Eliminating ether by using ice for *Drosophila* labs

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Abstract

This mini-workshop demonstrated an approach that can be adopted in a genetics lab to immobilize *Drosophila* using crushed ice rather than chemical anesthetization or chilling in a freezer. Vials can be cleared very quickly using this method. Offspring flies do not wake up, but rather, are kept immobile on a chilled metal plate for as long as the student keeps them cold. Flies for matings regain normal posture and movement in only a few minutes after being returned to a room temperature vial. The use of ice has helped our multiple lab sections entirely eliminate ether, which was both a health and a fire hazard concern. Instructor- and student-suggested improvements have helped reduce problems and streamline this method for quick immobilization, ease of examination, and rapid recovery for successful crosses with large numbers of offspring.

Introduction

There are a number of reasons that a genetics instructor may wish to find an alternative to the use of ether or other chemical anesthetization method for working with *Drosophila*. Those who have used these methods in classes may have heard student complaints about headaches or other concerns. Some lab manuals even caution students about not wearing contact lenses to lab when chemical anesthetization will be used to avoid potential student discomfort. Etherization requires a training period for students because flies can be killed or sterilized by over-etherization but may wake up too

soon if under-etherized. And, it would be difficult to calibrate all the etherizer units to act exactly the same. Students are often restless when waiting for the flies placed in a vial for a cross to regain consciousness, but it is essential that they check that the flies have not died before leaving lab that day. When there are many offspring, it is difficult to transfer all the flies into the small etherizer chambers and difficult to finish counting and sexing them before these flies regain consciousness. Killing the flies for counting is not a good option because over-etherized flies have their wings sticking out at an angle that makes it difficult to place them on their backs (ventral surface up) for proper sexing. Also, many genetics labs' curricula also contain bacterial labs that require the use of Bunsen burners for aseptic transfers. Those labs need to be physically or temporally separated from the often weeks-long *Drosophila* labs if ether is in use.

Some readers have used or may have thought about using chilling for the immobilization of flies. Some ice techniques have been taught (Geiger, 2002). I have been working to eliminate ether anesthetization and use only ice immobilization of flies in Saint Joseph's University's BIO 1021/1025 genetics labs for the last several years. During this time, there was some level of inconvenience or difficulties that kept this method from whole-hearted acceptance in the other instructors' and my point of view. The key factor that has now changed this to acceptance was the introduction of white metal outlet plates (Home Depot, $0.54 US) as the cold surface on which flies can be examined, sexed, and counted for the spring 2002 labs. It is a simple idea that has worked extremely well.

This workshop presents an approach using only crushed ice and chilled vials/plates for immobilizing and examining flies. These techniques are very easy to adopt, they save time and minimize student difficulties, and they eliminate ether from the classroom.

Procedures

How to clear stock vials, transfer flies, and start new stocks

1. Place an **empty** vial in ice (an ice bucket or styrofoam cup can be used). The open end is up and the vial should be dry inside. Be sure to allow this to chill for at least 5 min. *If flies are transferred into a plastic vial that has just been placed in the ice, it will not be cold enough to immobilize the flies; so, advise the students to wait a few minutes for it to chill.*
2. Tap down the flies to be transferred by knocking the edge of the stock vial against the tabletop and quickly open the plug and invert the stock vial over the chilled vial (Figure 1).
3. Holding the two vials mouth-to-mouth, tap the pair of vials down several times. This is analogous to the tapping of the flies down in stock vials against the tabletop. *Knocking with a finger on the top of the inverted vial will not make the flies drop down—it may, however, dislodge the food, so it is not advised. Instead, the whole vial should be moved up and down. The top vial can be tapped up and down independently by more experienced tappers.*
4. Remove and replug the stock vial. If clearing, check carefully for any other adults that may still be present. Stragglers can be allowed to walk up the sides of the vial and knocked down into the chilled vial by tapping the stock vial again over the opening of the chilled vial. *Difficult flies can be brushed into the waiting chilled vial.*
5. The flies that are in the chilled vial can then be discarded in the morgue (if clearing) or transferred into new vials, or placed on a chilled metal plate for examination/counting. *The*

chilled vial does not need to be plugged. Flies will stay at the bottom. Those few that try to climb up can be easily knocked down by tapping the chilled vial down once or twice.

