Taking the Headaches Out of Anesthetizing Drosophila

Authors: Artiss, Thomas, and Hughes, Bobby

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ONLINE ARTICLE

Taking the Headaches Out of Anesthetizing *Drosophila*

A Cheap & Easy Method of Constructing Carbon Dioxide Staging

THOMAS ARTISS BOBBY HUGHES

Drosophila spp. are excellent candidates for use in biology classes as model organisms to study a wide range of topics in biology including Mendelian genetics (see Lab 7 from the *Advanced Placement Lab Manual*), evolution (Salata, 2002), biochemistry (Sofer & Tompkins, 1994) and behavior (Forster, 1974), and they are well suited for open-ended and inquiry-based labs (Mertens, 1983). In addition, *Drosophila* are ideal study organisms for a variety of practical reasons: They are cheap and easy to maintain, they have short generation times, they are small enough that many individuals can be maintained even in small classrooms, and they are unencumbered by the ethical constraints of research on vertebrates (Sofer & Tompkins, 1994). Moreover, research using *Drosophila* has been conducted for more than a century, and in addition to a vast body of literature that exists on fruit flies (Roberts, 2006), there is an extensive community of experts and researchers generally willing to offer advice on their biology, care and maintenance. Despite these advantages, many teachers may be reluctant to conduct labs utilizing *Drosophila* in part because of difficulties anesthetizing and handling them and/or the prohibitive cost of the equipment.

Effective methods of anesthetizing *Drosophila* so they can be sorted, sexed, and scored generally involve the use of toxic chemicals that may affect the viability or sterility of the flies, and that pose a potential health risk to students. Diethyl ether is commonly used, but it is extremely volatile. Methylene chloride (dichloromethane) was suggested as an alternative because it is less toxic than ether and it is inflammable (Hedgley & Lamb, 1999), but it is still toxic to humans by inhalation or through contact with the skin. Similar problems may exist with triethylamine, the active agent in FlyNap®. Cooling *Drosophila* and sorting them on metal plates chilled on ice baths eliminates the use of toxic or flammable agents, and is a cheap and cost effective method of immobilizing fruit flies (Ratterman, 2003). However, manipulating flies when condensation forms on the plates may damage wings, thereby impeding courtship, and it is our experience that excessive condensation may be lethal to *Drosophila*.

Carbon dioxide is a non-flammable odorless gas which is an excellent alternative to volatile and toxic anesthetics, and has been used as an anesthetic in a wide range of insects including crickets, grasshoppers, cockroaches, honeybees, and fruit flies (see Nicolas & Sillans, 1989). For *Drosophila*, stages that have porous tops and are connected to a gas cylinder that allows for constant delivery of CO_2 keep the *Drosophila* immobilized while flies are manipulated by the observer. This system is superior to other chemical anesthetics because CO_2 is nontoxic in low doses, both to humans and *Drosophila*, and flies will remain anesthetized as long as they are kept on the staging apparatus. Flies tend to revive under other methods of anesthesia, so use of CO_2 allows students more time to observe and manipulate them.

Commercially manufactured CO_2 staging apparatus are costly; cheaper units are $85 per stage, and purchasing a classroom set of the staging alone is usually beyond the reach of a typical high school science budget. Instructions for building staging using Plexiglas exist (Melo Sene & Manfrin, 2001), however we developed an effective yet inexpensive alternative using materials likely found in a high school or college biology lab. Our staging apparatus were constructed using empty micropipet tip containers, plywood, rubber tubing, an aquarium valve, and felt. Carbon dioxide tanks may be rented fairly cheaply; typically a 20 lb tank with enough CO_2 for several labs can be rented for about $30, and a daily prorated rental fee of around 10 cents per day.

