Differences between *Drosophila melanogaster* and its sibling species *D. simulans* in sensitivity to acridine orange treatment*

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Summary. Larvae and adults of *D. melanogaster* and *D. simulans* were fed with acridine orange, in order to test sensitivity differences between the species. Our results show that, of the two species, *D. simulans* is more resistant in the larval stages, and *D. melanogaster* is more resistant in the adult stage. Furthermore, adult males of both species are more sensitive than adult females.

Acridine orange (AO) is a well known chemical agent widely used as a pigment and dyestuff. At the molecular level, AO has been shown to form complexes with DNA by intercalation between adjacent nucleotide pairs[1]. This binding process can induce a variety of biological effects. Thus, it has been reported that AO inhibits growth as well as DNA synthesis, causes reductions in RNA and protein synthesis and induces morphological changes[2,3]. A substantial amount of evidence attests to the mutagenic capability of intercalating agents. In particular, AO has been shown to be a mutagenic compound in several biological systems: viruses[4], bacteria[5,6], yeast[7], insects[8,9], plants[10] and mammals[11]. In spite of the well-documented mutagenic activity of AO and its ability to inhibit DNA repair, no malignancies were observed after skin applications with AO in mice[12].

In this paper we report the preliminary results on the toxic effects of AO in larval and adult stages of *D. melanogaster* and *D. simulans*.

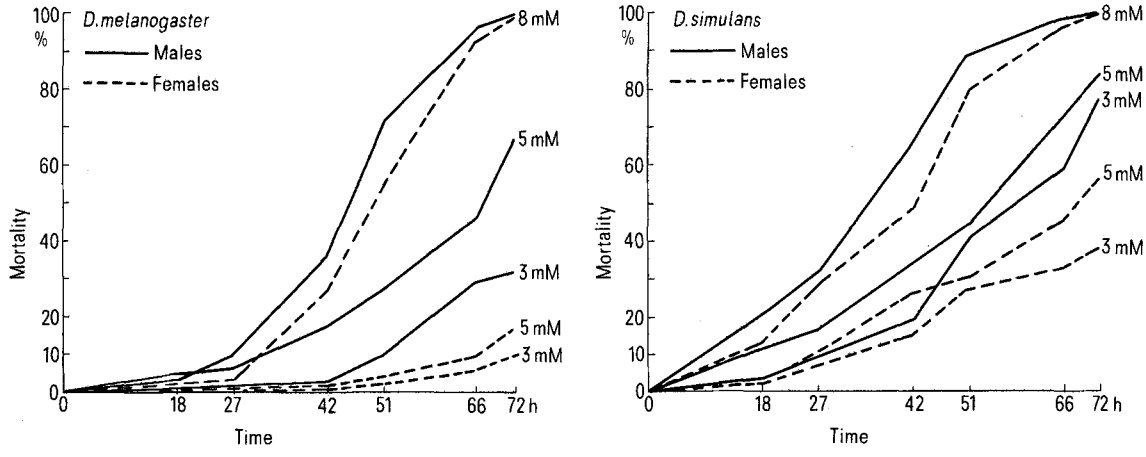
Material and methods. 1. Strains. The population of *D. melanogaster* used was a wild type stock maintained for a long time in the laboratory and designated Berlin-K. The *D. simulans* population used came from a large population caught in June 1979 in Mirasol (Barcelona)[13].

2. Treatment procedures. a) Toxicity in larvae. Eggs were collected in watch glasses with agar medium placed in special units[14]. Samples of 100 eggs were seeded in bottles with 25 ml of standard food medium enriched with living yeast to stimulate feeding of the larvae. After 24 h, when the bottles contained 1st-instar larvae, 1 ml of test solutions of AO (Sigma) containing 5% sucrose (Merck) was added to each bottle. Controls were treated with 5% sucrose only. The progeny in each bottle was counted and classified according to sex. b) Toxicity in adults. 3-day-old males and females in groups of 50 (200/sex/experiment) were put in special feeding units[15] and given different concentrations of AO in 5% sucrose. Prior to the treatment the flies were starved for 4 h in order to ensure immediate uptake. Dead flies were counted twice every day for 3 days. Control flies were fed with 5% sucrose only. All procedures were performed at 25 ± 1 °C.

