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EFFECTS OF CAFFEINE ON FECUNDITY, EGG LAYING CAPACITY, DEVELOPMENT TIME AND LONGEVITY IN *Drosophila prosaltans*

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ABSTRACT

In *Drosophila prosaltans* reared on culture medium with caffeine (50 µg/ml, 100 µg/ml, 1000 µg/ml and 1500 µg/ml), fecundity decreased with increasing dosage. Other effects were (a) an approximately one day increase in development time of flies at 1000 and 1500 µg/ml, (b) a decrease of egg laying capacity, with increasing dosage and (c) a decrease of longevity when virgin males and females or mated males were analyzed. Mated treated females, however showed, in most experiments, greater longevity than the controls, thus suggesting a benefit of the partial blockage of reproduction caused by caffeine.

INTRODUCTION

Caffeine (1,3,7-trimetilxantine, $C_8H_{11}N_4O_2$) is a stimulator of the central nervous system largely consumed by man in coffee, tea and a variety of medicinal products. It is produced by the enzymatic conversion of xantine (Timson, 1977). Only 1 to 3% of ingested caffeine is excreted in urine in unchanged form; most is N-demetilated at positions 7 and 3, yielding 1-metilxantine (1-MX), dimetiluric acid (7-MX), 1,7-dimetilxantine (1,7-MX) and dimetiluric acids (1,7-MU) (Scott *et al.*, 1988).

Prejudicial effects of caffeine have been demonstrated in several studied. For example malformations have been found in the rat (Bertrand *et al.*, 1965, Smith *et al.*, 1987), in *Biomphalaria glabrata* (Kawano and Simões, 1987), in *Arbacia puncutulata* (Druckrey and Schreiber, 1938) in *Telmatoscopus albipunctatus* (Sehgal *et al.*, 1977) and in *Drosophila melanogaster* (Sehgal and Simões, 1976). A significant departure from the

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1:1 sex-ratio in the chinese hamster, favoring females, has also been described (Weathersbee, 1975). Besides malformations, embryonic death was induced in mice (Nishimura and Nakai, 1960, in Timson, 1977).

Caffeine apparently inhibits different processes of DNA repair (MacPhee and Leyden, 1985; Sasaki *et al.*, 1989). In *Escherichia coli*, caffeine has been found to be a mutagen of the frameshift type (Clarke and Wade, 1973 in MacPhee and Leyden, 1985), but in *Salmonella typhimurium* it was found to block mutations due to base pair replacement by ultraviolet rays (MacPhee and Leyden, 1985).

Caffeine affects DNA synthesis (Chaudhuri and Ghosh, 1982), reduces the frequency of simple and double crossing-over (Loprieno *et al.*, 1974), and reduces the frequency of chromatid exchanges (Okoyama and Kitao, 1981). Chromatidic and chromosomal aberrations (De Marco and Polani, 1981, in *D. melanogaster* and Hernandez *et al.*, 1986, in *Allium cepa*, respectively) were also caused by treatment with caffeine. Also, some studies have shown that caffeine acts to reinforce the effect of physical and chemical mutagenic agents (ex. Dulout *et al.*, 1981; Kihlman *et al.*, 1982).

Caffeine inhibits cytokinesis (La Penã *et al.*, 1981; Espino and Vasquez, 1981) as well as the enzymatic activity of DNA polymerase (Balachandran and Srinivasan, 1982), phosphorilase (Kihlman and Overgard-Hansen, 1955), and phosphodiesterases (Sutherland and Rall, 1958 in Léger, 1985; Yokogoshi *et al.*, 1987).

A possible effect of caffeine on human reproduction decreasing fertility, was also discussed by Wilcox *et al.*, 1988 and Joesoef *et al.*, 1990 but their results conflict.

In the present study the effect of caffeine on reproductive performance, longevity and development time was analyzed in *D. prosaltans*.

MATERIALS AND METHODS

The strain of *D. prosaltans* (saltans subgroup, saltans group) used in this study was from Sangre Grande, Trinidad. It has no heterozygous inversions, according to studies of chromosomal polymorphism (Bicudo, 1973; Bicudo *et al.*, 1978).

The stocks of flies were maintained at 20°C ± 1°C, on banana-agar culture medium. Four different concentrations of caffeine were added: 50 (t_0 medium); 100 (t_1 medium); 1000 (t_2 medium); and 1500 µg/ml (t_3 medium). These caffeine dosages were chosen on the basis of a preliminary experiment, using techniques described by Bournias-Vardiabasis and Teplitz (1982) for determining LD50. Two thousand µg/ml of caffeine was found to be a lethal concentration for 50% of the adult flies after two days and for 100% after four days. Twenty-four crosses of five couples each were prepared using the same caffeine concentration. Only ten crosses yielded adult progeny. Among the 14 crosses which did not produce adult progeny, 11 produced larvae, showing that this dosage also strongly affects the larval stage. On the basis of these results, dosages

corresponding to 50% (1000 µg/ml) and to 5% (100 µg/ml) of the LD50 were initially used in this study. Concentrations corresponding to 75% (1500 µg/ml) and to 2.5% (50 µg/ml) were also included in the experiments. The caffeine was dissolved in hot distilled water at a proportion of 1 g/5.5 ml of distilled water (data obtained from the Merck Index) and mixed in hot culture medium before being distributed in the vials.

Two groups of experiments were prepared. In the first group, the control (C), t_1 and t_2 culture media were used, while the second group of experiments involved the C, t_0 and t_3 culture media. In both groups the procedure was the same: virgin seven day old male and female flies were put into 30 bottles (10 couples per bottle) for each experiment, totalizing 600 couples for each group.

Virgin six day old flies produced in the two groups of experiments were collected and crossed in tubes, 20 tubes for each of the following experiments:

GROUP 1

A - Flies produced in the C medium, transferred:
1. to the C medium; 2. to the t_1 medium; 3. to the t_2 medium.

B - Flies produced in the t_1 medium, transferred:
4. to the C medium; 5. to the t_1 medium, 6. to the t_2 medium.

C - Flies produced in the t_2 medium, transferred:
7. to the C medium; 8. to the t_1 medium, 9. to the t_2 medium.

