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 F_1 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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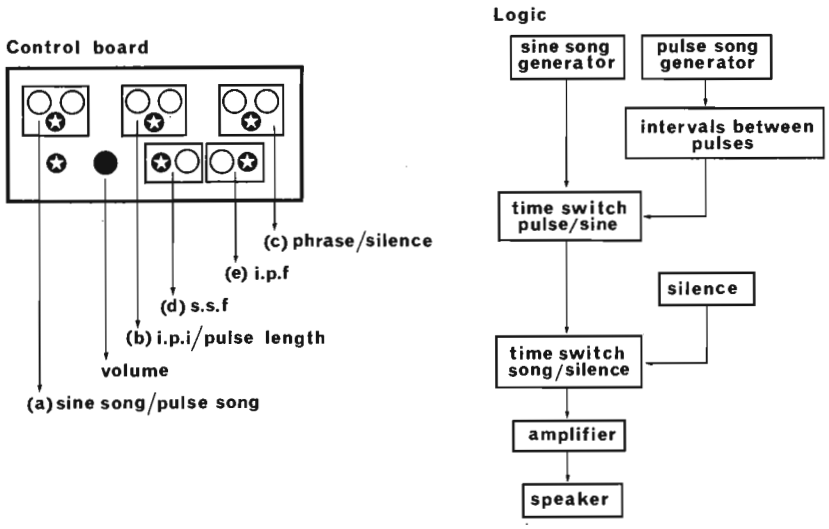
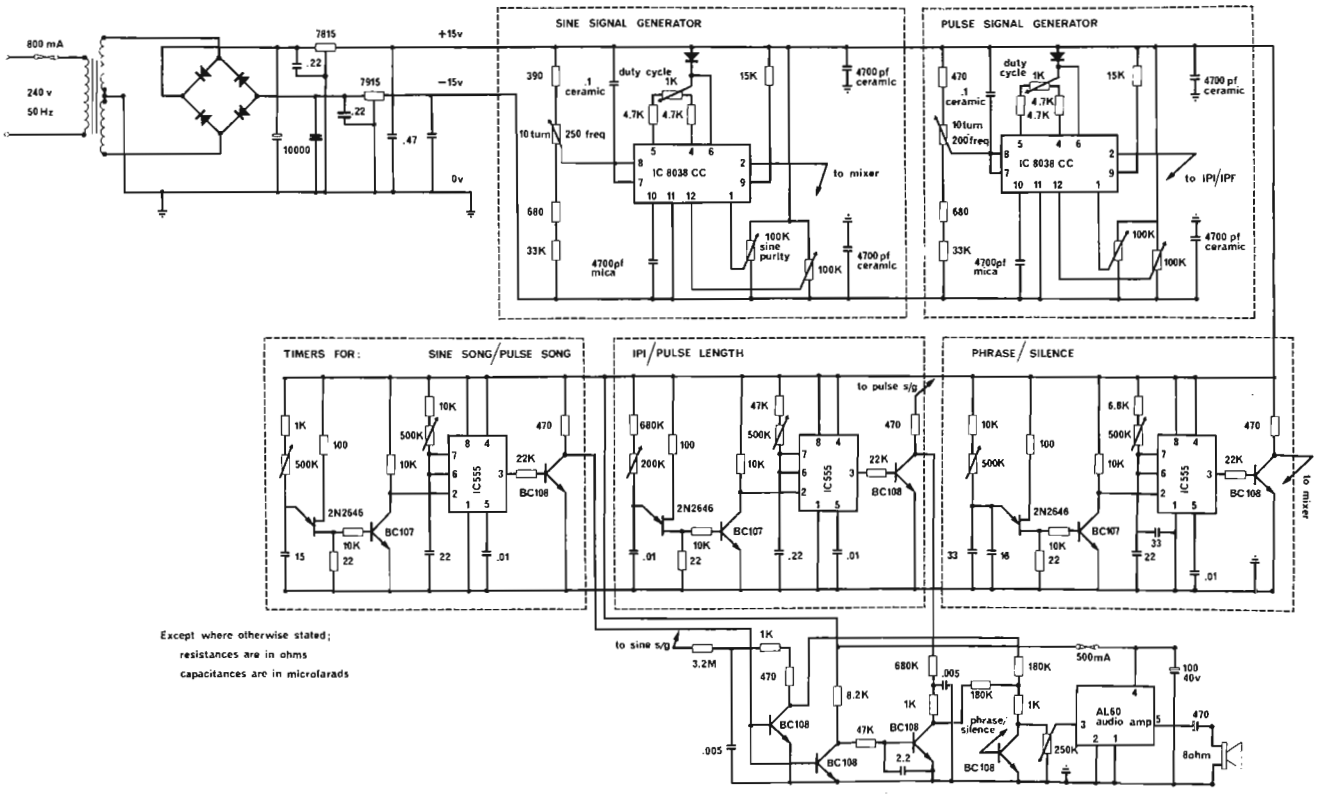
Johnson, P. and D.E. Cowling. University of Sheffield, England.
A courtship song simulator for Drosophila.

