from the insertion site towards 3' side. So, this P-element insertion could be affecting the *frizzled* gene and so its functions, and it is worth investigating at greater detail. The molecular function of this gene is described as: Wnt receptor activity; Wnt-protein binding; transmembrane receptor activity; non-G-protein coupled 7TM receptor activity; G-protein coupled receptor activity. It is involved in many biological processes like anatomical structure development; cell communication; sensory organ development; signal transduction; macromolecule localization; protein localization; regulation of cellular component organization and biogenesis; Wnt receptor signaling pathway. Thus, this gene is reported to be involved in sensory system development and signal transduction pathways which are important for mediating sense of smell and learning and memory.

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Exploring the mutagenic activity of colchicine in *Drosophila.*

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Introduction

Drosophila melanogaster has been used successfully to evaluate *in vivo* genetic endpoints resulting from DNA damage, and diverse mating systems have been employed to determine the effect of genotoxins on germinal and somatic cells. In the present study, we assayed colchicine (CO) for the induction of somatic mutation and mitotic recombination in the *Drosophila* Somatic Mutation and Recombination Test (SMART).

Material and Methods

Mating System

Standard cross (SC): 3-day-old virgin females from the $flr^3/TM3, Bd^S$ *stock* were mated with *mwh/mwh* males. From this cross, two types of progeny were recovered: inversion-free and inversion-carrier flies. During larval stage they are indistinguishable from one another. As adults, the presence of the Bd^S marker allows the classification of the progeny based on wing phenotype: wild-type wing borders in the inversion-free flies (+, flr^3 / *mwh,* +), and nicks in the wing border of inversion-carrier flies (*TM3,* Bd^S / *mwh,* +) (Figure 1a).

