Sloane, C. and E.B. Spiess. University of Illinois, Chicago, Illinois. Stimulation of male courtship behavior by female "odor" in D. pseudoobscura.

Evidence in favor of a sex pheromone in some Diptera has been demonstrated as a factor in stimulating courtship behavior in at least four laboratories: Rogoff et al., 1964, in Musca domestica; Adams & Mulla, 1968, in Hippelates collusor (eye gnat); Ehrman, 1969, in D. pseudoobscura; and Shorey & Bartell, 1970, in D. melanogaster. In contrast, Ewing & Manning, 1963, were unable to demonstrate the effect of scent from one sex upon the other in D. melanogaster. Using the method of Shorey & Bartell, we set about testing three strains of D. pseudoobscura for the influence of females upon male courtship elements. The olfactometer was blown from glass tubing (1" diameter) outfitted with 2 Teflon stopcocks so that air could be routed through one chamber or the other (see Shorey & Bartell's Fig. 1). Flies to be tested as an odor source were placed in a glass tube 5/8" in diameter open at both ends covered with cheesecloth and the tube was inserted in one of the entering air chambers. The males to be observed were placed in a similar tube inserted from the other end of the olfactometer. All flies were unetherized. The air source was the air jet on the lab bench filtered through distilled water containing activated charcoal in suspension; and the air flow was calibrated as close to 500 ml/min. as possible. The 3 strains of flies were obtained from Mr. Richard Sherwin in this laboratory from his 20th generation of selection for fast or slow mating propensity (Sherwin, 1970). One PP and one AR strain (FP2 and FA2) had been selected for fast mating, while one AR strain (SA1) had been selected for slow. Flies to be tested were sexed and isolated on emergence in lots of 6 or 30, depending on their use either in the observation chamber (6) or as the odor source (30).

Observations consisted of counting orientations and wing variations between males in a 6 minute period. Counts were made at 15 second intervals. The first 3 minutes of each test simply allowed air to flow through the empty chamber, then the stopcocks were reversed to allowed air to flow from the odor source (either 30 males or 30 females). Between trials the olfactometer was aired for at least 3 minutes. The first set of experiments was done using the same strain for both sexes while the second set interchanged fast and slow AR (FA ♂♂ had SA ♀♀, or SA ♂♂ with FA ♀♀). Observed orientations and wing vibrations during the 3 minutes of air flow (initial) served as a control for each test. All tests were replicated ten times.

Table 1
Means and st. errors of courtship elements in 3 minutes for 10 replicates per strain. ♀♀ or ♂♂=30 as odor source. Control = 3 initial min. air flow.

Strain Males	Orientations				Wing Vibrations			
	♀♀	Control	♂♂	Control	♀♀	Control	♂♂	Control
FA2	19.1±214	22.0±3.2	12.0±2.4	24.1±2.9	13.5±1.6	15.3±2.7	11.1±2.0	20.2±2.7
SA1	6.4±1.0	7.6±1.4	8.0±2.2	12.3±1.7	3.0±1.1	4.0±0.8	4.2±1.4	5.8±0.8
FP2	12.0±2.4	12.9±2.5	10.9±2.5	14.6±2.6	7.6±1.5	5.5±0.8	5.6±1.3	6.7±1.9

Mean courtship elements (orientations and wing vibrations) with standard errors are given in Table 1. Generally it is evident that the FA2 (AR) strain is most active, SA1 (AR) the least, with FP2 (PP) intermediate in both courtship elememts. Both fast strains (FA and FP) show an increase in these elements when the odor source is virgin females as opposed to males as a source, while the slow strain showed the reverse. Using "t" tests for significance between treatments of 30 ♂♂ vs. 30 ♀♀ as odor source, only in the fast AR (FA2) strain orientation ($t=2.08$) or in the pooled totals of orientations plus wing vibrations ($t=2.18$) were the differences significant (5% level), though the pooled totals for fast PP (FP2) approached significance ($t=1.68$, $p=.11$).

Controls were higher in each case than the odor source. It seems that in the initial 3 minutes before each odor trial flies were active merely in response to the air flow. Once accustomed to that, the values approached a more usual base level of activity for the odor tests.

Table 2
Means and st. errors of courtship elements of AR strains when tested with females of the opposite strain. (Controls, always greater, are omitted).

Strain	Orientations		Wing Vibrations	
Males	SA ♀♀	FA ♀♀	SA ♀♀	FA ♀♀
SA1	6.4±1.0	7.0±1.7	3.0±1.1	6.1±1.6
FA2	13.8±3.7	19.1±2.4	14.1±3.6	13.5±1.6

In the second study, fast and slow AR sexes were interchanged, and the results are given in Table 2. Comparison of males' response with their own strains is included with data from Table 1. FA females raise the orientation level of both types of male, though not significantly for either, while in wing vibration there is only an increment for SA males which approaches significance ($t=1.84$, $p=.07$).

These results indicate some increases in male activity due to presence of females but only for strains which are genetically of high mating propensity. Influence of fast-mating females upon slow-mating males is inconclusive though suggestive. These influences can only be attributed to airborne stimulation, presumably a volatile substance transmitted from the females.

References: Adams, T.S. and M.S. Mulla, 1968 J. Insect Phys. 14: 627-635; Ehrman, L., 1969 Evol. 23: 59-64; Ewing, A.W. and A. Manning, 1963 Anim. Behav. 11: 596-597; Rogoff, W.M., A.D. Beltz, J.O. Johnson and F.W. Plapp, 1964 J. Insect Phys. 10: 239-246; Sherwin, R. N., 1970 Genetics 64: s59; Shorey, H.H. and R.J. Bartell, 1970 Anim. Behav. 18: 159-164.

Picard, G. and Ph. L'Héritier.
Laboratoire de Génétique, Clermont-Ferrand, France. A maternally inherited factor inducing sterility in D. melanogaster.

It was found out recently that a laboratory strain bearing the gene sepia gives puzzling results when crossed with a standard Oregon strain. As shown in the table, females from the cross ♀ se x ♂ + lay eggs which hatch with a very low probability. Males from the same cross and both sexes in the reciprocal cross ♀ + x ♂ se behave normally.

Using flies from some other laboratory strains or flies caught in the wild in the crosses with the sepia strain leads to similar results, but with a somewhat variable degree of sterility.

original cross	back cross	total	eggs hatched	percentage of hatching
♀ se x ♂ +	♀ F_1 x ♂ se	360	34	9.4
♀ + x ♂ se	♀ F_1 x ♂ se	100	96	96.0

Eggs which do not hatch have been fecundated, since segmentation nuclei are readily observed, but most of them die before blastoderm stage. The probability of hatching for an egg laid by a sterile female has been found to be dependent upon two factors:

1) It rises with mother aging.

2) It gets nearly normal when temperature is raised to 30°C for a period of 48 hours located at the end of ovogenesis. Female offspring, which are allowed to reach imaginal stage owing to such a thermal treatment, are not genetically cured, and may show the same kind of sterility as their mothers.

The genetic determinism of the female sterility is presently under active investigation. The following points can be held as demonstrated:

1) The probability of hatching of an egg depends but poorly if at all upon its own genotype, the major factor being the genetic origin of the mother.

2) For a sterile female to arise, two genetic requirements must be filled. A maternally inherited factor of unknown nature normally propagated in the sepia strain and a genotype heterozygous for certain genes must be present.

3) Quite a number of genes located on all of the three major chromosomes seem to be involved in the phenomenon.