GROUP 2

A - Flies produced in the C medium, transferred:
1. to the C medium; 2. to the t_0 medium; 3. to the t_3 medium.

B - Flies produced in the t_3 medium, transferred:
4. to the t_0 medium.

C - Flies produced in the t_0 medium, transferred:
5. to the t_3 medium.

A total of 260 crosses were prepared in the 14 experiments. They were transferred three times to new medium, at three day intervals. The descendants were computed daily, separately by sex, as they were being produced.

In the study of caffeine effects on development time, virgin, six day old flies were put into five bottles (100 couples each), containing the C, t_0, t_1, t_2 and t_3 culture media. Thirty-six hours later the flies were transferred to population cages (Bicudo and Richardson, 1978) with Petri dishes having the same type of medium as in the bottles. In the two days preceeding the introduction of the flies into the population cages, the Petri dishes were changed twice a day: at 10 and 16 hours. The Petri dishes were maintained at 20°C ± 1°C. When the larvae started to eclode, those produced during one hour were collected as described in Bicudo and Richardson (1978) and put into tubes (10 larvae each) containing the same culture media as the population cages and bottles in which they and their parents were produced. A total of 100 larvae from each type of culture medium were collected and their development followed, daily till the complete emergence of adults. Males and females produced were also counted daily.

For studying the effect of caffeine on longevity, virgin, six day old flies were collected from the stocks and put into 50 bottles (10 couples each), divided into five groups of 10 bottles. Each of these groups contained one of the C, t_0, t_1, t_2 and t_3 culture media. Virgin, 6 day old flies produced in these bottles were collected and 10 couples placed in each bottle, as in the study of productivity. Thus 130 bottles were prepared, 10 of each of the following types: C → C, C → t_0, C → t_1, C → t_2, C → t_3, t_1 → C, t_1 → t_1, t_1 → t_2, t_2 → C, t_2 → t_1, t_2 → t_2, t_0 → t_3, t_3 → t_0.

Flies recently emerged in these bottles were collected and 16 females or 16 males, separately, or eight couples were introduced into vials containing the same type of culture medium on which the flies were produced. For each type of experiment, seven tubes with females, seven tubes with males and six tubes with couples were prepared, totaling 360 flies (112 females, 112 males and 48 couples in each type of experiment) in the 20 tubes. In the 13 experiments 4160 flies in 260 tubes were used. The flies were transferred every week to new culture medium till all had died.

In order to test a possible effect of caffeine on oviposition, virgin, seven day old flies from the stocks were put into 50 bottles (10 couples each), 10 bottles with each type of culture medium: C, t_0, t_1, t_2 and t_3. Virgin, six day old flies produced on the five types of medium were put into bottles (100 couples each) containing the same type of medium on which they were produced. Forty-eight hours later, these flies were transferred to population cages containing Petri dishes with the same culture medium. The Petri dishes were taken out of the cages 12 hours later and the eggs counted under the microscope. Another experiment was done using control flies, which were put into population cages having the four types of medium.

For testing the preference for medium with or without caffeine, virgin, six day old flies from the stocks were collected and 500 couples put into five bottles containing C medium, 100 in each bottle. Forty-eight hours later, the flies were transferred to five populations cages with two Petri dishes, both containing the C medium or one containing

the C medium and the other, t_0 or t_1 or t_2 or t_3. Twelve hours later the eggs in the Petri dishes were counted. In each experiment care was taken to provide homogeneous illumination around the population cages.

The caffeine used was Anhydrous, P.A. (1,3,7-trimetilxantina) - Reagen - Quimibras Indústrias Químicas S.A., Brazilian Industry.

RESULTS

The effect of caffeine on the number of progeny in the first group, involving the C, t_1 and t_2 media, is given in Table I. Control productivity was invariably greater than that of flies reared on caffeine.

Total progeny was lowest in the first vials in which the parent flies were contained, from the first to the third days, after mating (six to eight day old flies), greatest in the third vials, from the seventh to the ninth days (12 to 14 day old flies), and intermediate in the fourth vials, from the tenth to the twelfth days (15 to 17 day old flies). However, analyzing separately the experiments, three exceptions were found: experiments $t_1 \rightarrow t_2$ and $t_2 \rightarrow t_2$ where productivity was almost equal in the third and in fourth vials, and experiment $t_2 \rightarrow C$, whose productivity was greatest in the fourth vials. There also was little productivity in the first vials of experiments $t_1 \rightarrow C$, $t_1 \rightarrow t_1$ and $t_1 \rightarrow t_2$.

T test values showed no significant difference in the productivity of the sexes. Comparison of F_1 females, males and their sum among the nine experiments in the group showed significant values (at $P < 0.01$) for differences in the sexes and their sum between the control and each experiment using caffeine. For females, no other difference was significant. For males, the comparisons $t_1 \rightarrow t_2$ versus $C \rightarrow t_1$, $t_1 \rightarrow t_1$, $t_2 \rightarrow C$, $t_2 \rightarrow t_1$ and $t_2 \rightarrow t_2$ were also significant. In the comparison of the totals, another significant difference was found in $t_1 \rightarrow t_2$ versus $C \rightarrow t_1$.

Data on productivity of the second group of experiments are given in Table II. In this group the productivity of the controls was also greater than that of all experiments using caffeine. Also in this group, total productivity was smallest in the first vials, but greatest productivities occurred in the fourth vials, that is, 10 to 12 days after preparing the crosses. This might be due to the lower temperature at the time of preparing this group of experiments. Although the experiments were maintained in constant temperature chambers, manipulation (counting, transference to new vials, etc.) was done at room temperature.

Also in this group no significant difference was found in productivity between males and females. But comparison of the different experiments for productivity of

Table I - First group of experiments (mediums C, t_1 and t_2). Productivity of males and females in the 20 tubes in the sequence of 3 changes to new vials, mean productivity by tube, standard deviation separately for males and females, and t values for comparison of means of both sexes. F = females; M = males; T = total. t values were not significant.