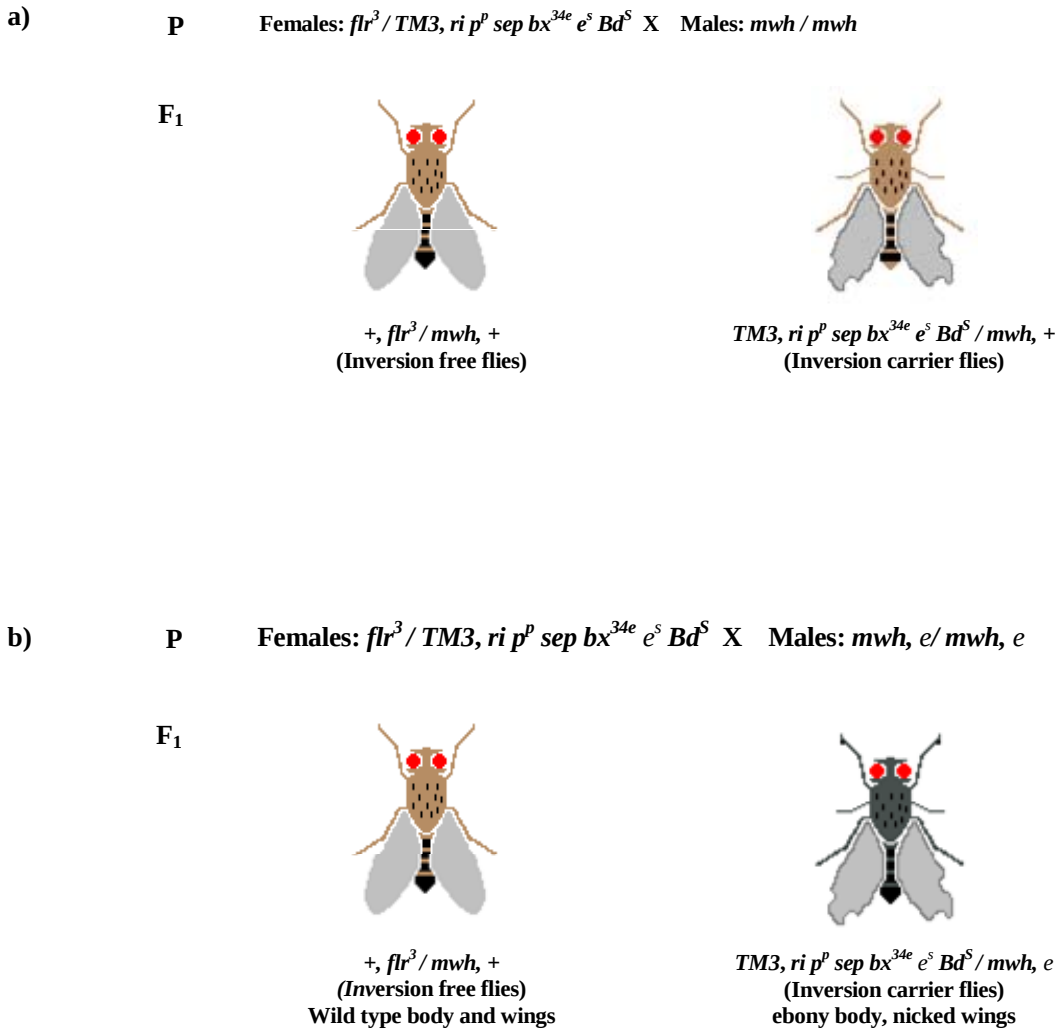

Figure 1. Cross and progeny obtained in the Mutation and Recombination test (SMART) in *Drosophila*. a) Standard Cross (SC); b) Modified cross (MC).

Modified cross (MC): 3-day-old virgin females from the flr^3/*TM3,* Bd^S stock were mated with *mwh e/mwh e* males. Two types of progeny were recovered. The *e* marker was used as a second morphological trait to confirm the inversion-carrier phenotype. Inversion-carrier flies which carry the *e* marker on the TM3, Bd^S chromosome became homozygous for the *ebony* marker and, in addition to nicked wings, had a dark body color (Figure 1b). For more detail of the markers, view Lindsley and Zimm (1992).

Chemicals

Colchicine (CO; CAS 64-86-8) was purchased from Sigma-Aldrich Química S. A. de C. V., Toluca, MX.

Treatments

Two types of treatments were performed.

a) Semi-chronic exposure. 72 ± 4 hr-old larvae from the SC were transferred to fresh medium supplemented with CO. Larvae remained feeding on this medium until pupation, for a total exposure period of 48 hr.

b) Interrupted exposure. 72 ± 4 hr-old larvae from the MC cross were transferred to medium supplemented with 0.125 mM CO. Groups of larvae were removed from the treatment medium at intervals of 6 hr, rinsed with tap water, and transferred to fresh standard food until adult emergence. The exposure times were: 6, 12, 18, 24, 30, 36, 42, and 48 hr (Figure 2) (Muñoz-Hernández, 1997).

In both treatments, the adult flies recovered were counted, sexed, classified by phenotype, and fixed in 70% ethanol.

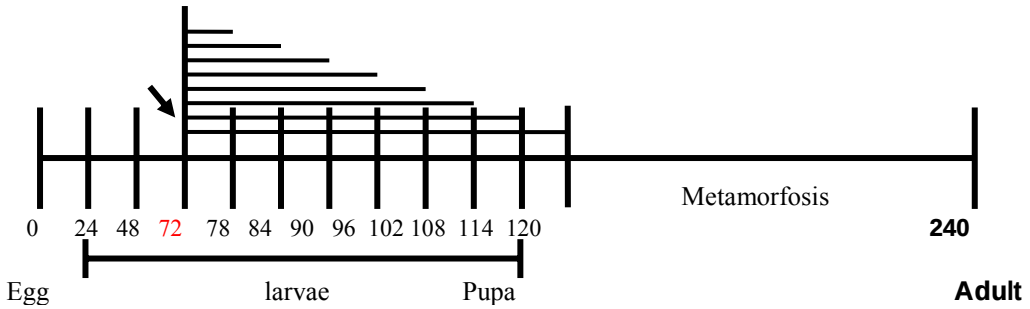

Figure 2. Interrupted treatment with colchicine.

Mounting of slides

Wings from 10 females and 10 males from each treatment/progeny combination were dissected in Faure´s Solution and wing pairs were mounted on glass slides (Graf *et al*., 1984). Slides were made from both types of the recovered progeny: inversion-free (wild type) and inversion-carriers (with nicks on the wing blade for the SC; or ebony body color and nicks on the wing blade for the MC).

Statistical analysis

SMART software was used for data processing (Frei and Wurgler, unpublished). The frequency of small, large, twin, and total spots from the experimental and control series were compared through the Multiple Decision Procedure (Frei and Würgler, 1988) in order to determine a positive, negative, inconclusive or weak positive diagnosis.

