

Crossley, S. and J. McDonald. Monash University, Clayton, Victoria, Australia. A method for permanently recording courtship song and courtship behavior simultaneously in *Drosophila*.

watching courtship and listening to amplified song at the same time (Bennet-Clark 1972) does not give this precise information because of the speed of interactions between courting flies and more importantly the inadequacy of our perceptual skills in attending to auditory and visual stimuli simultaneously.

To overcome these problems we have developed a method using video tape recording (Fig. 1). One camera (A) records the flies while another (B) records an oscilloscope trace (C) of any sounds produced. Each camera is fitted with a Macro-cosmicar V.T.R. lens ($f = 25$ mm, 1:1.4). Signals from both cameras are combined using a Special Effects Generator (National Model W1 546N/A) (D) and video taped (E). Digital indications of date (month, day), time (hours, mins, secs, 1/100 secs) are also superimposed on the recorded picture by a video timer (FOR-A Co. Ltd. Model VTG-33) (F). The audio-track of the video tape is used to record the sounds from the flies. The experimenter can also record via a microphone (G) information concerning the kind of pairing and the age of the participants, prior to each recording. A Video Monitor (H) shows the visual display recorded. In addition to recording sounds on video tape we also simultaneously record fly sounds and spoken information using an audio-tape recorder (Revox, Type A77) (I). We included this in the system because we find that it is more convenient to use the audio tape recorder for analyses of, for example, rapid events because playback can be made at half speed. There is no observable difference in the quality of sound recorded on video tapes and on audio tapes.

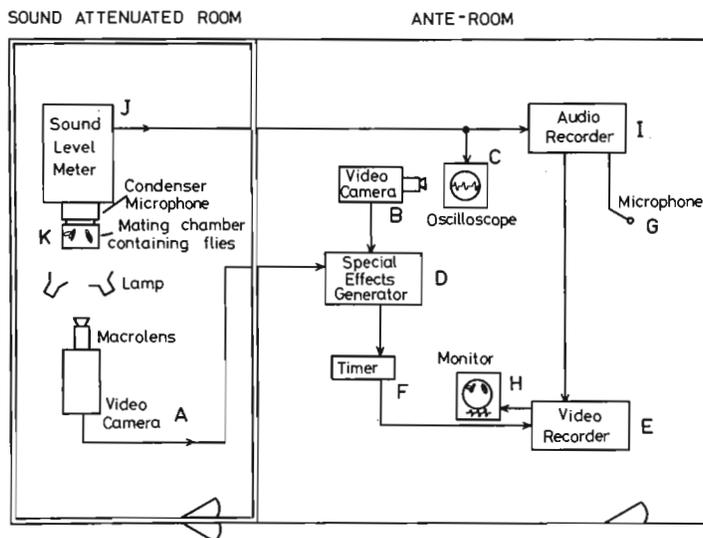


Fig. 1. Experimental set-up for recording courtship song of *Drosophila*.

floor of the mating chamber. Before recording, the original grid is removed from the microphone and the perspex chamber is pushed over the mesh grid. Flies are then introduced through one of two holes in the side of the chamber. The holes are stoppered with perspex plugs and the whole is then screwed onto the microphone base as far as it will go, which results in the mesh being separated from the diaphragm by about 1 mm.

Because of the high sensitivity of condenser microphones, we use a sound attenuated room and ante-room designed for reducing background noise. Fly song is recorded in the sound attenuated room and the experimenters and other apparatus occupy the ante-room. We have also obtained records using a ribbon microphone (Bennet-Clark 1972) which does not require quiet conditions. We prefer to use a condenser microphone, however, because this has known response

Courtship song is an important taxonomic character in *Drosophila* and provides evidence for establishing phylogenies. It is produced during wing displays which vary quantitatively and qualitatively within and between courtships. For comparisons within and between species it is necessary to know which aspects and how much of a total wing display produces sound. Simply

For recording and amplifying courtship song we use a one inch condenser microphone (Brüel and Kjaer Type 4145) attached to a sound level meter (Brüel and Kjaer Type 2203). The auditory signal from the sound level meter is led directly to the oscilloscope as well as to the audio tape recorder (I) and video tape recorder (E). The mating chamber (K) (Fig. 2) is a perspex tube one end of which is covered with glass. This tube slips over the condenser microphone up to an inner flange. This leaves a chamber 7 mm deep and 21 mm diameter between the microphone grid and the glass cover. The protection grid which is supplied with the microphone allows flies to touch the microphone diaphragm. The upper surface of this grid was therefore removed and replaced with another grid of very fine stainless steel wire mesh (48 squares per inch and with wire diameter .004 inches). This mesh grid forms the

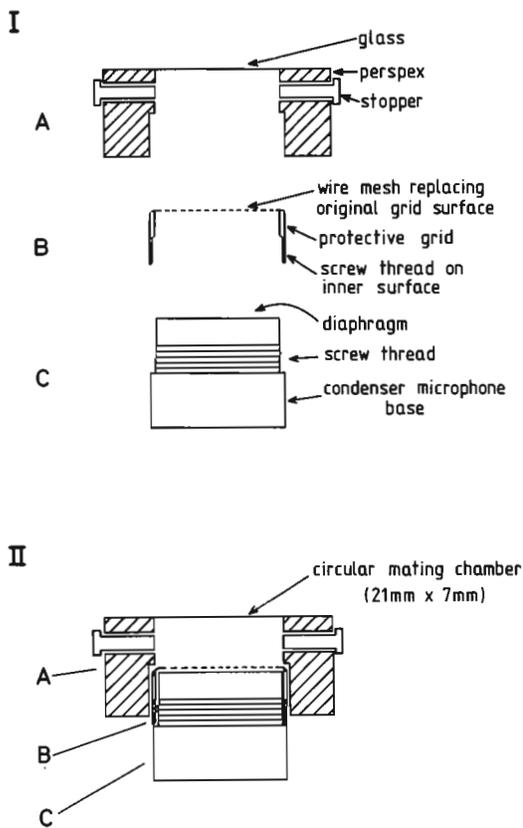


Fig. 2. Mating chamber used for housing pairs of flies during recording of courtship song. I - Mating chamber taken apart to show constituent parts; A cover, B floor, C condenser microphone. II - Mating chamber assembled.

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°C 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.