



Fig. 2. Mating chamber used for housing pairs of flies during recording of courtship song. I - Mating chamber taken apart to show constituent parts; A cover, B floor, C condenser microphone. II - Mating chamber assembled.

We use photographs for examining the overall pattern of fly song during bouts of courtship and video tape analysis for detailed study, e.g., (1) matching wing angle and courtship component; (2) identifying the source of sounds not produced by wings, (3) seeing how the position of the flies relative to the microphone diaphragm influences the signal recorded. These two methods of analysis complement each other in providing information about fly song.

These techniques were designed for work with *Drosophila*. However, they are also applicable, with little modification, to analyses of the behavioral context of sound emissions in other species and so may be of interest to a wider audience.

Reference: Bennet-Clark, H.C. 1972, DIS 49:127-128.

de Jong, F. and G.E.W. Thörig. University of Utrecht, The Netherlands. A simple test for intra-electromorphic variants of alcohol dehydrogenase in *D. melanogaster*.

Thörig et al. (1975) localized a genetic variant of Adh at the Adh locus, with the same electrophoretic mobility as Grell's Adh^F. However, while Adh^F-enzyme is rapidly inactivated at 40°C the new variant, Adh^{71K}, is still active. Moreover, Adh^F-enzyme is inhibited by high concentrations of ethanol when little NAD⁺ is present. Under these conditions Adh^{71K}-enzyme still shows high activity.

Considering these differences, the following method was developed to separate Adh^{F/F}, Adh^{F/71K} and Adh^{71K/71K} individuals, without the use of electrophoresis.

Single flies are homogenized in 0.025 ml 0.5M Tris-HCl buffer, pH 9.0, in grinding holes in ceramic tablets (at ±20°C). The tablets are wrapped in aluminum foil to prevent desiccation of the homogenates. Then Adh^F-enzyme is inactivated in an incubator at 50° during 15-20 minutes. After this 0.2 ml of the following staining solution is added:

characteristics and gives records with improved signal to noise ratio when compared with ribbon microphone recordings.

For recording and playback we use a video tape recorder (SANYO VTR 1100SL) with slow motion (1/5 normal speed) and stop action playback facilities. The latter permits frame by frame analysis of successive still scenes at 1/50 second intervals. Measurements of wing extensions, etc., and associated song patterns are made on these still scenes. Measurements of other behavioral parameters such as courtship duration and copulation length are obtained by reading the superimposed time at the beginning and end of a given behavior. During recording, brief notes are made of audio and video tape position indicator and timer information coincident with behavior under study. This information is necessary for quick access to record segments of interest in later analyses.

A Grass Kymograph Camera (Model C4R) is used for photographing fly song recorded on audio tapes. The camera is attached to a slave oscilloscope (Tetronix Type RM 561A) which is in turn attached to a storage oscilloscope (Tetronix Type RM 564). A 100 cps calibration signal is also filmed.

Fly song is also measured from video tapes by connecting the audio output of the V.T.R. to an oscilloscope (Hewlett Packard 1201B). Bursts of the song trigger the oscilloscope and are stored on the screen. The period of the waveform is measured (in msec) from the calibrated time base of the oscilloscope. Frequencies of the wave forms are then calculated from these measurements.

460 ml 0.05M Tris-HCl buffer, pH 7.1
 40 ml 96% ethanol
 25 mg NAD⁺
 25 mg MTT
 Δ PMS

The tablets are wrapped again in aluminum foil and put in an incubator at 35-40°C in staining the homogenates.

After 20 minutes, the solution is heavily stained by the Adh^{71K} individuals, whereas Adh^F individuals will not show any activity. Heterozygotes can be distinguished by their intermediate staining.

Reference: Thörig, G.E.W., A.A. Schoone and W. Scharloo 1975, *Biochem. Genet.* 11:721.

Gupta, A.P. Harvard University, Cambridge, Massachusetts (present address: Cidade Universitaria UFRJ, Rio de Janeiro, Brazil. A new technique for collecting *Drosophila* eggs.

Generally, *Drosophila* eggs are collected by having flies oviposit in bottles on spoons or in petri dishes containing colored food medium. The well fed adults are usually allowed to oviposit 24 to 48 hours to collect an adequate egg sample. It is difficult to collect eggs of sufficient sample size from a number of crosses or

strains simultaneously. To facilitate collecting large egg samples from a number of crosses simultaneously over a short period of time, I modified the prevailing techniques with excellent results. The success of this technique depends upon starving the flies shortly before permitting the flies to oviposit.

Twenty-five to 30 pairs of newly emerged *D. pseudoobscura* were allowed to mate in vials for 5 to 10 days at 24°C under optimal rearing conditions. They were then transferred to empty half-pint milk bottles and allowed to starve for 45 to 90 minutes at room temperature. The time of starvation is determined by noting when the activity of the flies diminishes. At this time, a teaspoon containing Carpenter's medium with food coloring and covered with a thin layer of dead or live Fleischmann's yeast suspension is put into the bottle. If dead yeast is used, prepare the solution 2-4 days before use. The thin layer of yeast suspension is allowed to dry before the spoon is put into the bottle. The back of the spoon must fit firmly against the side of the bottle to prevent females ovipositing between the spoon and the bottle. These bottles are put at 24°C, and the spoons with large numbers of eggs are removed after 6-14 hours.

It would appear that the starved females retain their eggs until they once again are able to feed. At that time they lay their eggs in profusion. For a research project I had to collect 1800 fertile eggs for each of two parental and two F₁ classes, for a total of 7200 eggs, to be tested simultaneously. Using this method I had no trouble collecting the required number of eggs in a short period of time. The technique was further tested using 25 to 30 pairs of *D. melanogaster*. Approximately 1000-2000 eggs were collected in 1-3 hours. Thus, this technique is probably generally useful for collecting large numbers of eggs in a number of species in a short time period.

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Johnson, P. and D.E. Cowling. University of Sheffield, England. A courtship song simulator for *Drosophila*.

Auditory stimuli play an important role in the sexual behavior of *D. melanogaster* and a number of other species (Bennet-Clark 1975). These take the form of a song produced by wing vibration by the male during courtship. The male

courtship songs are species specific and probably play a role in sexual isolation. The song of *D. melanogaster* consists of phrases which contain two discrete wave form elements known as sine song and pulse song (von Schilcher 1976a, 1976b; Burnet, Eastwood and Connolly 1977). A simulator which allows courtship and mating to be investigated using intact females paired with wingless males in the presence of artificial song of specified characteristics is described here.