Results and Discussion

i, Semi-chronic exposure of SC flies in the SMART

Table 1 and Figure 3 show the total spot frequency obtained after exposure of third instar larvae to different concentrations of CO. Significant increases in inversion free flies were found at

0.063 and 0.125. With inversion-carrier flies, treatment with 0.063, 0.25, and 0.5 mM CO increased the total spot frequency. A change in the behavior of the curve between two types of progeny was observed at 0.125 and 0.25 mM.

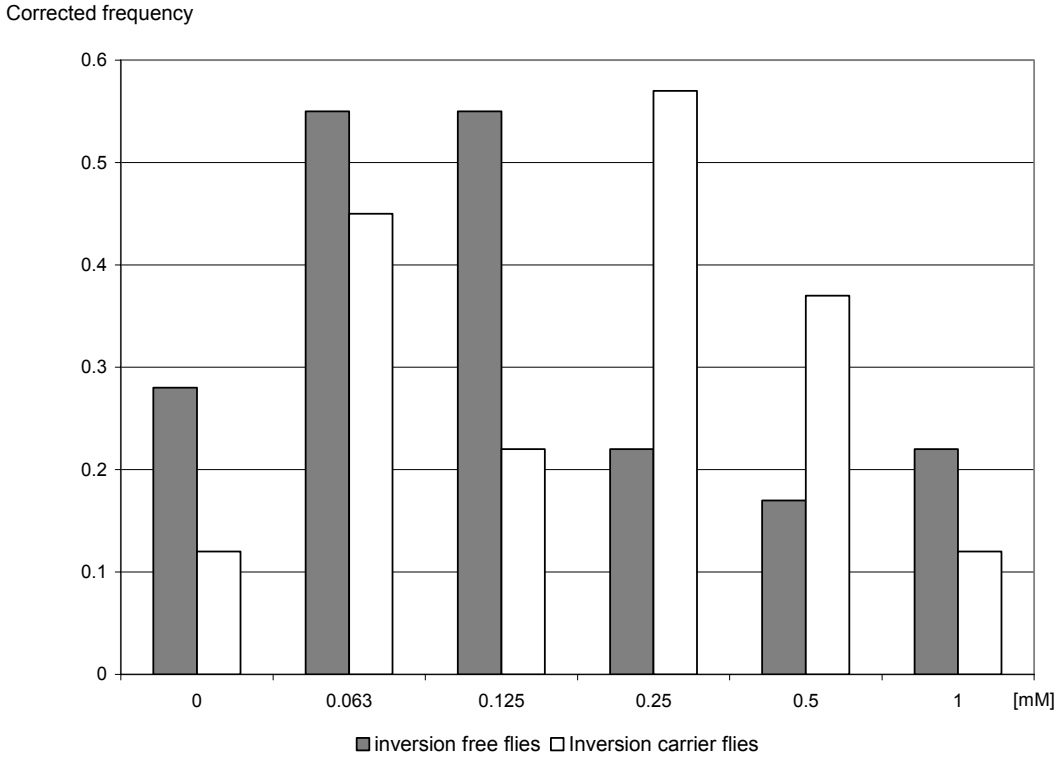

Figure 3. Corrected frequency of total spots from (SC) flies fed at different concentrations of colchicine.

Table 1. Frequency of total spots recovered from flies from the standard cross (SC) exposed to CO. Subchronic exposure (72 x 48 h).

Concentration [mM]	Number of Wings N	Total spots Fr.	n
Inversion free flies			
0	80	0.28	(22) -
0.063	40	0.55	(22)+
0.125	40	0.55	(22)+
0.25	40	0.22	(9) --
0.5	80	0.17	(14) -
1.0	80	0.22	(18) -
Inversion carrier flies			
0	80	0.12	(10)
0.063	40	0.45	(18) +
0.125	40	0.22	(9) i
0.25	40	0.57	(23) +
0.5	80	0.37	(30) +
1.0	40	0.12	(5) -

Fr = Frequency; (n) number of spots. Statistical diagnosis according to Frei and Wurgler (unpublished). α = β= 0.005. +, positive; - negative; i, inconclusive; two side test. Distilled water was used as solvent and negative control

