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Induction and genotypic verification of recombinants in males of *Drosophila melanogaster*

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Abstract. Ethyl methanesulphonate (0·75%) was mixed with food (1:9) and fed to developing F_1 (Oregon-K + /*dumpy black cinnabar, dp b cn*) individuals in second one-third part (54–86 h after egg deposition) of larval life, at 25 ± 1°C, for inducing recombination in males of *Drosophila melanogaster*. In control and 0·75% ethyl methanesulphonate experiments, pooled test cross progeny comprised 3475 and 13887 flies, respectively, out of which 9 and 236 were recombinants. From these, 9 and 192 recombinants were further test-crossed and 55·5% and 87·0% were verified, respectively. Non-reciprocal recombination was observed in *dp-b* but not in *b-cn* region with 0·75% ethyl methanesulphonate as evidenced by high frequency of + *b cn* male recombinants over its complementary class *dp* + +. Majority of the + *b cn* male recombinants verified did not produce in their test cross progeny the two phenotypes (+ *b cn* and *dp b cn*) in expected 1:1 ratio contrary to + *b cn* female recombinants, suggesting influence of cytoplasm in transmission of recombinant (+ *b cn*) phenotype from male.

Keywords. Induced recombination; genotypic verification; males; *Drosophila melanogaster*.

1. Introduction

Normally, the two complementary recombinant products resulting from a crossing-over event are expected to appear in a 1:1 ratio; non-reciprocal recombination is said to occur when these products fail to appear in this fashion (Strickberger 1979). Non-reciprocal recombination has been found in *Aspergillus*, yeast *Ustilago*, *Drosophila* and *Escherichia coli* (Mohindra 1984). Miglani and Thapar (1983a) observed non-reciprocal recombination with ethyl methanesulphonate (EMS) in males of *D. melanogaster*; the genotypes of the induced recombinants were, however, not verified. The present investigation was, therefore, undertaken on *D. melanogaster* to verify the genotypes of induced recombinants and to study their performance in the next generation.

2. Materials and methods

Two stocks of *D. melanogaster*-a standard 'wild-type' laboratory strain (Oregon-K) and a 'mutant' stock homozygous for 3 second chromosome recessive markers, *dumpy* (*dp*: 2–13·0), *black* (*b*: 2–48·5) and *cinnabar* (*cn*: 2–57·5)–were used. Gene symbols and the map distances are as given by Lindsley and Grell (1968). EMS (Sigma Chemical Co., Batch No. 89c-0439) was used as a probe. For EMS, 0·75% concentration was determined as LD_{50} for the second one-third part of 96 h larval life of *D. melanogaster*, at 25 ± 1°C (Miglani and Thapar 1983b) and was found to be most efficient in inducing recombination in males of *D. melanogaster* (Miglani and Thapar 1983c). It was therefore decided to treat the developing F_1 (Or-K + /*dp b cn*)

individuals with 0·75% EMS, mixed with food in ratio 1:9, in the second one-third part of larval life (i.e., 54–86 h after egg deposition) following the method of Miglani and Thapar (1983c). A two-day old F_1 male was crossed with 3 *dp b cn* females to get first testcross (TC_1) progeny. Each of the TC_1 recombinant was again test-crossed to obtain second test cross (TC_2) progeny for verifying its genotype. A TC_1 recombinant was considered as verified if it produced recombinant type flies in addition to *dp b cn* type in TC_2 progeny. All the experiments were done at 25 ± 1°C.

In order to test the difference in the proportion of recombinants in the progeny of control and 0·75% EMS experiments and to determine whether a particular progeny fits into a particular ratio, *z*-test and χ-square tests were used respectively (Gupta 1980).

3. Results

3.1 *Induction of recombination in males*

Out of 14 and 39 F_1 *D. melanogaster* males randomly selected from control and 0·75% EMS experiments, 5 (35·7%) and 32 (82·05%) individuals, respectively, produced a total of 9 and 236 recombinants (table 1). In all these experiments the most prevalent male recombinant phenotype was *+ b cn*. Non-reciprocal recombination was thus observed only in *dp-b* region. The number of *+ b cn* male recombinants was strikingly higher than that of *+ b cn* female recombinants.

Out of 3475 flies produced by control F_1 males, 0·178% were *+ b cn* and 0·089%, *dp + +* type recombinants. The 0·75% EMS-treated F_1 males yielded TC_1 progeny

Table 1. Number of second chromosome recombinants recovered (R) in first test cross progeny of *D. melanogaster*, number of recombinants further test crossed (T) and verified (V).