Experiments	1st tubes			2nd tubes			3rd tubes			4th tubes			Subtotal			Mean productivity and standard deviation		t
	F	M	T	F	M	T	F	M	T	F	M	T	F	M	T	F	M	
C → C	219	237	456	333	338	671	370	352	722	282	305	587	1204	1232	2436	60.2 ± 13.7	61.6 ± 11.9	0.35
C → t_1	86	89	175	211	214	425	322	306	628	258	232	490	877	841	1718	43.8 ± 18.7	42.0 ± 16.3	0.32
C → t_2	120	100	220	201	196	397	258	248	506	225	199	424	804	743	1547	40.2 ± 16.5	37.2 ± 16.4	0.59
t_1 → C	31	31	62	220	188	408	278	283	561	173	183	356	702	685	1387	35.1 ± 16.2	34.2 ± 15.7	0.17
t_1 → t_1	22	34	56	244	249	493	322	277	599	196	212	408	784	772	1556	39.2 ± 10.1	38.6 ± 11.8	0.17
t_1 → t_2	53	34	87	182	171	353	221	199	420	233	199	432	689	603	1292	34.5 ± 14.8	30.1 ± 12.8	0.98
t_2 → C	161	158	319	216	223	439	206	198	404	232	248	480	815	827	1642	40.8 ± 17.7	41.3 ± 16.3	0.11
t_2 → t_1	74	69	143	240	280	520	252	285	537	233	228	461	799	862	1661	40.0 ± 14.0	43.1 ± 18.3	0.61
t_2 → t_2	114	148	262	197	224	421	203	242	445	219	227	446	733	841	1574	36.7 ± 13.1	42.0 ± 13.9	1.27
Total	880	900	1780	2044	2083	4127	2432	2390	4822	2051	2033	4084	7407	7406	14813			

Table II - Second group of experiments (culture mediums C, t_0 and t_3). Productivity of males and females in the 20 tubes in the sequence of 3 changes to new vials, mean productivity by tube, standard deviation separately for males and females, and t values for comparison of means of both sexes. F = females; M = males; T = total. t values were not significant.

Experiments	1st tubes			2nd tubes			3rd tubes			4th tubes			Subtotal			Mean productivity and standard deviation		t
	F	M	T	F	M	T	F	M	T	F	M	T	F	M	T	F	M	
C → C	60	77	137	261	238	499	317	324	641	374	393	767	1012	1032	2044	50.6 ± 9.1	51.6 ± 9.0	0.35
C → t_0	67	78	145	234	234	468	272	263	535	324	324	648	897	899	1796	44.8 ± 15.2	45.0 ± 17.1	0.02
C → t_3	47	49	96	189	164	353	215	222	437	250	206	456	701	641	1342	35.0 ± 12.2	32.0 ± 14.4	0.71
t_3 → t_0	38	39	77	161	178	339	265	267	532	348	338	686	812	822	1634	40.6 ± 12.8	41.1 ± 14.1	0.12
t_0 → t_3	107	100	207	187	153	340	208	158	366	168	122	284	664	533	1197	33.2 ± 11.7	26.6 ± 10.0	1.90
Total	319	343	662	1032	967	1999	1277	1234	2511	1458	1383	2841	4086	3927	8013			

females, males and their sum with the control gave significant t values, except C → t_0. Also significant were the differences of female productivity between experiments C → t_0 and C → t_3, and between experiments C → t_0 and t_0 → t_3, and differences of male productivity in the comparisons C → t_0 versus C → t_3 and t_0 → t_3, and t_3 → t_0 versus t_0 → t_3. The results of comparing the sums were the same as obtained in the comparison of males.

The study of the effect of caffeine on development time showed no difference in the larval stage reared on C, t_0 and t_1 media. Larvae from t_2 and t_3 media, however, showed slower development when compared to the other media. This difference remained as development continued. At the eleventh day only pupae were visible in tubes with C, t_0 and t_1 media, while in the vials with t_2 and t_3 media, larvae were still predominant.

Further stages of development in media t_2 and t_3 were delayed in relation to those in C, t_0 and t_1. As shown in Figure 1, in general, the distributions of flies reared on C, t_0 and t_1 media were more similar to each other than with those of t_2 and t_3, which in turn were more similar to each other. The imagos began to emerge on day 20 in C, t_0 and t_1 media and continued till the 24th and 25th days. In all experiments only males were produced on the last day of emergence, while on the first day only females were obtained in the control, and both males and females in the media containing caffeine. The distributional mode was, respectively, 21 and 22 days for the two groups of media (C, t_0, t_1 and t_2 and t_3).

No malformations were observed in the progeny of flies treated with caffeine in this study.

Data on the effect of caffeine on longevity are presented in Table III. Both virgin treated females and males had shorter lifespans than the respective controls. In every case, except t_3 → t_0 mean female longevity was greater than that of males.

Mean longevities of mated males and females are also given in Table III.

The t values for comparison of mean longevities of mated males and females, in each experiment, were significant in the control and in six of the experiments with caffeine. Male longevities were greater than those of females.

For mated females, the controls lived significantly longer in five of the 12 cases, involving t_1 → C, t_2 → C, t_2 → t_1, t_3 → t_0 and t_1 → t_1. In C → t_3 the control had a significantly shorter lifespan.

Comparisons of mean longevities of mated males from the experiments using caffeine with that of control males showed only two significant t values, involving C → t_0 and t_0 → t_3. In both cases, mean longevity of mated control males was greater than those of mated males from the treated experiments. Of the 66 comparisons involving experiments using caffeine, the mean longevity of mated males showed significant differences in 29.