Comparison between the total spot frequencies in the two types of progeny indicated that the induction of spots was generally higher in inversion-carrier flies. These data apparently indicate that the CO effects could be associated with the presence of chromosomes with multiple inversions. Zordan (1989), using the *Drosophila* Somatic Mutation and Recombination Test (SMART), reported that multiple inversion-carrier flies seemed to have a greater sensitivity towards the genotoxicity of the aneuploidogen vinblastine than inversion-free flies. On the other hand, Lynch *et al*. (1991) reported that in *Drosophila* the exposure to diverse compounds induced among other malformations, nicked wings. Consequently, there was a possibility that this feature, which is used to classify the inversion-carrier flies in the SMART, would be expressed in inversion-free flies as a result of treatments with CO (which is a known aneuploidogen) and, therefore, some organisms would be misclassified as inversion carriers flies (named phenocopies) and an apparent mutagenic effect would be obtained.

Table 2. Frequency of spots per wing recovered from flies of the modified cross (MC) with different hours of exposure to [0.125 mM] colchicine. Interrupted exposure (72 x 6 h).

Length of exposure (h)	Number of Wings N	Type of Spots					
		Small Single		Large Single		Total spots	
		Fr.	n	Fr.	n	Fr.	n
Inversion free flies							
0	80	0.44	(35)	0.09	(7)	0.52	(42)
6	80	0.54	(43) -	0.10	(8) -	0.66	(53) -
12	78	0.72	(56)+	0.15	(12) -	0.91	(71)+
18	62	0.45	(28) -	0.08	(5) -	0.56	(35)-
24	80	0.60	(48) i	0.25	(20)+	0.86	(69)+
30	78	0.90	(70)+	0.10	(8) -	1.00	(78)+
36	40	0.65	(26) i	0.17	(7) i	0.85	(34)+
42	80	0.96	(77)+	0.09	(7) -	1.08	(86)+
48	40	0.62	(25) i	0.12	(5) -	0.75	(30) i
Inversion carrier flies							
0	80	0.32	(26)	0.06	(5) -	0.39	(31)
6	40	0.37	(15) -	0.05	(2) -	0.43	(17) -
12	78	0.49	(38) i	0.08	(6) -	0.56	(44) i
18	40	0.28	(11) -	0.25	(10)+	0.52	(21) i
24	40	0.37	(15) -	0.25	(10)+	0.62	(25) i
30	62	0.50	(31) i	0.10	(6) i	0.60	(37)+
36	112	0.27	(30) -	0.05	(6) -	0.32	(36) -
42	80	0.35	(28) -	0.06	(5) -	0.41	(33) -
48	64	0.50	(32) -	0.08	(5) -	0.58	(37) i

Fr = Frequency; (n) number of spots. Statistical diagnosis according to Frei and Wûrgler (unpublished). $\alpha = \beta = 0.005$. +, positive; - negative; i, inconclusive; two side test. Distilled water was used as solvent and negative control for CO.

ii, Interrupted exposure of MC flies in the SMART

In order to evaluate this possibility, we carried out experiments using 0.125 mM CO, since with this concentration we find differences between the genotoxicity response in both two types of progeny using the SC, and:

a. Larvae from the MC (which carried the additional marker for body color, *ebony*) to identify the correct genotype of the progeny and discard phenocopies induction as the cause for the higher activity of CO on inversion-carrier flies. With this cross, inversion-free flies were heterozygous for the recessive marker (*e*) and had a wild-type body color, whereas inversion-carrier flies were homozygous for this marker and had a dark body color and nicks on the wing border (Figure 1b).

b. Different lengths of exposure to explore CO effect. Two vials containing larvae were withdrawn from treatment every 6 hours (beginning at 12 hr of exposure), rinsed, and put into fresh medium until development was complete; the maximum time of exposure was 48 hr.

Table 2 shows the spots per wing frequency obtained from flies from the modified cross (classified according to two markers: Bd^S and *e*). With this cross, the treatment of MC larvae yielded significant increases in the frequency of small (12, 30, and 42 hr of exposure), large (24 hr of exposure), and total spots (12, 24 to 42 hr of exposure) in inversion-free flies ($P < 0.05$). For inversion-carrier flies, small spots were not significantly increased by any exposure period, the frequency of large spots increased after 18 and 24 hr of exposure, and the increase in the frequency of total spots was significant only following 30 hr of exposure.

The fact that it is not possible to recover mutant spots through recombinogenic events in inversion-carrier flies leads us to the conclusion that the spots recovered in these flies were produced by a weak mutagenic activity of CO.

