residue were assayed for inhibitor activity. In the next step, the SS was dialyzed extensively against 0.0001 M phosphate buffer pH 7.0 at 10 °C overnight. The SS was passed through a column (15 cm × 1.1 cm ø) of Dowex 50 W × 8 (200–400 mesh) and the inhibitor eluted with 1 column-volume of distilled water at about 3 °C. The eluate thus obtained was treated with activated charcoal (0.25 g/ml eluate) at 2 °C. This was filtered and the filtrate was assayed for the inhibitors.

Analysis of data was done by repeating all the experiments in series 5 times. Each observation was made in duplicate keeping its control and blank side by side, and each datum was expressed as mean ± SD. Almost all observations were found to fall within ± 2 SD.

Results and discussion. Pea DAO is reported to be absent from resting seeds in general[15] and it is said to appear in the cotyledon of pea seeds on the 2nd day of germination[12, 17, 18] In the present study, it was also detected on the 2nd day. *Sorghum vulgare* shoot and root homogenates obtained from seeds germinated for 5 days were incubated with pea DAO, showing the presence of the inhibitors. The shoot supernatant of the embryo obtained from 50 mg of the tissue showed the maximum inhibition of 21.7 units/g fresh tissue; while the shoot residues had practically no inhibitor (table 1). The inhibitor seems to be thermolabile as 15 min of treatment destroyed about 50% of the inhibitor, and a further treatment gave a final reduction to about 25% of the original value. The inhibitor does not seem to be a protein since after trichloroacetic acid treatment the TCA supernatant, after neutralization, contained all the detectable inhibitor activity (21.3 units). Shoot supernatant, after dialysis overnight, no longer contained the inhibitor whilst the control solution had the same initial activity. The inhibitor could be eluted with water from Dowex-50 but was adsorbed on activated charcoal.

The action in vivo was then studied by soaking pea seeds in the shoot supernatant and germinating them on filter papers moistened by the same extract in a petri dish. The germination of the seeds was found to be diminished in the extract-treated seeds which also had reduced enzyme activities. Heated extract could not show any reversal effect on the germination or on the enzyme synthesis. Various fractions at different stages of purification could not restore the normal germination nor the enzyme synthesis (table 2); this indicates the presence of more than one inhibitor - one acting in vitro blocking the enzyme activity and the other acting in vivo blocking the germination of the seeds and the enzyme synthesis.

The 2 types of inhibitors, one active under in vitro conditions and the other on germinating seeds could be separated by adsorption on active charcoal. The latter inhibitor is heat stable and is neither inactivated by TCA nor adsorbed on activated charcoal.

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4 T.A. Smith, Endeavour *31*, 22 (1972).
5 U. Bacharach, in: Functions of naturally occurring polyamines p. 96. Ed. U. Bacharach Academic Press, New York 1973.
6 J.M. Hill and P.J.G. Mann, Biochem. J. *91*, 171 (1964).
7 E.F. Yamaski, R. Swindell and D.J. Read, Biochemistry *9*, 1206 (1970).
8 S.K. Srivastava and V. Prakash, Phytochemistry *16*, 189 (1977).
9 A.J. Clarke and P.J.G. Mann, Biochem. J. *65*, 763 (1957).
10 R.H. Phelps and L. Sequira, Pl. Physiol., Lancaster *42*, 1161 (1961).
11 T.A. Smith, Phytochemistry *14*, 865 (1975).
12 S.K. Srivastava, V. Prakash and B.I. Naik, Phytochemistry *16*, 185 (1977).
13 R.K. Sindhu and H.V. Desai, Indian J. Biochem. Biophys. *17*, 194 (1980).
14 B. Holmstedt, L. Larsson and R. Tham, Biochim. biophys. Acta *48*, 181 (1961).
15 B. Joshi, unpublished observation.
16 R.K. Sindhu and H.V. Desai, Phytochemistry *19*, 317 (1980).
17 R.H. Kenten and P.J.G. Mann, Biochem. J. *50*, 360 (1952).
18 E. Werle, G. Baucampand and U. Schirren, Planta *53*, 125 (1953).