No method of anesthetizing *Drosophila* is without its risks. While the use of carbon dioxide is non-toxic, it may induce hypoxia and cause headaches or dizziness through prolonged exposure. Additionally, care must be taken to ensure that the CO_2 tank is safely secured to a desk or other solid object with a chain because the gas is under pressure. Finally, studies on the effects of CO_2 on adult *Drosophila* indicate that longevity and fecundity of young adult flies is affected, and that reproductive behavior may decrease, even after flies have recovered from the anesthesia for 20 hours (Barron, 2000). A recent study on

THOMAS ARTISS (thomas.artiss@lakesideschool.org) is a biology teacher and Science Department Head, and BOBBY HUGHES (bobbyh@stanford.edu) is Lab Assistant, both at Lakeside School, Seattle, WA 98125.

the effects of CO_2 on *Drosophila* larvae suggests that exposure to CO_2 for longer than 30 minutes is fatal, and that even less time may be fatal to adults who are not burrowed into their food (Badre et al., 2005). So while the use of CO_2 may be more forgiving than other chemical anesthetics, there are still behavioral, reproductive and physiological effects associated with this method of anesthesia.

Materials

Materials needed for each stage include:

- empty micropipet tip container (size of tips is not an issue)
- hot glue gun
- ¾″ plywood for the base
- drill with a ¾″ drill bit
- #2 rubber stopper with a hole in the center to receive the tubing.

In order to allow several students to work from a single tank of CO_2, stages can be connected to a five-gang aquarium valve (Figure 1) that has the added benefit of allowing students or instructors to regulate gas flow to individual stages. Color coding the stages and the aquarium valve with colored tape is helpful. Flexible aquarium tubing is used to connect the stages to the aquarium valve (lengths will vary according to individual needs). Rubber tubing and polypropylene connecting tubes may be needed depending on the size of the regulator on the CO_2 tank.

Constructing the Stages

Remove the plastic base from the pipet tip holder using a flathead screwdriver (Figure 2). Drill a ¾″ hole in the side of the pipet tip holder for the rubber stopper. Insert the rubber stopper and secure it in place with hot glue (Figure 3).

Micropipet tip containers are made of light plastic and we found them to be unstable, so a solid base is needed to stabilize the staging. This is achieved by attaching some plywood to the base. Cut a piece of ¾″ plywood to 7.94 cm (3 1/8″) x 11.59 cm (4 9/16″) (or the appropriate dimensions if your micropipet tip containers are a different size) with rounded corners to substitute for the plastic piece removed earlier from the bottom of the micropipet tip container. The wood adds weight to the apparatus so that it doesn't flop around if someone knocks the tubing, and it keeps the platform stable under the dissecting microscope. Secure the wood piece in place with a line of hot glue around the edges (Figure 4).

The top of the staging is covered with white felt or similarly-porous fabric to prevent *Drosophila* from falling through the holes in the micropipet container while permitting CO_2 to flow through. White is the best color to provide contrast needed for viewing the flies under a microscope. The fabric needs to be roughly half an inch larger than the top of the micropipet tip container so that the fabric folds over the top, and can be secured with hot glue (Figure 5).

Figure 1. Photo of completed apparatus used to anesthetize *Drosophila*. The tank of carbon dioxide has been connected to a color coded five-gang aquarium valve that feeds five anesthetizing stages with aquarium tubing.

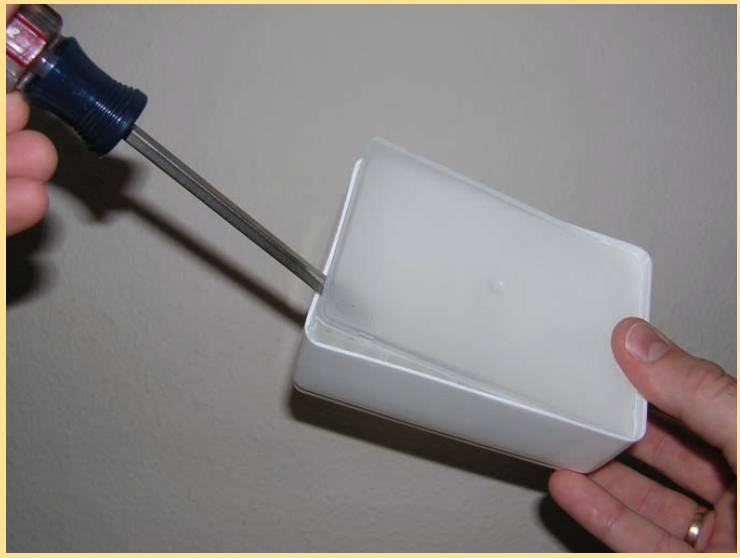

Figure 2. The base of the pipet tip holder is removed with a flathead screwdriver.

