

EFFECT OF ACRIDINE ORANGE AND ACRIFLAVINE ON FERTILITY IN *Drosophila melanogaster* AND *D. simulans*

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ABSTRACT

Acridine orange (AO) and acriflavine (AF) were fed to adult *Drosophila melanogaster* and *D. simulans* in order to test their effects on fertility. The two compounds produced a significant decrease in the fertility of both species, the effect of AO being more pronounced. Furthermore, *D. simulans* seems to be more sensitive.

INTRODUCTION

The acridine derivatives acridine orange (AO) and acriflavine (AF) are chemicals widely used for medical, industrial and scientific purposes (Albert, 1966; Nasim and Brychcy, 1979). Both compounds form complexes with DNA by intercalation between adjacent nucleotide pairs and this binding process can induce multiple biological effects. Thus, it has been reported that AO and AF inhibit cell growth as well as DNA, RNA and protein synthesis (Lasnitzki and Wilkinson, 1948; Yamagata and Uchida, 1969; Barker and Hardman, 1974), induce morphological changes (Lasnitzki and Wilkinson, 1948), inhibit DNA repair (Bernheim and Falk, 1981), cause curing of plasmids (Hirota and Iijima, 1957; Hirota, 1960), and induce mutations in a wide variety of organisms (for more detailed information see the extensive review of Nasim and Brychcy, 1979).

In previous papers we demonstrated that in *Drosophila* AO and AF exert a toxic effect on different developmental stages: egg, larva and adult (Alba, 1983; Alba *et al.*, 1983a) and also induce mutations in both germinal (Alba *et al.*, 1983b) and somatic cells (Xamena *et al.*, 1984).

Several studies have pointed out that *D. simulans* is usually more sensitive to many environmental stresses than its sibling species *D. melanogaster* (Parsons, 1975, 1979). However, this finding cannot be generalized since in previous experiments we reported that the differences in sensitivity to several intercalating agents between the two species depend on the chemical tested and on the developmental stage analyzed (Marcos *et al.*, 1981; Alba, 1983; Alba *et al.*, 1983a).

Fertility variations, measured as a decrease in offspring production after treatment of adults, provide a good estimate of the biological effects, both of a physiological and genetical nature, that a compound induces in a defined organism. In this paper we report the effects of AO and AF on fertility in *D. melanogaster* and *D. simulans*.

MATERIALS AND METHODS

Strains

The population of *D. melanogaster* used was a wild-type *Berlin-K* stock maintained in the laboratory for a long time. The *D. simulans* population used came from a large population caught in June 1979 in Mirasol (Barcelona) and maintained under laboratory conditions since then (Marcos *et al.*, 1981).

Culture medium

A standard food medium enriched with living yeast was used to rear the flies and for the tests.

Chemicals and treatment procedure

Acridine orange (3,6-bis (dimethylamino)-acridine hydrochloride) and acriflavine (a mixture of 3,6-diamino-10-methylacridinium chloride and 3,6-diamino-acridine) were purchased from Sigma and administered in an aqueous solution containing 5% sucrose.

The test solutions were fed to 2-3-day-old males and females in special glass filter feeding units, by the method of Vogel and Lüers (1974). The treatment period was 72, 48 or 24 hours, depending on the concentration used and on the species tested (Table I). Before treatment, the flies were starved for 4 hours to ensure immediate uptake. Control flies were fed 5% sucrose only.

Table I - Treatments applied.

Species	Chemical	Concentration	Duration of treatment
<i>D. melanogaster</i>	AO	0.2%	72 h
	AO	0.3%	72 h
	AF	5.0%	48 h
<i>D. simulans</i>	AO	0.2%	48 h
	AO	0.3%	48 h
	AF	5.0%	24 h

Fertility measurements

Untreated and treated males and virgin females were crossed individually in small vials containing 8 ml of standard food medium. All the combinations were performed using approximately 40 replicates/group. The control group consisted of untreated flies. Every 2-3 days, the pairs were transferred to new vials, and the experiment lasted a total of 15 days (7 broods). The number of offspring produced in each vial was recorded.

All procedures were performed at $25 \pm 1^{\circ}\text{C}$.

RESULTS AND DISCUSSION

The study of the effects of chemical treatments on fertility is of particular relevance. The relationship between concentration and fertility rate can provide information about the effects of a particular chemical on the general metabolic rate and/or on the genetic material, since extensive genetic damage occurring in the germ cells of either males or females would prevent development of the zygotes into viable progeny, causing a depression in fertility.

In our experiments, fertility was measured as the average number of offspring obtained from the different pairs during the 15 day-period. Figures 1 and 2 show the results obtained for *D. melanogaster* and *D. simulans*, respectively. It is interesting to observe the different fertility patterns in the controls of the two species, *D. melanogaster* showing a high fertility rate during the first days, which decreased during the course of the experiment, and *D. simulans* showing a lower, but constant, fertility throughout the experiment. Differences between the treated groups and the controls were analyzed by the *t* test.