Influence of the feeding conditions on competition between *Drosophila melanogaster* and *Drosophila simulans* for their oviposition site

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Summary. Under low feeding conditions, the number of eggs laid by the female *Drosophila melanogaster* or the female *Drosophila simulans* is frequency dependent: it increases simultaneously with the increase of the frequency of the species to which the female belongs. This result could be explained by the role of scents in the choice of the oviposition site in *Drosophila*.

Populations of both sibling species, *Drosophila melanogaster* and *Drosophila simulans*, are often sympatric[1]. An important component of competition between *Drosophila* is the amount of eggs laid by each female per unit of time. Both species employ the same kind of oviposition sites which are also the feeding sites of the adult flies. Owing to the limited food supply available at certain periods of the year, these closely related species may compete for the use of a common resource. This work will be devoted to an experimental study dealing with competition for an oviposition under low feeding conditions, at various frequencies of the two species.

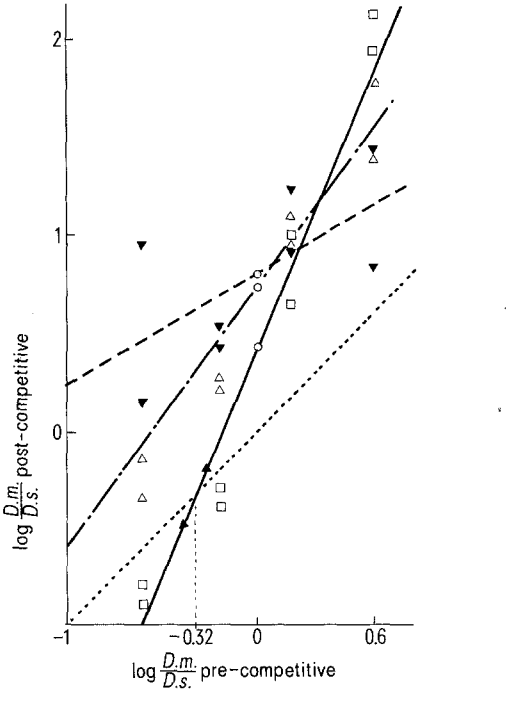
Material and methods. A *sepia* mutant strain of *D. melanogaster* and a wild strain of *D. simulans*, captured in 1971 and 1977 respectively, have been kept in the laboratory by mass-replications at 20 °C. 6 populations of 1000 fertilized, 3-day-old females were initiated with these strains, the relative frequencies of each species in each population being respectively 0.00, 0.20, 0.40, 0.60, 0.80, 1.00. Each population was placed in a population cage with 12 vials containing 30 cm^3 of Pearl's food medium[2]; the age of each population was 2 days in order to suppress the effects of anaesthesia. The cages were stored under conditions commonly recognized as optimal for both species[3–5]:

20±0.5 °C, 12:12 illumination so that during the experimental periods the flies were in darkness.

After this 2-day period, the 12 vials were removed and replaced by a single new one, containing Pearl's medium. The flies were further aged for 2 more h in darkness in order to preclude distorting results that could emerge due to the numerous ovipositions used by *D. melanogaster* at the beginning of the dark period[4]. Every h over a period of 10 h, the vial was replaced by a new one. There were never more than 90 eggs laid on a vial so that the expectation of the larval competition did not play a role here. The emerging flies were scored in each species. This experiment was replicated 36 h later. Meanwhile the flies could feed on 6 vials of food medium replaced every 12-h period; the 1st and last periods correspond to light, whereas the 2nd corresponds to darkness. At the end of this 2nd period, the 6 vials were kept in an attempt to test for a possible influence of darkness on this competition. The contents of each vial was divided into 2 parts, each of which was placed in a bottle providing an oversupply of food and therefore eliminating larval competition. In order to detect a possible effect of light on competition, the 6 vials, introduced at the beginning of the 3rd period, were also transferred into 2 bottles of food medium.

t-test to unity, (t_b), ordinate at the origin (A) and slope (b) of the regression line $y=bx+A$ of the logarithms of the pre-competitive population size ratio involving both species on the post-competitive one

	Starvation	Food in darkness	Food in light
t_b	4.1963	0.9585	0.8934
	Significant	(NS)	(NS)
A	0.4409	0.8134	0.7520
b	2.4536	0.5775	1.3200



Regression diagram of the logarithms of the pre-competitive population size ratio involving both species on the post-competitive one in starvation in darkness (—), in oversupply of food in light (---) and in oversupply of food in darkness (-.-) compared to the bisectrix (...). The average points of each of the replications were only plotted, respectively with symbols □, △ and ▽.