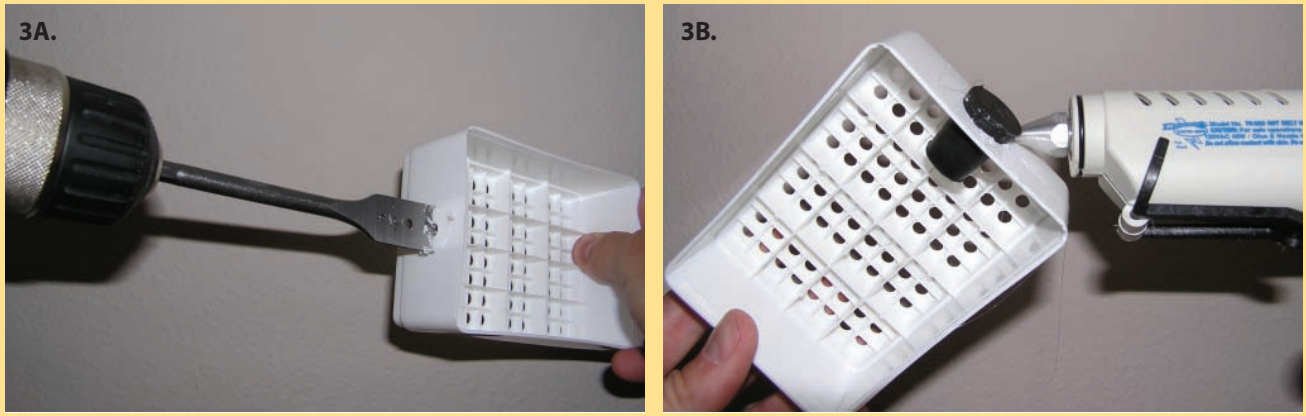

Figure 3A. A ¾″ hole is drilled in the side of the pipet tip holder. **3B.** The rubber stopper is inserted and secured with hot glue.

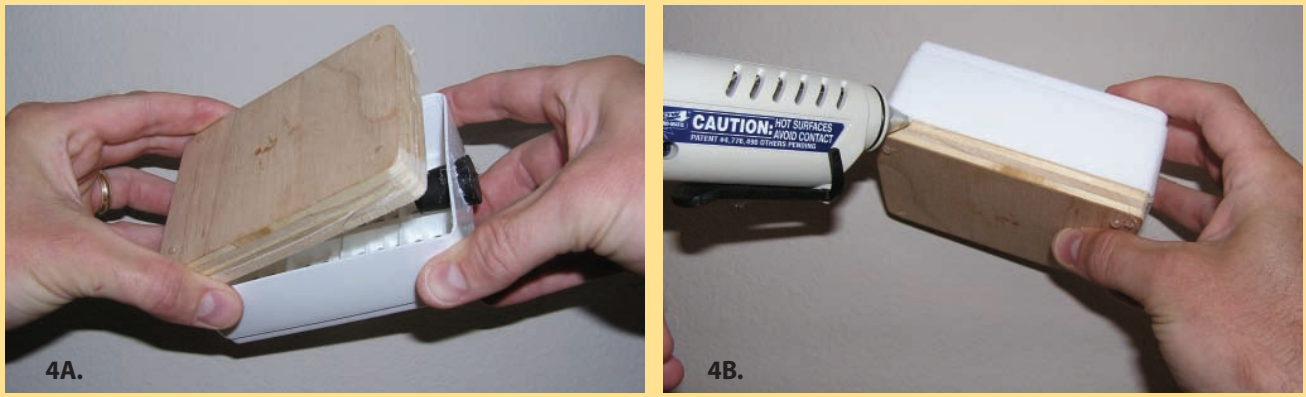

Figure 4A. The pipet tip box is placed over a piece of ¾″ plywood cut to size. **4B.** It is secured in place with hot glue.