Results and discussion. The experimental data for preimaginal lethality observed after treatment of 1st-instar larvae populations of *D. melanogaster* and *D. simulans* with AO are summarized in the table. The data were corrected for control lethality by the use of Abbott's formula[16]. These results indicate that, in both species, AO produced a significant decrease in the viability of larvae and that there was a dose-effect relationship in which lethality increased with the concentration. At the concentrations tested, there were no significant differences in sex ratio between AO-treated and control larvae. Equal sensitivity of male and female larvae with respect to caffeine[17] or cycloheximide[14] has also been reported with the Berlin-K stock.

As the table indicates, in spite of the higher spontaneous lethality found in *D. simulans*, this species is more resistant to acridine orange than *D. melanogaster* in preimaginal stages.

Exposure-mortality relationships to AO of adult males and females of both species during feeding are shown in the figure. Induced mortalities were calculated according to Vogel and Natarajan[18]. These data constitute evidence that, in both species, there was a concentration-mortality relationship in adults, *D. simulans* being more sensitive than *D. melanogaster*. This finding is in sharp contrast to the situation found in larvae; thus it seems that the mechanisms responsible for resistance can act more effectively in larvae in *D. simulans* and in adults in *D. melanogaster*. Furthermore, adult females of both species are more resistant than adult males. This is in agreement with the results obtained by other authors with different chemicals[14,19,20]. In spite of the concentration-resistance pattern of *D. melanogaster* adults found in this experiment, which agrees with the results found for environmental stress traits[21], this



Exposure-mortality relationships to AO of *D. melanogaster* and *D. simulans* males and females during adult feeding.

Preimaginal lethality after treatment of larval populations of *D. melanogaster* and *D. simulans* with acridine orange

Species	Concentration (mM)	No. flies emerged Males	Females	Total	Sex-ratio (♀ : ♂)	Lethality (%) Observed	Induced*
D. melanogaster	Control	865	822	1687	0.95	15.67	-
	0.1	568	618	1186	1.08	40.70	29.68
	0.5	210	209	419	0.99	79.05	75.16
	1.0	64	55	119	0.85	94.05	92.94
D. simulans	Control	726	770	1496	1.06	25.20	-
	0.1	623	621	1244	0.99	37.80	16.84
	0.5	365	365	730	1.00	63.50	51.20
	1.0	289	291	580	1.01	71.00	61.23

*Corrected for the spontaneous lethality. - For each treatment 2000 eggs were scored.

finding cannot be generalized, since in a previous experiment[13] we reported that *D. melanogaster* was more sensitive to ethidium bromide than *D. simulans* after treatment of adults. Thus, the concentration-resistance pattern may differ considerably, depending on the chemical tested and on the population analyzed.

The essence of this paper can be summarized as follows: acridine orange is toxic for *D. melanogaster* and for *D. simulans*, and the sensitivity of the two species is not the same. More experiments on physiological and genetic hazards of AO on these species are in progress.

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Modulation of membrane permeability by amino acids in *Vinca* petals

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Summary. Exogenously supplied l-amino acids, including proline, differentially reduce the permeability of *Vinca* petal membranes to acid and alkali. It is suggested that these amino acids, which accumulate naturally under drought conditions, may help to sustain drought effects by stabilizing the membranes.

It is well known that free amino acids accumulate under water stress conditions[1]. Enormous accumulation of proline has been repeatedly shown during water stress[2], as well as salt and temperature stress[3]. Proline also accumulates during starvation and mineral deficiency[4]. Various roles have been assigned to this accumulating pool of amino acids, especially proline, including that of nitrogen storage under stress conditions and as an osmoregulator to overcome stress effects[5]. Proline has also been shown to delay wilting and maintain a higher relative water content in wheat and barley seedlings[6]. In an attempt to explore the positive role of these accumulating amino acids in sustaining drought effects, we have investigated the effects of exogenously supplied amino acids on the permeability of membranes in *Vinca major* petals.

Vinca major petals were collected from a wild population and infiltrated with different concentrations of amino acids for 24 h. Amino acids were supplied as the HCl salt in aqueous solutions. Permeability of membranes to acid and base was measured by noting the time taken by 0.5 N HCl or NaOH to pass into the petal[7]. Acid changed the color of the petal from purple to red and alkali from purple to yellow. Though change of color took some time, the time noted is the one for complete change of color. Experiments were conducted in 10 sets in triplicate.

It is clear that different amino acids affect membrane permeability differentially (table 1). L-Proline, L-leucine, L-asparagine, L-arginine and L-alanine double the time taken for alkali to pass through the membrane, and thus reduce the permeability to about half. Similarly, L-phenyl-