Results. No significant difference was found between the 2 experiments, the data were therefore pooled. The standard method to analyze a competition with various frequencies of 2 species is that suggested by De Witt[6], and consists of plotting the regression diagram of the logarithms of the pre-competitive population size ratio, involving both species on the post-competitive one. The regression slope was tested to unity by a t-test, with the theoretical expectation in lack of competition as one (table and curves).

It can be seen from the figure that:

- under low competition for an oviposition site, the regression slope does not significantly differ from unity, either under light ($t=0.8934$, p 0.40) or dark ($t=0.9585$, p 0.40) conditions. Under these conditions, no influence of species-specific frequencies can be shown on competition for oviposition.
- under strong competition for an oviposition site, the regression slope is significantly higher than one ($t=4.1963$, p 0.001). There is an unstable equilibrium point at the point of intersection between the regression line and the bisectrix. This point, whose abcissa is log *D.m./D.s.* (introduced individuals) $=-0.32$ correspond to a ratio *D.m./D.s.* $=0.48$, i.e. a frequency of 0.33 *D.m.* and 0.66 *D.s.* Below $\frac{1}{3}$, *D.m.* will be eliminated by *D.s.*, whereas over that frequency, *D.m.* will be favored in the competition against *D.s.* for the oviposition site.

Conclusion. Many recent works have been devoted to demonstrating that pheromones could play an important role in the choice of an oviposition site, as well in presence of males and females belonging to the same species[7,8] as in presence of males of other species[8]. Furthermore, Laudien[9] stressed the tendency of *Drosophila* to oviposit on media providing a scent similar to that of the medium from which they emerged. Yet these authors did not take into consideration the consequences of such an oviposit behavior on the interspecific competition. This paper aims to outline some aspects of this problem. It appears from the above-described experiment that the number of eggs, laid by each female per unit of time, is species-specific frequency dependent, a given female laying more eggs as the frequency of the species to which it belongs increases. This oviposition stimulation through the presence of numerous flies of the same species is worth comparing with the results of Del Solar[7] who observed in *D. melanogaster* a pronounced tendency to oviposit on sites yielding conspecific eggs. If *D. simulans* is shown to display the same ovipositing behavior as its sibling species, this would account for our results. The threshold ($\frac{1}{3}$ *D.m.*, $\frac{2}{3}$ *D.s.*), from which one of the species disappears, depends more probably on experimental conditions than on intrinsic properties of the relevant species. The mechanism of attraction to a previously used oviposition site may proceed either from the action of a chemical (species-specific or not), left by the ovipositing female or given off by eggs, or from any other phenomenon.

1 M.T. Rocha-Pité and L. Tsacas, Bolm. Soc. Port. Ciênc. nat. *19*, 37 (1979).
2 R. Pearl, A.L. Allen and W.B.D. Penniman, Am. Nat. *60*, 357 (1926).
3 A.O. Tantawy and G.S. Mallah, Evolution *15*, 1 (1961).
4 R. Allemand, J. Insect Physiol. *22*, 1031 (1976).
5 R. Allemand, Genetica *47*, 1 (1977).
6 C.T. de Witt, Versl. landbouwk. Onderz. *66*, 8 (1960).
7 E. Del Solar and H. Palomino, Biologica *39*, 27 (1966).
8 M. Mainardi, F. Le Moli and L. Grisendi, Ateneo parmense, Acta nat. *10*, 205 (1974).
9 H. Laudien and H.H. Iken, Z. Tierpsychol. *44*, 113 (1977).