1000R x-rays [12], 0.25% and 2.5% FA [5, 13], 0.5% HAS [4], 0.3% EMS [4], and 0.75% EMS [8] for inducing male recombination in *D. melanogaster*.

So far as phenotypic spectrum of recombinants was concerned, in the TC_1 progeny of *D. melanogaster* F_1 (+/*dp b cn*) males treated with chloroquine phosphate [8], the recombinant *b cn* was produced in highest frequency. With 0.5% HAS, the recombinant *dp b* was recovered in TC_1 progenies of F_1 (+/ *al dp b*) males in highest frequency, followed by *al dp*. The 0.25% and 2.5% FA-treated F_1 (+/ *al dp b pr*) males of *D. melanogaster* yielded *al dp* as the most frequent and *b pr* as the next most prevalent recombinant in their TC_1 progenies [15]. In the present study, 0.75% EMS- treated F_1 (+/ *al dp b cn*) males of *D. melanogaster* yielded *al dp* as the most frequent and *b cn* as the next most prevalent recombinant in their TC_1 progenies (Table 1).

Different regions of chromosome 2 of *D. melanogaster* revealed differential susceptibility for induction of male recombination with EMS in the present study. EMS in 0.75% dose induced 0.0386% male recombination in the *al–dp*, 1.3318% in *dp–b*, and 0.0965% in *b–cn* regions of *D. melanogaster* when the chemical was fed to F_1 (+/ *al dp b cn*) larvae during their second 32-h period of life.

With 0.5% HAS, higher frequency of male recombination were observed in the *al–dp* (0.81%) and *b–cn* (0.27%) regions [4] than those with 0.75% EMS in the corresponding regions, but in the case of *dp–b* region, the recombination frequency with EMS was higher than that with HAS (0.90%). Within 0.25% FA, male recombination were observed at lower

rate in the *al–dp* (0.063%) and *dp–b* (0.347%) regions [15] than those with 0.75% EMS. With 2.5% FA, lower frequency of male recombination were observed in the *al–dp* (0.018%) and *Dp–b* (0.887%) regions [4] when compared to those with 0.75% EMS. Male recombination with 0.75% EMS in the *dp–b* region (1.3508%) is slightly lower than reported earlier (1.483%) [8].

The number of different complementary recombinant classes observed in this study when compared for 1:1 ratio revealed male recombination to be nonreciprocal in the *dp–b* and *b–cn* regions but reciprocal in *al–dp* region (Table 1). Induction of nonreciprocal male recombination with 0.75% EMS in the *dp–b* region of *D. melanogaster* has been reported earlier [14, 16]. With bleomycin also, nonreciprocal recombination was observed in the *dp–b* region [11]. Nonreciprocal recombination was reported earlier with FA in the *b–pr* region [17]. HAS-induced nonreciprocal recombination has been reported in the *al–dp* and *dp–b* regions [4]. FA-induced male recombination was shown to be nonreciprocal in the *dp–b* and *b–pr* but reciprocal in *al–dp* regions [5]. Our observations are thus in agreement with some while in disagreement with certain other observations.

Male recombination could occur by the system of the classical crossing over, as in female, or by chromosome breakage and reunion events [18]. There is some evidence, however, which suggests that male recombination does not occur by the same mechanism as female recombination does [7]. High frequency of male recombination in the *dp–b* region may be due to preferential chromosome breakage by EMS [7]. It was objective of the present study to explore the possible association of EMS- induced male recombination with chromosomal aberrations. The TC_1 larvae of *D. melanogaster* were found to possess two deletions and two inversions in the salivary chromosomes. Five inversions and two deletions were detected in the progenies of TC_1 male recombinants produced with 0.75% EMS [3]; three of these inversions overlapped the *dp–b–cn* region. To explain these observations, role of chromosomal aberrations in male recombination with EMS in *D. melanogaster* was suggested. Four inversions and one deletion were detected in the progeny of three HAS-induced TC_1 recombinant males; one of those inversions, 2L (26F–29F), overlapped the *dp–b* chromosomal region [4].

No chromosomal aberration was, however, detected in the TC_2 and FS_1 larvae in the present study. Further, when a male/female from a homozygous male recombinant line (HMRL) derived from an EMS- induced TC_1 male recombinant was crossed with a female/male from an aberration-free complementary stock, no chromosomal aberration was detected in their F_1 larvae. The present study thus showed no chromosomal aberration after TC_1 generation. No chromosomal aberration was likewise detected from the heterozygotes of HMRLs constructed from one spontaneous and 7 HAS-induced [4] and

five control and 18 FA-induced male recombinants [5]. One inversion, 2L (31A–34C), observed with 0.25% FA in F_1 (+/ *al dp b pr*) larvae may be involved in the induction of male recombination as it was included in the *dp–b* region in which the frequency of male recombination was highest [5].

The inversion 2L (32A–35C) observed in the present study in a TC_1 larva may be involved in the induction of male recombination as it is included in the *dp–b* region in which the highest frequency of male recombination was observed. At present, we have no evidence to prove this point. We also do not have any reason to rule out direct or indirect involvement of other chromosomal aberrations in the induction of male recombination because intrachromosomal effects on male recombination are known [19].

If the hypothesis [18] about the involvement of chromosome breakage and reunion in male recombination is valid, our contention is that the breaks responsible for male recombination in *D. melanogaster* may not lead to the formation of chromosomal aberrations as such. If at all the chromosomal aberrations play a role in the induction of male recombination, it must be indirect.

Regarding the mechanism through which EMS may have induced male recombination and chromosomal aberrations in *D. melanogaster*, one may speculate that since EMS is known to produce mutations through alkylation it may have induced male recombination as well as chromosomal aberrations through the same process.

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