

SHORT COMMUNICATION

# Effects of caffeine on mitotic index in *Drosophila prosaltans* (Diptera)

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## ABSTRACT

The effect of two concentrations of caffeine (1500 µg/ml and 2500 µg/ml) on mitotic indices of *Drosophila prosaltans* was analyzed in larval brain cells. Although the differences detected between treated and control cells were not significant, the percentages obtained suggest a possible effect of caffeine in slowing the process of cell division.

## INTRODUCTION

The effect of caffeine on reproductive parameters of *Drosophila prosaltans* was previously described (Itoyama and Bicudo, 1992; Itoyama *et al.*, 1995). In short, this substance decreased productivity, longevity, mating frequency and copula duration, and increased development time and pre-copula duration. Besides these results, other data such as the knowledge that caffeine affects DNA synthesis in Sarcoma-180 mouse ascite cells (Chaudhuri and Ghosh, 1982), inhibits DNA repair and blocks cells at the G<sub>2</sub> phase in human cells (Bates *et al.*, 1985) and in mouse embryos (Müller *et al.*, 1993) and inhibits cytokinesis in plants (La Peña *et al.*, 1981; Hepler and Bonsignore, 1990) stimulated the present study on the mitotic index in the same *Drosophila* species.

## MATERIAL AND METHODS

*Drosophila prosaltans* (saltans group, saltans subgroup) is maintained in our laboratory at 20 ± 1°C, on banana-agar culture medium. The strain used is from

Sangre Grande, Trinidad and is inversion free (Bicudo, 1973).

Cells in mitosis were studied in brain preparations of third instar larvae. Two treatments were used: 1500 µg or 2500 µg per ml of culture medium, designated t3 and t5, respectively. Virgin males and females (six days old) were mass-crossed in bottles containing banana culture medium with or without caffeine (control). F<sub>1</sub> virgin males and females were placed in 10 tubes (one couple per tube) containing the same type of medium present in the bottle of parental flies. Ten larvae produced in the tubes (one larva per tube) were used for preparations in each treatment and in the control.

Brains were removed from larvae and transferred to slides containing a drop of lacto-acetic orcein (1 min), washed in 45% acetic acid aqueous solution, transferred to a drop of 50% lactic acid aqueous solution (2 min) and squashed under coverslip. Colorless nail polish was used to seal the coverslip.

## RESULTS AND DISCUSSION

The mitotic indices of larvae, submitted or not to the treatment with caffeine, are in Table I. The mitotic index of flies t5 was higher than that of flies t3. In turn, the mitotic index of flies t3 was higher than that of control flies. In the control as in the t3 and t5 flies, cells

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in prophase predominated (67%, 58% and 40%, respectively), while the percentage of cells in telophase was the lowest among phases in every case (2.8%, 0% and 3.8%, respectively). The percentage of cells in metaphase was the same for t3 and t5 treatments (25%) and lower in the control (17%). Increasing percentages of anaphase cells were observed in the control, t3 and t5 flies (14%, 17% and 31%, respectively).

Analysis of variance using Fisher angular transformation of data (Bishop *et al.*, 1984) for comparison of mitotic indices showed that the differences between treated and control experiments were not significant ( $P = 0.405$ ). However, we think that the statistical analysis used (or any other available) may not be sufficiently sensitive and thus the percentage values and the mitotic indices obtained possibly indicate some effect of caffeine. In accordance with data of other authors, mentioned in the Introduction, this effect might be a slowing of the process of cell division that could justify the greater number of dividing cells found in t5 treatment. The retardation in development time of flies observed in both treatments with 1000 and 1500  $\mu\text{g}$  of caffeine by Itoyama and Bicudo (1992) could also be the consequence of a greater duration of the process of cell division. Other experiments are necessary in order to verify this hypothesis.

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## RESUMO

O efeito de duas concentrações de cafeína (1500 e 2500  $\mu\text{g}/\text{ml}$ ) sobre o índice mitótico em *Drosophila prosaltans* foi analisado em células de gânglios cerebrais de larvas. Embora as diferenças detectadas entre células controle e tratadas não sejam significativas, as porcentagens obtidas poderiam ser sugestivas de algum efeito da cafeína ampliando a duração do processo de divisão celular.

**Table I** - Number of brain cells in mitosis and interphase per larva and mitotic indices for the control and the treated flies.

Flies	Number of analyzed cells	Number of cells					Mitotic index (%)	
		In mitosis				In interphase		
		P	M	A	T			Total
<b>Control</b>								
1	110	2	4	1	0	7	103	6.36
2	100	2	0	0	1	3	97	3.00
3	105	1	0	0	0	1	104	0.95
4	116	2	1	0	0	3	113	2.59
5	100	2	0	0	0	2	98	2.00
6	109	2	0	1	0	3	106	2.75
7	115	3	0	1	0	4	111	3.48
8	107	6	0	1	0	7	100	6.54
9	104	1	0	0	0	1	103	0.96
10	112	3	1	1	0	5	107	4.46
Total	1078	24	6	5	1	36	1042	$\bar{X} = 3.31$
<b>t3</b>								
1	97	3	0	0	0	3	94	3.09
2	100	0	1	0	0	1	99	1.00
3	103	1	0	0	0	1	102	0.97
4	99	2	1	0	0	3	96	3.03
5	106	4	0	1	0	5	101	4.72
6	104	2	1	1	0	4	100	3.85
7	97	4	1	0	0	5	92	5.15
8	101	0	2	1	0	3	98	2.97
9	102	3	2	3	0	8	94	7.84
10	105	2	1	0	0	3	102	2.86
Total	1014	21	9	6	0	36	978	$\bar{X} = 3.55$
<b>t5</b>								
1	96	1	0	3	0	4	92	4.17
2	93	5	0	2	1	8	85	8.60
3	99	3	0	0	0	3	96	3.03
4	105	5	0	2	0	7	98	6.67
5	101	0	0	0	0	0	101	0.00
6	111	0	2	1	0	3	108	2.70
7	108	1	5	2	0	8	100	7.41
8	102	2	1	2	0	5	97	4.90
9	95	2	4	1	1	8	87	8.42
10	99	2	1	3	0	6	93	6.06
Total	1009	21	13	16	2	52	957	$\bar{X} = 5.20$

P = Prophase; M = metaphase; A = anaphase; T = telophase.

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