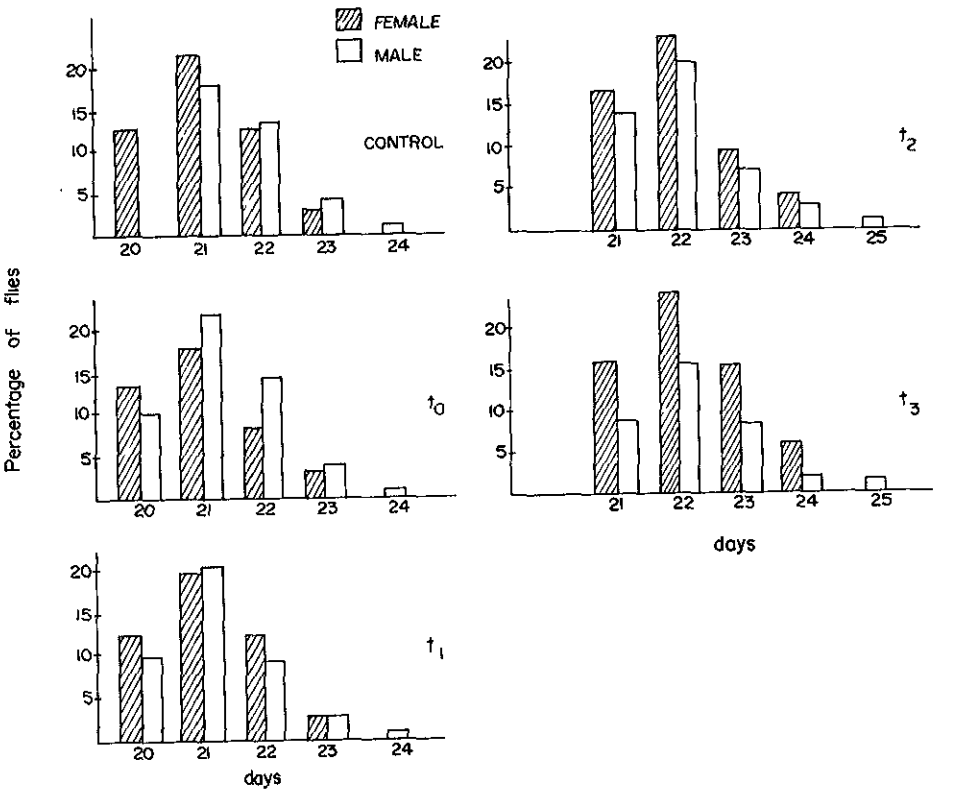

Figure 1 - Distribution of emergence of imagos in the mediums C, t_0, t_1, t_2 and t_3.

Comparing the mean longevity of virgin females with that of mated females, in each experiment, significant differences were found in C → C, C → t_0, C → t_3, t_2 → C and t_2 → t_1. The same comparison involving virgin and mated treated males gave significant differences in C → C, C → t_0, C → t_1, C → t_2, t_1 → t_2, t_2 → C and t_2 → t_2. Thus, significant differences were found for both sexes in experiments C → C, C → t_0 and t_2 → C (Table IV).

Figures 2 and 3 show the longevity distributions for virgin and mated males and females in the experiments involving flies from control transferred to media with caffeine. The most evident feature observed in treated virgin or mated flies compared to the controls is the shortening of the distribution accompanied by a decrease in the number of peaks of the curve denoting, respectively, a decrease of longevity and an increase in uniformity of the longevity of treated flies.

Table III - Mean longevity, standard deviation and t values calculated for virgin females (VF) versus virgin males (VM), for mated females (MF) versus mated males (MM), for virgin versus mated females, and for virgin versus mated males for all experiments. * = P < 0.05, ** = P < 0.01.

Experiments	Mean longevity							
	Virgin female	Virgin male	t	t	Mated female	Mated male	t	t
	$\overline{X} \pm S$	$\overline{X} \pm S$	(VF x VM)	(VF x MF)	$\overline{X} \pm S$	$\overline{X} \pm S$	(MF x MM)	(VM x MM)
C → C	60.20 ± 46.60	58.92 ± 43.04	0.23	7.05**	28.58 ± 13.69	40.54 ± 26.88	2.75**	3.27**
C → t_0	31.68 ± 15.89	36.33 ± 20.42	1.90	3.04**	24.86 ± 11.49	29.46 ± 15.14	1.67	2.36*
C → t_1	34.40 ± 15.29	25.89 ± 15.60	4.12**	1.86	29.29 ± 16.25	34.29 ± 17.63	1.44	2.86**
C → t_2	35.23 ± 16.08	33.61 ± 21.33	0.64	1.75	31.10 ± 12.51	40.39 ± 14.40	3.38**	2.34*
C → t_3	37.59 ± 14.42	32.84 ± 23.62	2.47*	7.14**	23.62 ± 9.73	36.48 ± 19.51	4.08**	1.16
t_1 → C	35.58 ± 18.52	33.41 ± 20.81	0.82	0.41	34.46 ± 14.29	34.27 ± 16.32	0.06	0.28
t_1 → t_1	41.53 ± 19.12	40.97 ± 18.34	0.22	1.63	36.35 ± 18.09	45.06 ± 22.13	2.11*	1.13
t_1 → t_2	38.08 ± 14.19	31.80 ± 16.84	3.02**	1.35	34.39 ± 16.53	37.69 ± 15.48	1.01	2.14*
t_2 → C	41.97 ± 18.59	39.99 ± 22.87	2.11*	2.57*	41.83 ± 16.63	47.92 ± 21.00	1.57	2.13*
t_2 → t_1	47.14 ± 17.45	42.16 ± 18.91	2.27*	3.41**	38.06 ± 14.47	47.77 ± 20.35	2.69**	1.79
t_2 → t_2	30.08 ± 15.22	25.36 ± 18.88	2.43*	0.42	29.15 ± 11.77	42.37 ± 17.89	4.28**	5.88**
t_3 → t_0	35.37 ± 19.02	44.50 ± 22.02	3.32**	0.16	34.94 ± 14.58	41.02 ± 20.01	1.70	0.98
t_0 → t_3	27.26 ± 10.66	28.26 ± 10.72	0.70	0.88	25.92 ± 7.91	30.52 ± 7.65	2.90**	1.51

Table IV - Egg laying capacity of control flies transferred to population cages with two Petri dishes, both containing one of the different culture mediums.

Petri dishes	Number of eggs in the mediums				
	C	t_0	t_1	t_2	t_3
1	306	382	264	235	49
2	421	185	258	150	43
Total	727	567	522	385	92

Table IV shows egg laying capacity of flies grown on the control culture medium and put into population cages with two Petri dishes, both with the same culture medium. Egg laying capacity was smaller in the treated media than in the control. The degree of decrease followed the concentration of caffeine. The decrease relative to the control was about 22% in t_0, 28% in t_1, 47% in t_2 and 87% in t_3.

Tests for preference using the control and one other culture medium in each population cage and flies grown on control medium (Table V) showed that flies apparently prefer to lay eggs in media with caffeine, since the number of eggs was greater in these than in the control. However, a decrease in the number of eggs was found as caffeine concentration increased. This decrease was also observed in the Petri dish containing the C culture medium in the same cages.