	Control	0·75% EMS
F_1 males test crossed	14	39
Pooled test cross population size	3475	13887
F_1 males yielding recombinants	5	32
Recombinants recovered	9	236
Percent recombinants	0·267	1·699
Male recombinants	R–T–V	R–T–V
+ b cn	5–5–1	143–130–106
dp + +	3–3–3	20– 14– 14
+ + cn	0–0–0	4– 2– 2
dp b +	0–0–0	12– 12– 12
+ b +	0–0–0	1– 1– 1
	8–8–4	180–159–135
Female recombinants		
+ b cn	1–1–1	16–12–11
dp + +	0–0–0	16–12–12
+ + cn	0–0–0	6– 2– 2
dp b +	0–0–0	8– 7– 7
Total	1–1–1	46–33–32

of 13887, out of which 1·145% were $+b\ cn$; 0·331%, $dp+\ +$, 0·072%, $+\ +\ cn$; 0·144%, $dp\ b\ +$ and 0·007%, $+b\ +$ type recombinants. Overall per cent recombinants (males plus females) in TC_1 progeny of control (0·267%) and 0·75% EMS experiments (1·699%) were significantly different from each other ($P<0·001$).

3.2 *Genotypic verification of TC_1 recombinants*

Number of TC_1 male and female recombinants further test-crossed and verified in TC_2 generation is also given in table 1. The percentages of TC_1 male recombinants verified in control (50%) and 0·75% EMS (84·91%) experiments differed significantly ($P<0·01$) from one another. The only class of male recombinants which were not verified was $+b\ cn$. The only one $+b\ cn$ female recombinant observed in pooled TC_1 progeny of control F_1 males was further test crossed and was verified (table 1). In control and 0·75% EMS experiments combined, irrespective of the phenotype, almost all the TC_1 female recombinants further test crossed were verified.

3.3 *Frequency of recombinant type flies in TC_2 progenies*

In 0·75% EMS experiment, out of a total of 159 male recombinants further test crossed, 24 (all having the phenotype $+b\ cn$) did not produce any recombinant type flies in TC_2 progeny; only *dp b cn* flies appeared. Such $+b\ cn$ recombinants were considered as unverified. The TC_2 progeny of only the verified recombinants were further analysed. The per cent of flies showing TC_1 recombinant phenotype in TC_2 progeny of a $+b\ cn$ male recombinant from control experiment was 12·2. This figure varied from 0·0–63·6 in 0·75% EMS experiments. An overwhelming majority (100% in control, and 78·3% in 0·75% EMS experiments) of $TC_1+b\ cn$ female recombinants verified produced the recombinant and *dp b cn* type flies in equal frequency in TC_2 generation (table 2). Thus, in reciprocal crosses (*dp b cn* × $+b\ cn$ recombinant and $+b\ cn$ recombinant × *dp b cn*) differences were conspicuous with regard to the proportion of recombinant type flies in TC_2 progenies.

The *dp b cn* and recombinant type flies appearing in TC_2 progeny of TC_1 recombinants were individually tested for 1:1 ratio (table 2). Combining all the recombinants in control and 0·75% EMS experiments, out of a total of $107+b\ cn$ male recombinants verified, TC_2 progeny of only 10 (9·3%) fitted into 1:1 ratio, while out of a total of $12+b\ cn$ female recombinants verified, TC_2 progeny of as many as 10 (83·3%) fitted into 1:1 ratio. Quite a high frequency of male and female recombinants of other classes, verified, revealed in TC_2 progeny, the parental and recombinant types in 1:1 ratio (table 2). Thus majority of the $+b\ cn$ male recombinants failed to produce the parental and recombinant type flies in TC_2 progeny in 1:1 ratio.

4. Discussion

4.1 *Spontaneous recombination*

In the present investigation, the control *D. melanogaster* F_1 (Oregon-K+/*dp b cn*) males yielded 0·133% verified recombinants in *dp-b* region, at 25°C. Spontaneous

Table 2. Testing the TC_2 progenies of verified *D. melanogaster* TC_1 male recombinants for 1:1 ratio.

TC_1 phenotype	No. of TC_1 recombinants verified	No. of TC_2 progenies showing recombinants		
		= dp b cn	*< dp b cn*	*> dp b cn*
Control				
Males				
+ b cn	1	0	1	0
dp + +	3	2	1	0
Females				
+ b cn	1	1	0	0
0·75% EMS				
Males				
+ b cn	106	10	93	3
dp + +	14	12	2	0
+ + cn	2	1	0	1
dp b +	12	10	1	1
+ b +	1	1	0	0
Females				
+ b cn	11	10	1	0
dp + +	12	10	0	2
+ + cn	2	2	0	0
dp b +	7	6	0	1

induction of crossing-over was reported in *D. melanogaster* wild-type laboratory strains (Whittinghill and Lewis 1961; Hannah-Alva 1968; Hiraizumi 1973). While studying the effect of temperature on recombination in *D. melanogaster*, no recombinant was observed in test cross progeny of F_1 (Oregon-K + /*dp b cn*) males, at 25°C (Miglani and Thapar 1982).