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.



The circuit diagram and control panel are illustrated in the figure. The controls are 20-turn potentiometers with modified multi-turn dial mechanisms. There are two signal generator circuits. The sine signal generator specifies the sine song frequency (s.s.f.) which can be varied directly by a single control (d). An adjacent coaxial socket facilitates direct measurement of the frequency. The pulse signal generator specifies the intrapulse frequency (i.p.f.) which can be varied via control (e), and monitored directly through an adjacent coaxial socket. There are three timer circuits. Timer (i) "ipi/pulse length" controls the interpulse interval and number of cycles per pulse to give monocyclic or polycyclic pulses. These parameters are varied using the pair of controls (b) after prior setting of the ipf (control e), because altering ipf affects the number of cycles per pulse. The ipi/pulse length timer together with the two signal generators specify the basic characteristics of the song.

Timers (ii) "sine/pulse" and (iii) "phrase/silence" specify the overall phrase structure of the song via control pairs (a) and (c), respectively. Timer (ii) sets the maximum required duration of continuous pulse and continuous sine song by having two inputs (sine and pulse) but only one output which alternates between the two. The timing of the alternation is controlled by (a). This forms the input of the phrase/silence timer (iii) which specifies the phrase



length and periods of silence between phrases (interphrase interval). The output of timer (iii) is to an 8", 8 ohm loudspeaker with frequency range 30 Hz - 4 K Hz, and power handling capacity 25W r.m.s., which resonates in free air at 30 Hz. The amplitudes of the sine and pulse song are regulated by a volume control.

The apparatus is switched on for a 30 min. warming up period to stabilize before use. For each trial 10 pairs of virgin flies are placed in a 20 x 45 x 45 mm cage with Perspex sides and cotton gauze roof and floor. The cage is suspended 5 cm above the speaker cone on four wire suspensors rigidly fixed to the speaker casing.

The simulator produces courtship song with any desired combination of characteristics with respect to ssf, ipf and ipi, with phrases of a specified duration and composed of a desired mixture of sine and pulse song and interphrase periods of silence.

References: Bennet-Clark, H. 1975, Verh. Dtsch. Zool. Ges. 18-25; Schilcher, F. von 1976a, Anim. Behav. 24:18-26; Schilcher, F. von 1976b, Anim. Behav. 24:622-625; Burnet, B., L. Eastwood and K. Connolly 1977, Anim. Behav. 25:460-464.

Johnston, J.S. Texas A&M University, College Station, Texas. Hawaiian Drosophila with colored headlamps: a new mark-recapture technique.[1]

Hawaiian Drosophila, which pupate in moist soil, must emerge by repeatedly inflating and deflating the ptilinum. When the soil is covered with a thin layer of fluorescent pigment (5 mg/mm^2) followed by a 2-4 mm layer of dry sand, the ptilinum picks up a layer of pigment. This layer of pigment gets pulled, upon emergence, into the ptilinal suture. Here, it forms a permanent fluorescent layer which cannot be rubbed off, cleaned, or removed. Under ultraviolet light, the area in and around the ptilinal suture fluoresces brightly, like a "colored headlamp". The headlamp is visible, under a dissecting scope, throughout the life of the fly. In 20 day and older flies, I found the headlamps more visible when the flies were compressed lightly between two pieces of glass.

Twenty-seven quart jars of D. mimica pupae, moist sand and pigment were used to test for marking effectiveness using a variety of pigments and colors (Table 1).

Table 1

Total Jars	Pigment Source	Marked Flies	Unmarked Flies
2	Tinopal[2]	42	0
2	Helecon[3]	56	0
6	Poster paint[4]	169	1
17	None (control)	0	410

One fly in 268 emerged without obvious markings. Emergence rate was not significantly different between marked and control jars. (χ^2 = 4.9, $p > .30$). Marked and control flies were next divided into 5 and 10 vials respectively and scored for survival and marking effectiveness. Fifteen survival curves were determined for the flies in the 5 marked and 10 control vials. These survival curves were then compared using an analysis of covariance. The adjusted mean survival for marked and control flies were 64 and 63 days, respectively. The survival rate for marked flies was not significantly different from that of control flies ($p = .28$).

For field studies, poster paint and sand could be spread directly onto the soil at selected sites. To obtain larger numbers and provide more experimental control, field caught flies could be used to produce a population of pupae in the laboratory. Then, jars, pupae, sand and pigment could be set out at selected sites in the field. Preliminary studies suggest that pigment can be changed daily by scraping away the old sand and color, and replacing with a new color and new sand. This would permit distinct daily marking. After emergence, the numbers marked can be measured by filling the jar (or tray) with water. Empty pupae cases float and can be counted.

(1) Supported in part by NSF Grant DEB-76-19879, to J.S. Johnston; (2) Tinopal SFG, CIBA-GEIGY Corp., Ardsley, NY 10502; (3) Helecon Fluorescent Pigments, U.S. Radium Corp., Hackettstown, NJ 07840; (4) Blacklight Pigments, Barnett Mfg. Co. Inc., Los Angeles, CA 90035.