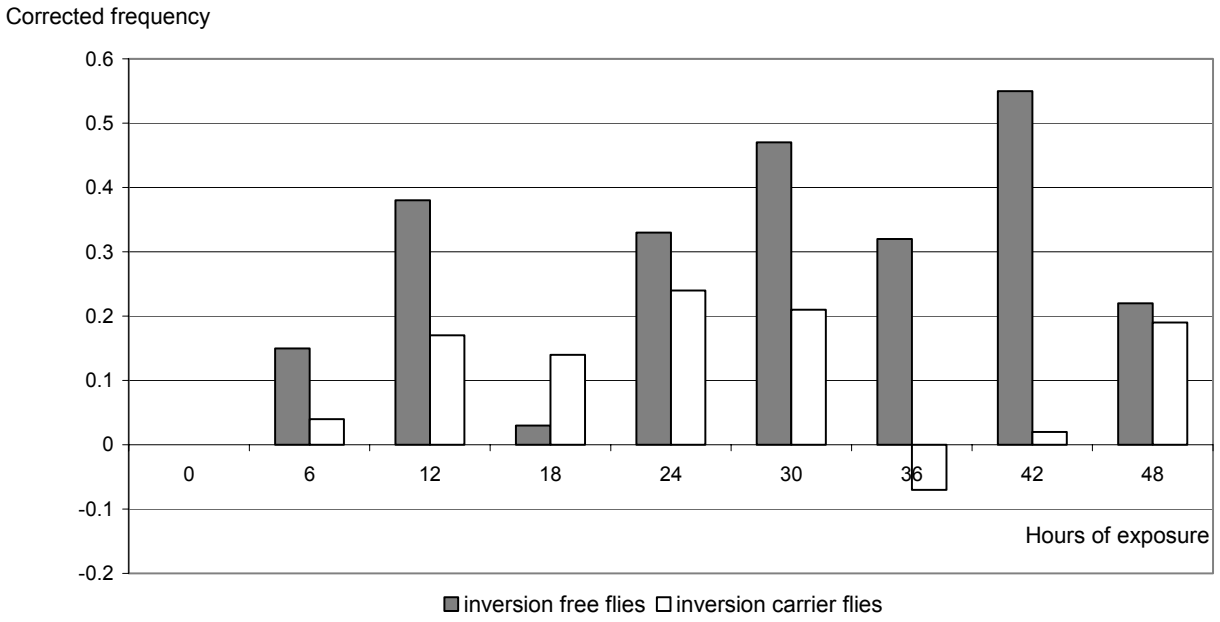

Figure 4. Corrected frequency of total spots from MC flies fed at different time of exposure with 0.125 mM of colchicine.

Figure 4 shows the corrected frequencies of total spots from the two types of progeny. The corrected spot frequencies in experimental series were obtained by subtracting the number of spontaneous spots; hence, the corrected frequencies correspond to an estimate of the mutant spots actually induced by the compound. Using both two markers to identify the progeny phenotype, data show that the corrected frequency of total spots in inversion-free flies was clearly higher than the corrected frequency in inversion-carrier flies.

In the present study, the observed genotoxic effect suggests that CO, in addition to aneuploidogenic activity, is a mutagen that induces spots in the SMART of *Drosophila*. The use of a second marker (ebony) improved the classification of the phenotypes of the progeny analyzed.

More experimental evidence needs to be obtained to explore whether the nicks on the wings induced in treated flies could be associated to aneuploidogenic activity and, in consequence, whether the alteration in the border of wings could be an auxiliary tool to identify compounds with aneuploidogenic activity.

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Mammals replacement: *Drosophila* is a reliable option for the screening of anti-inflammatory activity.

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Introduction

Inflammation induced by chemical, physical, or biological agents implies both vascular and cellular reaction mediated by chemical factors. Chronic inflammation has been associated with several steps preceding cancer as cellular transformation, cellular proliferation, tissue invasion, angiogenesis, and metastasis (Mantovani, 2005). The cancer's risk increases in patients showing inflammatory processes (Ohshima *et al*., 2003), and in cancerous patients the inflammation accelerates tumor growth and cancer progression.

Methods for screening compounds for anti-inflammatory activity use rodent models, which previously were injected with croton oil or similar agents as swollen inductors. After a period of