In *D. melanogaster*, after treatment with 0.2% AO, a significant decrease of

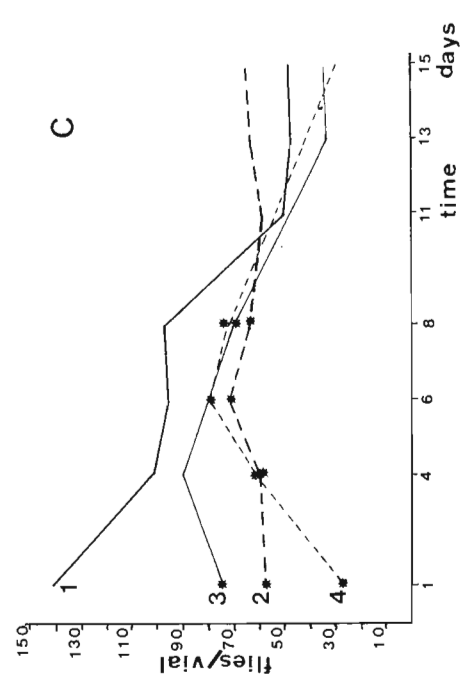
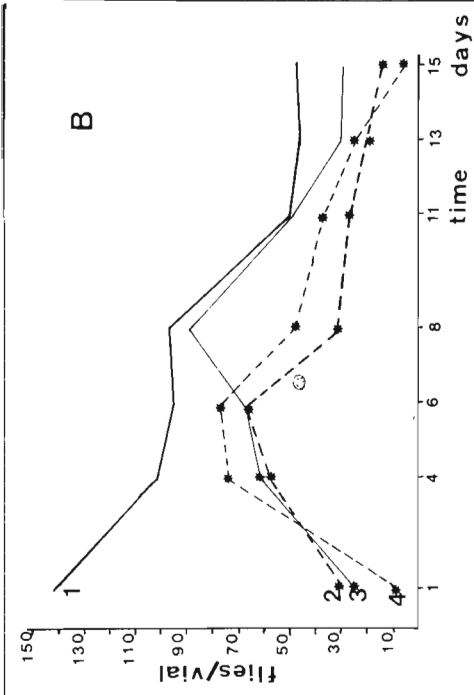
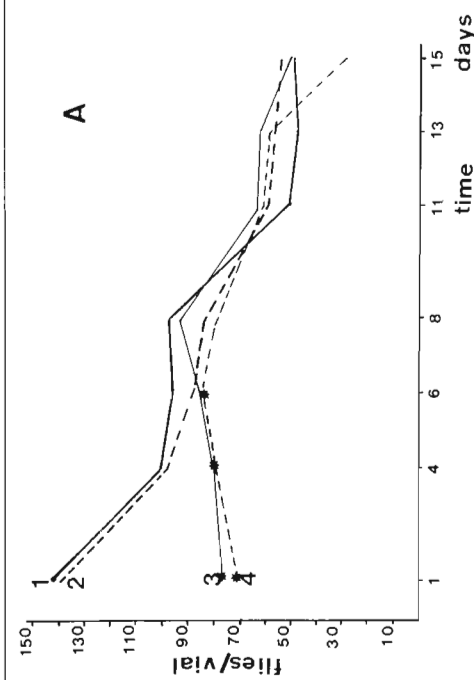


Figure 1 - Fertility of *D. melanogaster* after treatment of adults by feeding. A, B, and C show the results for 0.2% AO, 0.3% AO and 5.0% AF, respectively. 1, Control; 2, treated males x untreated females; 3, untreated males x treated females; 4, treated males x treated females. *, Significantly different from control based on the *t*-test ($P < 0.05$).