Connect a length of rubber tubing from the regulator on a CO_2 tank to the aquarium valve using polypropylene connecting tubes as needed (Figure 1). Run plastic tubing from the aquarium valve to individual platforms. In some cases, a fitting reducer is needed if the CO_2 tank valve and the aquarium valve are different sizes. We color code the aquarium valve and platforms with colored tape so we can quickly identify which valve feeds which platform. Adequate CO_2 flow exists if you can feel the gas lightly diffusing through the platform with the back of your hand or if you hear it lightly hissing if you hold the platform next to your ear. If the flies are visibly moved by the gas diffusing through the platforms, the flow may be too high. Conversely, if flies resuscitate and begin to walk around, adjust the valve to increase the flow of gas which should immediately immobilize them. Once the gas level is set, the platforms may be placed under dissecting microscopes for observation and manipulation of *Drosophila*.

Figure 5. White felt or similarly porous fabric is placed over the top of the pipet tip box and secured around the edges with hot glue.

Anesthetizing & Transferring Fruit Flies

Drosophila in culture tubes or stock vials must be anesthetized before they are transferred to the staging apparatus. First remove the tubing from the rubber stopper on one of the stages. Carefully invert the culture tube or stock vial so anesthetized flies don't fall into the culture medium, insert the rubber tube into the culture vial between the foam stopper, and turn on the gas. The flies should be immobilized within a matter of seconds. Be careful not to turn the gas on too high as it could dislodge culture media or bounce the flies around. Remove the tubing and insert it back into the rubber stopper on the staging. Remove the foam stopper from the culture tube, and shake the flies onto the platform.

An alternative apparatus for initially anesthetizing *Drosophila* in their culture tubes can be constructed from an empty culture tube, a rubber stopper with a hole in it, a glass tube about 8 cm in length, and a disposable plastic pipet tip cut to about 8 cm in length (Figure 6). Insert the glass tubing into the hole in the stopper and fit the plastic pipet tip over the tube. Add water to the empty culture tube until it is roughly 1/4 full. Add half of a tablet of Alka-Seltzer and quickly seal with the rubber stopper. As the Alka-Seltzer dissolves, it releases CO_2. Carefully turn upside down the vial containing the fruit flies (ensuring that the fruit fly culture medium doesn't dislodge). Insert the pipet tip into the culture tube containing the *Drosophila* between the foam stopper. As the CO_2 is produced, it is vented into the culture tube containing the *Drosophila*. When all the flies are anesthetized, remove the pipet tip, remove the foam stopper, and carefully shake the flies onto the anesthetizing platform.

Figure 6. An alternative apparatus for initially anesthetizing *Drosophila*. Alka-Seltzer and 100 ml of water are added to culture tube on the left. The rubber stopper (with a hole into which a glass tube and pipet tip have been inserted) is placed over the culture tube. The CO_2 generated may be directed into a culture tube containing *Drosophila*.

Concluding Remarks

We have found that students become proficient with the staging very quickly, and we have virtually no problems with over-anesthetizing flies or with student complaints of noxious odors. Still, the staging should be used in a well-ventilated room. We have also found that having a spare CO_2 tank on hand was helpful in the event that one tank runs out.

The total cost for the project will depend on what is purchased. Micropipet tip containers are usually thrown out or recycled when they are empty, and we have boxes of them. Plastic five- gang aquarium valves and plastic aquarium tubing are relatively inexpensive and both are available at most pet stores. Cloth and plywood are needed in small quantities, and we used scrap wood and cloth. Number 2 rubber stoppers are usually found in chemistry labs, but can be purchased fairly inexpensively from biological or chemical supply companies. The biggest recurring expense is the rental fee for carbon dioxide tanks, but given the benefits of this system, we feel it is well worth it.

Acknowledgments

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