Table V - Egg laying capacity of control flies transferred to population cages containing 2 Petri dishes with control medium or 1 Petri dish with control medium (Petri dish 1) and other containing medium with caffeine (Petri dish 2).

Population cages	Number of eggs in the Petri dishes		
	1	2	Total
1	C - 1000	C - 303	1303
2	C - 386	t_0 - 597	983
3	C - 151	t_1 - 397	548
4	C - 278	t_2 - 373	651
5	C - 15	t_3 - 99	114

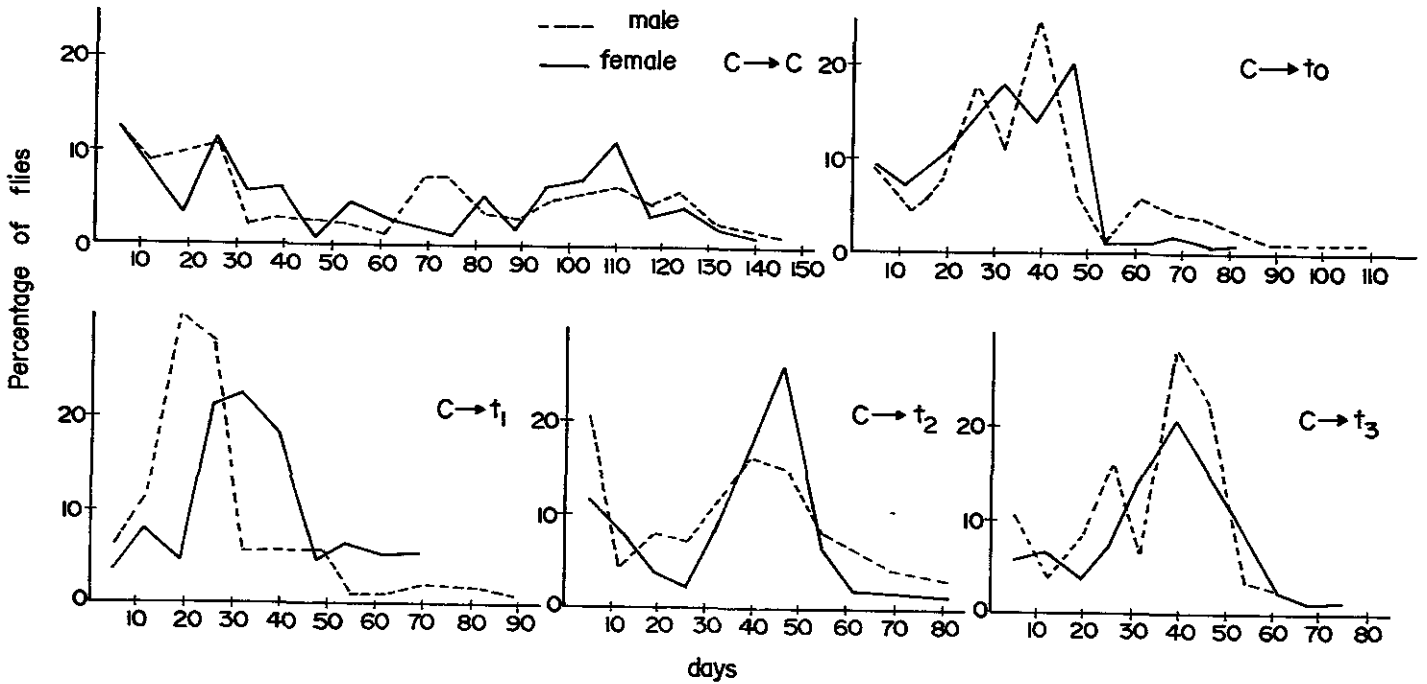

Figure 2 - Longevity distribution for virgin males and females.

