

Technique Notes



A multi-chamber device for observing courtship behaviors of fruit flies without using anesthesia.

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Researchers commonly measure courtship behaviors in *Drosophila* (Parsons, 1974), which usually requires many measurements over a short period of time. Several designs exist for constructing mating chambers (*e.g.*, Elens and Wattiaux, 1964; McRobert and Tompkins, 1985; Welbergen, 1991), but these chambers do not facilitate the large number of measurements required by many studies. Drapeau and Long (2000) overcame this problem when they developed the Copulatron—a multi-chamber apparatus enabling researchers to simultaneously observe the behaviors of many pairs of flies. Using multiple, identical chambers within a single unit helps to control for environmental factors (Drapeau and Long, 2000), which can impact results obtained through the use of single-chamber designs. However, the Copulatron does not provide a means for easily inserting flies without the use of anesthesia. Consequently, flies must be afforded a period of recovery after being placed in the chamber (Ruedi and Hughes, 2008), which substantially lengthens the time needed for measurements. Here, we describe an alternative design that builds on the Copulatron's design.

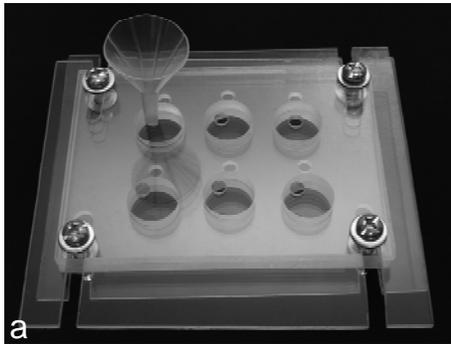
Our goal was to design a multi-chamber unit that enables a researcher to load flies without anesthesia, accurately view courtship behaviors, and subsequently transfer flies to individual vials. Our unit differs from the Copulatron in a few key ways. First, our unit is much smaller, containing only six mating chambers. This permits a single researcher to observe courtship behaviors in real time. Second, we have included sliding cover sheets that enable the insertion and removal of flies without anesthesia. This facilitates speedy measurements and avoids adverse side effects associated with anesthesia. Further, because our unit is inexpensive and easy to construct, one can make several units to increase the number of flies that can be observed simultaneously.

Our unit (Figure 1) consists of four pieces of plexiglass held together with a nut and bolt on each corner. We used thicker sheets of plexiglass for the two inner pieces (105 × 145 × 5 mm), which contain the circular mating chambers (23 mm in diameter). The outer sheets (140 × 165 × 3 mm) serve as the top and bottom. Both cover sheets have six entrance holes (6 mm in diameter), which are each centered over a corresponding mating chamber. These holes enable one to insert flies independently into each chamber using a funnel (Figure 1a & b). We designed the cover sheets to slide approximately 17 mm to shift positions between the insertion and observation of flies (open vs. closed position, Figure 1a & c).

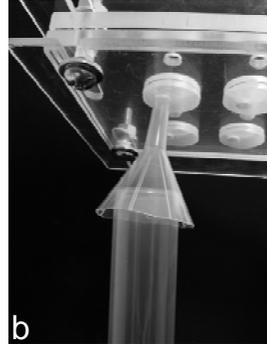
Similar to the Copulatron, we separate the two inner sheets of plexiglass with a piece of paper or thin plastic to serve as a barrier between the male and female fly in each chamber. We insert flies by turning the unit upside down and placing a funnel in the entrance hole (Figure 1b). We allow flies to climb upward into the chamber from a vial, remove the funnel, and then cover the hole with a

piece of masking tape. We repeat this process for both males and females on opposite sides. Once all flies are inserted, the top and bottom cover sheets can be slid in a way that allows unobstructed viewing of each chamber (Figure 1c). The barrier between males and females can then be removed in the same way as in the Copulatron exposing males to females in all chambers at once. One researcher can easily view the courtship of six pairs at a time. Fewer flies can be inserted into the unit if necessary, or our design can be adapted for viewing more matings with the aid of a video camera.

Open



Loading



Closed

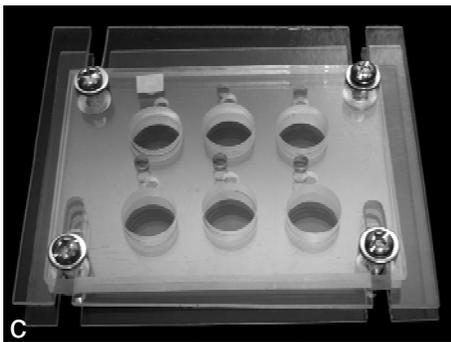


Figure 1. Our device has six mating chambers with sliding cover sheets on each side, enabling the insertion of non-anesthetized flies. We use a funnel to insert flies through openings in the cover sheet (a). We invert the unit, place the funnel through the hole in the cover sheet, and allow a fly to climb up into a chamber (b). We then remove the funnel and cover the hole with masking tape. We repeat this process for males on one side and females on the other side. Once all flies have been inserted, the cover sheets can be slid in a way that permits unobstructed viewing (c).

At the end of observations, we remove flies with the aid of anesthesia. The cover sheets are slid upward such that the taped holes are back in the “open” positions. We penetrate the tape with a CO₂ gun and anesthetize the flies. Once knocked down, we place flies into individual containers for further analysis. After the removal of flies, the next run can begin immediately as the cover sheets are in the original position. Flies can be removed without anesthesia if necessary. In this situation, we remove a piece of tape and quickly place a vial over the hole. Flies tend to climb upward into the vial, which can then be plugged with cotton. This process requires little time, but removal with anesthesia remains the fastest method. While our unit was designed for observing courtship in *Drosophila* spp., it could easily be adapted for studies using other insects.

Literature Cited: Drapeau, M.D., and A.D. Long 2000, *Dros. Inf. Serv.* 83: 194-196; Elens, A.A., and J.M. Wattiaux 1964, *Dros. Inf. Serv.* 39: 118-119; McRobert, S.P., and L. Tompkins 1985, *Dros. Inf. Serv.* 61: 194; Parsons, P.A., 1974, *Behav. Genet.* 4: 395-404; Ruedi, E.A., and K.A. Hughes 2008, *Behav. Genet.* 38: 424-436; Welbergen, Ph., 1991, *Dros. Inf. Serv.* 70: 262-263.