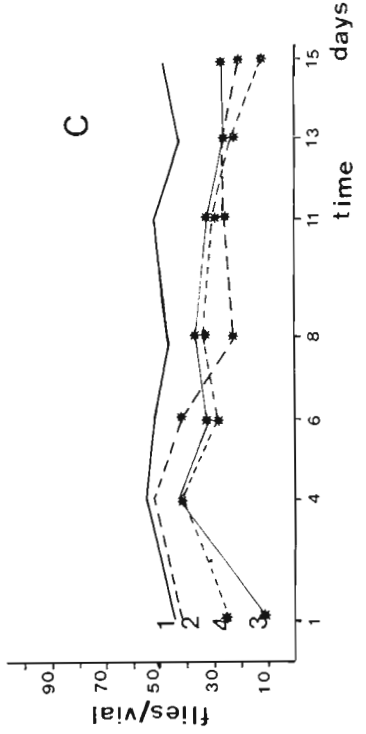
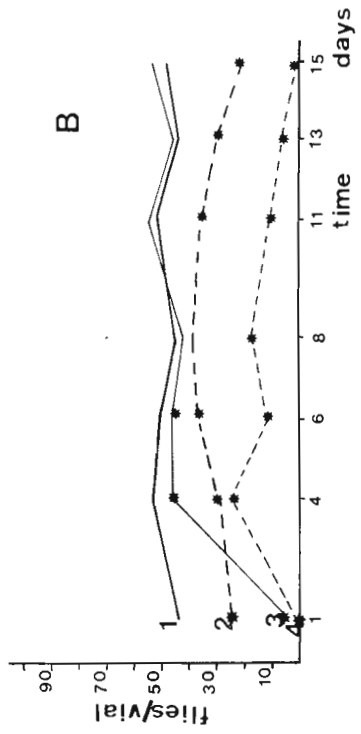
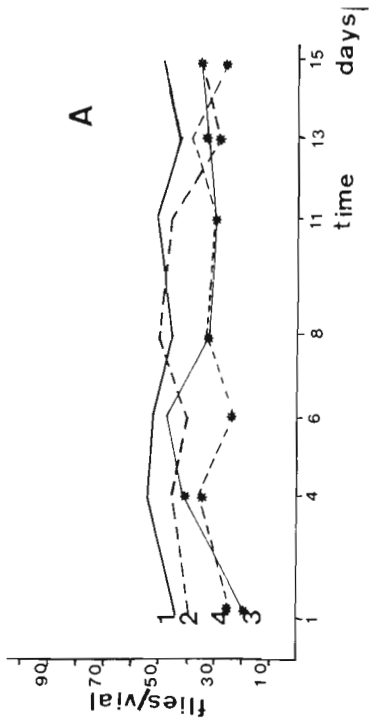


Figure 2 - Fertility of *D. simulans* after treatment of adults by feeding. A, B, and C show the results for 0.2% AO, 0.3% AO and 5.0% AF, respectively. 1, Control; 2, treated males x untreated females; 3, untreated males x treated females; 4, treated males x treated females. *, Significantly different from control based on the *t*-test ($P < 0.05$).

fertility was found during the first 6 days after treatment, when treated females were mated with treated and untreated males. After this period, the fertility of treated females approached the control value. The effect of 0.3% AO was more drastic and permanent, mainly affecting the fertility of the pairs with treated males. After treatment with 5% AF, the depression in fertility was significant only during 8 days and was less strong than with 0.3% AO, and particularly evident in crosses with treated males.

The qualitative effects of both doses of AO on *D. simulans* were in general agreement with those on *D. melanogaster*. Thus, while 0.2% AO had a greater reducing effect on the fertility of crosses with treated females, 0.3% AO seemed to affect mainly the fertility of crosses with treated males. On the other hand, at the concentration of 0.3% AO, there was a drastic depression in offspring production in treated pairs. With 5% AF, the decrease of fertility was less strong than with 0.3% AO. It is interesting to point out that, in *D. simulans*, the initial depression in fertility found after treatment was less drastic than in *D. melanogaster* but continued throughout the experiment.

Taking into account that the concentrations of acridine orange tested were lower than that of acriflavine, from our results we can deduce that AO was more effective than AF in reducing the fertility of both species. The loss of fertility observed in our experiments may have been due to physiological and/or genetic factors. Thus, the accumulation of AO and AF into the fly can exert toxic effects which disturb the metabolic functions and cause a disruption in oogenesis and/or spermatogenesis. Likewise, the physiological weakness due to the extreme stress imposed on the flies might have reduced sexual activity in general. The gradual return to normal fertility mainly observed in *D. melanogaster* may have been due to recovery from the lesion induced by treatment.

With respect to genetic factors, we may consider the possibility of induction of dominant lethals. In a previous paper (Marcos *et al.*, 1981) we reported that ethidium bromide, another typical intercalating compound, produces dominant lethals to a significant extent in *D. melanogaster* and *D. simulans*. Dominant lethals are generally caused by chromosomal breakage (Bateman and Epstein, 1971; Vogel and Sobels, 1971) and intercalating agents were found to induce DNA double-strand breaks in treated cells (Ross and Bradley, 1981). In addition, we demonstrated that AO and AF are effective clastogenic agents in *D. melanogaster*, giving positive results in the sex-chromosome loss test (Alba *et al.*, 1983b). These findings support the view that the loss of fertility found in our experiments may be partially due to the induction of dominant lethals.

We conclude that treatment of adults of *D. melanogaster* and *D. simulans* with AO and AF produces a significant decrease in the fertility of both species, the effect of AO being more pronounced. Immediately after treatment the effect of both

chemicals was more drastic in *D. melanogaster*, but the gradual recovery generally found in this species was not apparent in *D. simulans*. The reason for the greater sensitivity of *D. simulans* to AO and AF is not obvious. The fact that the experiments were performed over two different treatment periods could be a contributing factor to the reduced response of *D. simulans*.

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RESUMO

Adultos de *Drosophila melanogaster* e de *D. simulans* receberam laranja de acridina e acriflavina em experimentos visando a testar os efeitos destas substâncias na fertilidade. Ambos os compostos provocaram uma diminuição significativa da fertilidade em ambas as espécies, sendo o efeito da laranja de acridina mais marcante. Além disso, *D. simulans* parece ser mais sensível.

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