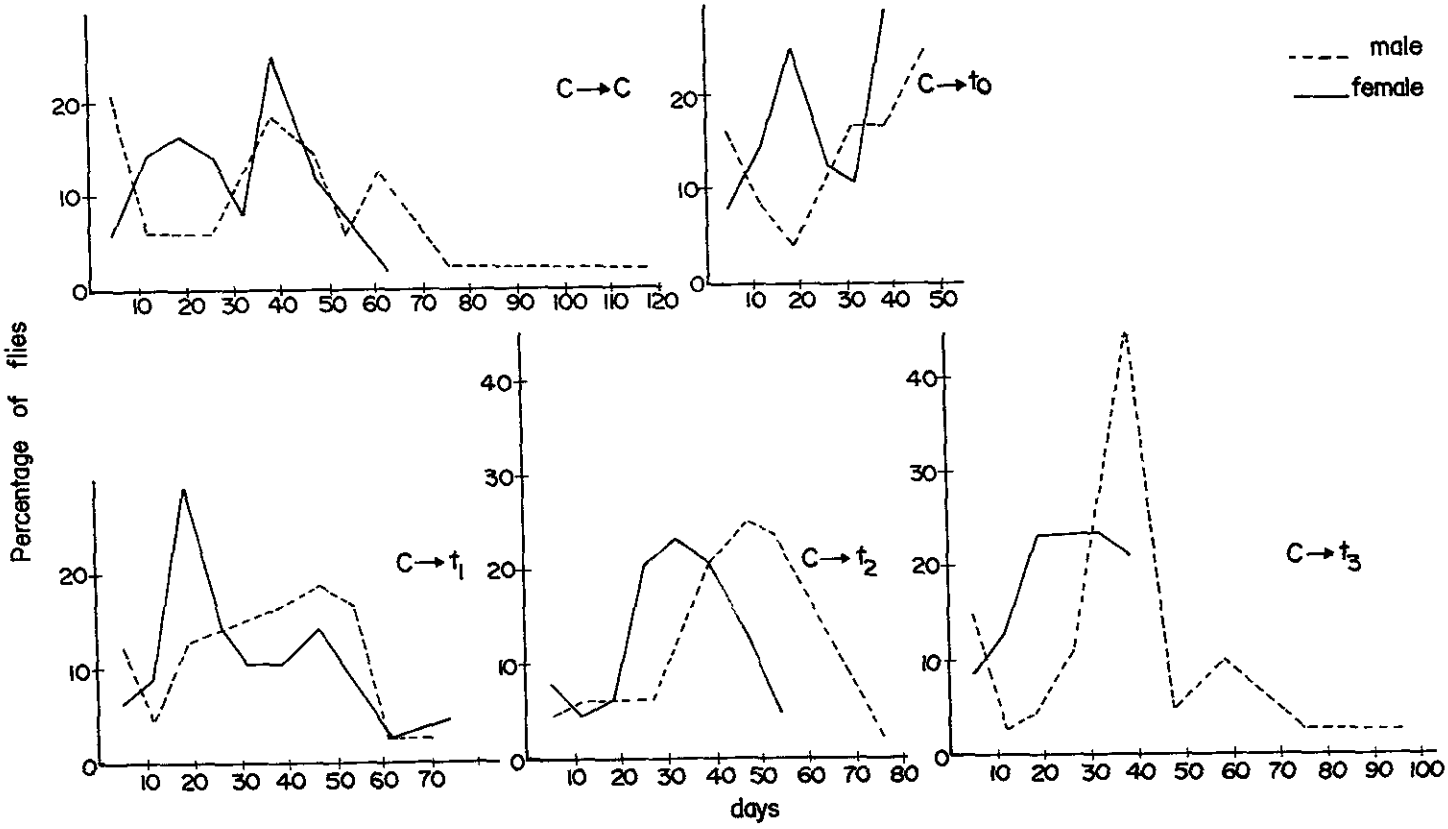

Figure 3 - Longevity distribution for mated males and females.

The experiments using flies grown on different media and transferred to the C culture medium (Table VI) showed that treated females lay less eggs than the control. The number of eggs became smaller as the concentration of caffeine in the medium on which the females were reared increased. The decrease relative to the control was about 75% in t_3, 53% in t_2, 37% in t_1 and 19% in t_0.

Tabela VI - Egg laying capacity of females reared in different mediums and transferred to population cages containing 2 Petri dishes with control culture medium.

Petri dishes with control medium	Number of eggs of females grown in the 5 mediums				
	C	t_0	t_1	t_2	t_3
1	767	533	260	316	268
2	582	566	590	314	74
Total	1349	1099	850	630	342

DISCUSSION

Using culture media t_0, t_1, t_2 and t_3, with caffeine concentrations respectively equal to 50, 100, 1000 and 1500 µg/ml, productivity (number of progeny) of treated flies was significantly smaller than that of the control. This was observed in every experiment except $C \rightarrow t_0$.

The productivity observed in $C \rightarrow C$, $C \rightarrow t_0$, $C \rightarrow t_1$, $C \rightarrow t_2$ and $C \rightarrow t_3$ was negatively correlated with the concentration of caffeine in the culture medium. Additionally, when flies were transferred from a greater to a lesser concentration of caffeine, productivity increased. The opposite was also true (for example $t_2 \rightarrow C$ versus $C \rightarrow t_2$, $t_2 \rightarrow t_1$ versus $t_1 \rightarrow t_2$ and $t_3 \rightarrow t_0$ versus $t_0 \rightarrow t_3$). A single exception was found involving $t_1 \rightarrow C$ versus $C \rightarrow t_1$.

Two of the 34 comparisons of the number of female progeny reared on caffeine showed significant differences, while the same comparison for male progeny showed eight significant differences. This may indicate that male progeny is more impaired by caffeine than female, reinforcing data in the literature showing greater sensibility of males to adverse environmental conditions.

In spite of the inverse relationship between productivity and caffeine concentration, variations in productivity did not follow, in magnitude, variations of

caffeine concentration. Probably phenomena such as resistance and variations in amount of ingested medium (and, consequently, ingested caffeine dosage) are involved.

Resistance to mutagenic and toxic agents has been described in several organisms (for example, radioresistance in *Drosophila* - Nöthel and Abdalla, 1982; resistance to caffeine in mice - Petersen, 1969 and resistance to caffeine in *Saccharomyces cerevisiae* - Bard *et al.*, 1980). In every case, resistance was found to be under genetic control. A temporary tolerance to caffeine relative to a behavioral effect has also been described in rats treated daily. Complete sensibility returned two or three weeks after stopping the treatment (Holtzman and Finn, 1988).

How can the productivity decrease induced by caffeine in *D. prosaltans* be explained? Caffeine decreases the number of functional tubes in the testes of mice and affects spermatogenesis, as shown by Petersen (1969). However, in progeny of crosses involving strains C57BL x C3H, the same author found no sperm anomaly. In rabbits, Stieves (1928, in Timson, 1977) also found that doses above 0.15 g per Kg of body weight, given daily, caused retrogressive changes in gonads and even complete sterility. In the Chinese hamster Weathesbee *et al.* (1975) showed that sensibility of ovaries to caffeine is greater than that of testes. Doses lower than those which cause gonad atrophy have effects on reproduction. Changes in the sex ratio, with a significant increase in female progeny, were also found in that study. Ovary malformations and female sterility were also caused when eggs and larvae of *D. melanogaster* were treated with caffeine (Sehgal and Simões, 1976).

In the present study we found that caffeine affects oviposition. When mated females reared in the C medium were allowed to oviposit during 12 hours in media with caffeine, the number of eggs deposited decreased with increased caffeine concentration. Flies reared in media with caffeine also had decreased oviposition in the C medium. Such a decrease was also greater as caffeine concentration in the medium increased.

A decrease in oviposition was also found in choice tests which showed that flies from C medium prefer to lay eggs in caffeine medium, when both C and treated media are available in a population cage. The decrease occurred in both the treated and C media in every test, and was greater with increased caffeine concentration.

Heppner *et al.* (1986) found that rats prefered caffeine solution at a low concentration to water. Preference for caffeine solution at a higher concentration was obtained after 14 days of forced ingestion of relatively high caffeine dosages (Vitiello and Woods, 1975).

Flies in our preference tests were from C medium and, thus, they had no anterior experience of caffeine ingestion.