4.2 *Chemically-induced recombination*

When 0·75% EMS was fed, in the present experiments, to the developing F_1 males in their second one-third part of larval life, 32 out of 39 flies (82·05%) produced recombinants in their progenies. Formaldehyde (0·25% concentration) induced male recombination in third chromosome of *D. melanogaster* in 10% of males tested (Whittinghill and Lewis 1961) while 70·59% of 17 x-irradiated (3,000 r) curly males produced recombinants in their progeny (Whittinghill 1947). The above comparisons revealed 0·75% EMS when given in the second part of larval life to be more effective than formaldehyde and x-rays, at the doses tested, in producing recombinants in higher frequency of treated *D. melanogaster* males.

The overall frequency of recombinants (1·699%) detected with 0·75% EMS, in the present investigation, in *dp-b-cn* region is less than that (3·355%) observed earlier under similar conditions (Thapar 1982). The 0·75% EMS, when given in second part of larval life produced higher frequency of male recombination in *dp-b-cn* region than that (0·189%) with 0·3% EMS given intra-abdominally to adult males (Sharma and Swaminathan 1965).

The present results also indicate superiority of 0·75% EMS (1·699%) over formaldehyde (0·076%) (Sobels *et al* 1959), bleomycin (0·26%) (Demopolous *et al* 1980) and x-rays (0·559%) (Zimmering *et al* 1966) at the doses tested, in inducing male recombination in *dp-b-cn* region of *D. melanogaster.*

4.3 *Reciprocal versus non-reciprocal recombination*

Failure of complementary classes to appear statistically in 1:1 ratio in test cross progeny was interpreted as non-reciprocal recombination (Strickberger 1979). Non-reciprocal recombination was observed with 0·75% EMS in *dp-b* but not in *b-cn* region of *D. melanogaster,* in the present study (table 1). Spontaneous non-reciprocal recombination was observed by various workers using various second and third chromosome genetic markers (Kidwell and Kidwell 1975; Woodruff and Thompson 1977). In our preliminary experiments also, we observed non-reciprocal recombination with 0·75% EMS in *dp-b* but not in *b-cn* region (Miglani and Thapar 1983a). Sharma and Swaminathan (1965) reported non-reciprocal recombination with 0·3% EMS in *dp-b* region. An equal recovery of complementary classes was observed with x-rays in region *b-cn* (Vander Wielen 1979).

With x-rays, non-reciprocal recombination was observed using various second and third chromosome markers (Hannah-Alva 1968; Schaçht 1958; Puro 1964). With radio-frequency (Mickey 1963) and neutrons (Kale 1980) also, certain cases of non-reciprocal recombination were reported. Incidence of both non-reciprocal and reciprocal recombinations were observed in various second and third chromosome markers when unfractionated genomic DNA from M-strain or P-strain was injected into M-strain egg (Cronmiller and Cline 1983). Bleomycin induced non-reciprocal recombination in both *dp-b* and *b-cn* regions (Demopolous *et al* 1980). With formaldehyde, non-reciprocal recombination was observed in *Gl-Sb* (Lewis 1957) and *b-pr-vg* regions (Sobels and Van Steenis 1957). Thus various chemicals appear to behave differently in inducing reciprocal/non-reciprocal recombination in various chromosomal regions of *D. melanogaster.*

Non-reciprocal recombination in males of *D. melanogaster* may arise due to induction of recombination, not by classical crossing-over, as in females, but by chromosome breakage and reunion events, as suggested by Woodruff and Thompson (1977). Alternatively, induction of recombination by classical crossing-over, as in females, but preferential elimination of one of the two complementary products may result in what we here observed as non-reciprocal recombination (Sharma and Swaminathan 1965).

4.4 *Confirmation of recombinant genotypes*

In control and 0·75% EMS experiments, respectively, 55·5% and 87·0% of TC_1 recombinants were verified in the present study, as they produced recombinant type flies, in addition to *dp b cn*, in TC_2 progeny. Schacht (1958) further bred 18 x-ray-induced cross-overs involving second chromosome markers in *D. melanogaster* and all of them were verified when bred again. In the present experiments, all recombinants further test crossed were however, not verified. The only class of recombinants some members of which were not verified was + *b cn*. About those + *b*

cn male recombinants that were not verified, it may be suggested that these individuals may not genotypically be $+b$ *cn*, but they might have looked like $+b$ *cn* flies because of certain developmental modifications.

4.5 *Performance of TC_1 recombinants in TC_2 progeny*

More than 90% of the $TC_1 + b$ *cn* male recombinants, verified in TC_2 generation, did not produce the recombinant ($+b$ *cn*) and the triple homozygous recessive (*dp b cn*) flies in 1:1 ratio (table 2). In fact, in 78·3% of $TC_1 + b$ *cn* male recombinants verified in EMS experiments, recovery of recombinant type flies was below 15% in TC_2 generation. But on the other hand almost all the $TC_1 + b$ *cn* female recombinants verified produced the two phenotypes in equal frequency (table 2). Thus, in these reciprocal crosses differences were noted with regard to the proportion of recombinant type flies in TC_2 progeny. This suggests that cytoplasm may be playing some role in transmission of recombinant ($+b$ *cn*) phenotype from male. Cytoplasmic influence has been suggested by Luning (1981) with reciprocal crosses over the level of recombination.

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