According to Bouletrean-Merle (1973, in Ashburner and Wright, 1980) mated flies lay eggs rapidly and if well fed continue to produce new follicles at a rate equal to the rate of egg laying, while virgin females retain their mature eggs and do not begin to

lay in significant numbers till the 5th or 7th days. In these flies the rate of follicle production is delayed. On the basis of these data we suppose that the caffeine media are atractive for the flies. Ingested caffeine blocks the production of new follicles, causing a delay or decrease in oviposition.

The failure of production of new follicles might be caused by a disturbance in DNA duplication and repair, mentioned frequently in the literature (for example, MacPhee and Leyden, 1985).

Several studies in vertebrates (including rats and humans) have been performed in order to detect the effect on development of maternal ingestion of caffeine. Effects found include a decrease of placental, fetal body and brain weights (Tanaka *et al.*, 1987), delayed fetal development involving both sexes, and a decreased rate of subsequent development and high mortality at birth (Pollard *et al.*, 1987).

In the present study development time was also affected by caffeine at concentrations of 1000 μg/ml (t_2 medium) and 1500 μg/ml (t_3 medium). Flies produced in these media had development delayed by one day when compared to flies produced in C, t_0 (50 μg caffeine/ml) and t_1 (100 μg caffeine/ml) media.

The mean longevity of virgin flies of both sexes treated with caffeine was significatively shorter than that of the control. However the decrease was not correlated with caffeine concentration.

The mean longevity of virgin males and females in the control medium did not differ significantly, but in seven of 12 experiments involving virgin treated flies the mean differed significantly between sexes; in six of the seven cases the mean longevity of females was higher than that of males. This observation is possibly related to the fact that *Drosophila* males are more sensitive than females to harmful environmental conditions.

In C medium reproduction decreased mean longevity of both sexes, but affected females more strongly. Corradi (1979) also found that the mortality of *Drosophila hydei* mated females was greater than that of mated males, while the mortality of virgin males was greater than that of virgin females.

Caffeine generally increased the mean longevity of mated females. We suppose that blockage of ovogenesis by caffeine could result in an energy economy that would benefit mated females. Maynard Smith (1958) found *Drosophila* females without ovaries or rendered sterile by high temperature lived longer than inseminated females allowed to lay eggs. Females sterilized with radiation gave similar results (Lamb, 1964).

Thus caffeine affects fecundity, egg laying capacity, development time and longevity of *D. prosaltans*. Since the capacity to convert xenobiotics in *Drosophila* seems to be similar to that in mammals (Foerster and Wurgler, 1984), the results obtained may be useful for a better understanding of caffeine effects in man.

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RESUMO

Quatro concentrações de cafeína (t_0 = 50 µg/ml, t_1 = 100 µg/ml, t_3 = 1500 µg/ml) foram administradas, no meio de cultura, a *Drosophila prosaltans*, objetivando detectar possíveis efeitos sobre a fecundidade, a oviposição, a longevidade dos adultos e a velocidade do desenvolvimento ovo-adulto. Os resultados mostraram (a) redução da fecundidade em todas as concentrações, numa relação direta dose-efeito, (b) aumento em um dia do tempo de desenvolvimento nas concentrações 1000 e 1500 µg/ml, (c) redução da oviposição, também em relação direta à concentração de cafeína no meio e (d) redução da longevidade das fêmeas e machos virgens e dos machos cruzados. As fêmeas cruzadas, tratadas, mostraram, na maioria dos experimentos, maior longevidade que o controle, sugerindo um certo benefício do bloqueio parcial da oviposição, causado pela cafeína.

REFERENCES

Ashburner, M. and Wright, T.R.F. (1980). The Genetics and Biology of *Drosophila*. Volume 2d, Academic Press Inc (London) Ltd, 702 pp.

Balachandran, R. and Srinivasan, A. (1982). Caffeine inhibits DNA polymerase I from *Escherichia coli*: Studies *in vitro*. *Carcinogenesis 3*: 151-153.

Bard, M., Neuhauser, J.L. and Lees, N.D. (1980). Caffeine resistance of *Saccharomyces cerevisiae*. *J. Bacteriol. 141*: 999-1002.

Bertrand, M., Schwam, E., Frandon, A., Vague, A. and Alary, J. (1965). Sur un effet tératogène systématique et spécifique de la caféine chez les rongeurs. *C.R. Soc. Biol. 159*: 2199-2202.

Bertrand, M., Girod, J. and Rigaud, M.F. (1970). Electrodactylie provoquée par la caféine chez les rongeurs, rôle des facteurs spécifiques. *C.R. Soc. Biol. 164*: 1488-1489.

Bicudo, H.E.M.C. (1973). Chromosomal polymorphism in the saltans group of *Drosophila*. I. The saltans subgroup. *Genetica 44*: 520-552.

Bicudo, H.E.M.C. and Richardson, R.H. (1978). Morphological and developmental studies of *Drosophila mulleri, D. arizonensis* and their hybrids. *Biol. Zbl. 97*: 195-203.

Bicudo, H.E.M.C., Hosaki, M.K., Machado, J. and Marquez, M.C.N. (1978). Chromosomal polymorphism in the saltans group of *Drosophila*. II. Further study on *Drosophila prosaltans*. *Genetica 48*: 5-15.

Boumias-Vardiabasis, N. and Teplitz, R.L. (1982). Use of *Drosophila* embryo cell cultures as an *in vitro* teratogen assay. *Teratogenesis, Carcinogenesis and Mutagenesis* 2: 333-341.

Chaudhuri, M.M. and Ghosh, S. (1982). Effect of caffeine on macromolecular synthesis in Sarcoma-180 mouse ascites cells. *Ind. J. Exper. Biol. 20*: 433-436.

Corradi, M.A.S. (1979). Esforço reprodutivo e idade em populações experimentais de *Drosophila hydei*. Doctoral Thesis, USP, São Paulo.

De Marco, A. and Polani, S. (1981). Cell-stage-specific enhancement by caffeine of the frequency of chromatid aberrations induced by X-rays in neural ganglia of *Drosophila melanogaster*. *Mutat. Res. 84*: 91-99.

Druckrey, H. and Schreiber, D.E. (1938). Die wirkung des coffeins auf die zellteilung und das wachstum. *Archiv. Exptl. Pathol. Pharmacol. 188*: 208-214.

Dulout, F.N., Larramendy, M.L. and Olivero, O.A. (1981). Effect of caffeine on the frequency of chromosome aberrations induced *in vivo* by triethylenemelamine (TEM) and adriamycin (ADR) in mice. *Mutat. Res. 82*: 295-304.

Espino, F.J. and Vasquez, A.M. (1981). Chromosome number of *Saintpaulia ionantha* plantlets regenerated from leaves cultured *in vitro* with caffeine and colchicine. *Euphytica 30*: 847-853.

Foerster, R.E. and Würgler, F.E. (1984). *In vitro* studies on the metabolism of aflatoxin B1 and aldrin in tests of genetically different strains of *Drosophila melanogaster*. *Arch. Toxicol. 56*: 12-17.

Heppner, C.C., Kemble, E.D. and Cox, W.M. (1986). Effects of food deprivation on caffeine consumption in male and female rats. *Pharmacol. Biochem. Behav. 24*: 1555-1559.

Hernández, P., Mingo, R., González-Fernández, A. and López-Sáes, J.F. (1986). Relationship of chromosomal damage induced by caffeine to growth temperature and ATP level in proliferating cells. *Mutat. Res. 164*: 327-333.

Holtzman, S.G. and Finn, I.B. (1988). Tolerance to behavioral effects of caffeine in rats. *Pharmacol. Biochem. Behav. 29*: 411-418.

Joesoef, M.R., Beral, V., Rolfs, R.T., Oral, S.O. and Cramer, D.W. (1990). Are caffeinated beverages risk factors for delayed conception? *The Lancet 335*: 136-137.

Kawano, T. and Simões, L.C.G. (1987). Morphogenetics effects of caffeine on *Biomphalaria glabrata* (Pulmonata, Planorbidae). *Developmental Biology 90*: 281-302.

Kihlman, B.A. and Overgaard-Hansen, K. (1955). Inhibition of muscle phosphorylase by methylated oxypurines. *Exptl. Cell Res. 8*: 252-255.

Kihlman, B.A., Hansson, K. and Andersson, H.C. (1982). The effects of post-treatments with caffeine during S and G_2 on the frequencies of chromosomal aberrations induced by thiotepa in root tips of *Vicia faba* and in human lymphocytes *in vitro*. *Mutat. Res. 104*: 323-330.

Lamb, M.J. (1964). The effects of radiation on the longevity of females *Drosophila subobscura*. *J. Insect. Physiol. 10*: 387-497.

La Pena, A., Puertas, M.J. and Merino, F. (1981). Biomeiosis induced by caffeine. *Chromosoma 83*: 241-248.

Léger, L. (1985). La caféine, un insecticide? *La Recherche 16*: 554-555.

Loprieno, N., Barale, R. and Baroncelli, S. (1974). Genetic effects of caffeine. *Mutat. Res. 26*: 83-87.

MacPhee, D.G. and Leyden, M.F. (1985). Effects of caffeine on ultraviolet-induced base-pair substitution and frameshift mutagenesis in *Salmonella*. *Mutat. Res. 143*: 1-3.

Maynard Smith, J. (1958). The effect of temperature and of eggs laying on the longevity of *Drosophila subobscura*. *J. Exp. Biol. 35*: 832-842.

Nöthel, H. and Abdalla, H.I. (1982). Investigations on radiosensitive and radioresistant populations of *Drosophila melanogaster*. *Mutat. Res. 92*: 123-132.

Okoyama, S. and Kitao, Y. (1981). Inhibition of chromosome repair by caffeine or isonicotinic acid hydrazide on chromosome damage induced by mitomycin C in human lymphocytes. *Mutat. Res. 81*: 75-80.

Petersen, K.W. (1969). The effect of caffeine consumption on spermatogenesis in the rat. *Diss. Abstr. B. 29*: 3168.

Pollard, I., Jabbour, H. and Mehrabani, P.A. (1987). Effects of caffeine administered during pregnancy on fetal development and subsequent function in the adult rat: prolonged effects on a second generation. *J. Toxicol. Environ. Health 22*: 1-15.

Sasaki, Y.F., Imanishi, H., Ohta, T. and Shirasu, Y. (1989). Modifying effect of components of plant essence on the induction of sister-chromatid exchanges in cultured Chinese hamster ovary cells. *Mutat. Res. 226*: 103-110.

Scott, N.R., Stambuk, D., Chakraborty, J., Marks, V. and Morgan, M.Y. (1988). Caffeine clearance and biotransformation in patients with chronic liver disease. *Clin. Scien. 74*: 377-384.

Sehgal, S.S. and Simões, L.C.G. (1976). Preliminary results on the effects of neurotropic drugs on *Drosophila melanogaster. Ciênc. Cult. 28*: 917-920.

Sehgal, S.S., Simões, L.C.G. and Jurand, A. (1977). Effects of caffeine on growth and metamorphosis of moth fly *Telmatoscopus albipunctatus* (Diptera, Psychodidae). *Ent. Exp. Appl. 21*: 174-181.

Smith, S.E., Mcelhatton, P.R. and Sullivan, F.M. (1987). Effects of administering caffeine to pregnant rats either as a single daily dose or as divided doses four times a day. *Fd. Chem. Toxic. 25*: 125-133.

Tanaka, H., Nahazawa, K. and Arima, M. (1987). Effects of maternal caffeine ingestion on the perinatal cerebrum. *Biol. Neonate 51*: 332-339.

Timson, J. (1977). Caffeine. *Mutat. Res. 47*: 1-52.

Vitiello, M.V. and Woods, S.C. (1975). Caffeine: Preferential consumption by rats. *Pharmacol. Biochem. Behav. 3*: 147-150.

Weathersbee, P.S., Ax, R.L. and Lodge, J.R. (1975). Caffeine-mediated changes of sex ratio in Chinese hamster, *Cricetulus griseus. J. Reprod. Fertil. 43*: 141-144.

Wilcox, A., Weinberg, C. and Baird, D. (1988). Caffeinated beverages and decreased fertility. *The Lancet II*: 1453-1456.

Yokogoshi, H., Tani, S. and Amano, N. (1987). The effects of caffeine and caffeine-containing beverages on the disposition of brain serotonin in rats. *Agric. Biol. Chem. 51*: 3281-3286.

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