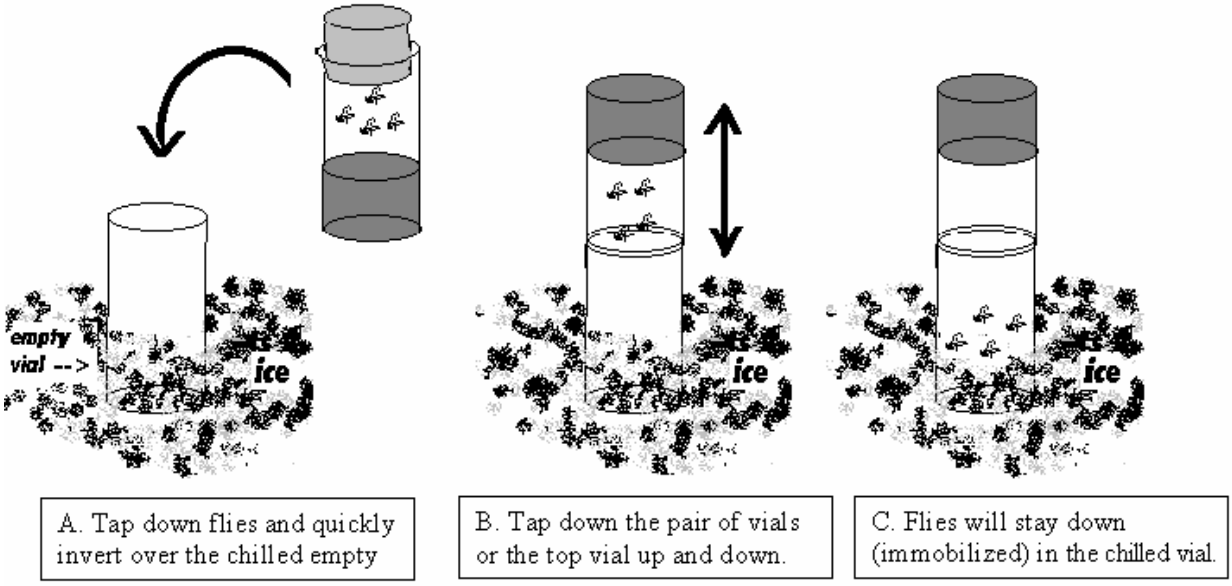

Figure 1. Procedure for moving flies into a chilled empty vial.

Clearing stock vials

1. Knock all adults into a chilled empty vial (Fig. 1), carefully checking that all adults have been removed. If necessary, remove any last few adults with a paintbrush or a forceps. Replug the stock vial.
2. Discard all the flies in the chilled vial by knocking them into the morgue (a can with about 1 inch of vegetable or mineral oil in the bottom).

Advantages of using the ice method for clearing vials:

a. Students, once trained, can come in to clear their stock vials without supervision. Stock vials, a bucket filled with ice, empty vials, some paint brushes/forceps, and a morgue are all that is necessary. *Students typically need to clear stock vials at least 3 hours before they will be setting up their parental crosses to obtain virgins. Freshmen do need some help if unclear about <u>what</u> they need to clear, but they do pick up the method quickly!*

b. If vials are pre-chilled, clearing takes less than 5 min. *This is good news for students who must stop in between classes to clear stock vials.*

c. If the instructor or TA clears many vials at once for backup, this is very quick and easy. Many vials can be cleared in rapid succession.

d. Compared to a small etherizer chamber, the chilled vial has a much wider mouth that makes it easy to knock the flies into the vial.

e. Because the flies do not fly out of the chilled vial, it is easy to knock additional flies down into it, if not all flies were removed in the first transfer. *When using etherizers, a second etherizer must be used for this second transfer.*

Transferring flies to empty vials

This may be used to give each student team some mutant to examine, with the transfer being made just prior to class. Food is not necessary if the flies will be examined later that afternoon.

1. Knock the desired flies into a chilled empty vial (Fig. 1).
2. Taking the chilled vial out of the ice, pour the flies into the empty vials, and plug the vials. Flies will regain consciousness shortly thereafter. *It is easy to have enough time to separate the flies into several empty vials and then go back and plug these vials without losing flies if you work quickly. If you have difficulty, try keeping the target vials on ice too – then plug and allow to warm.*

Starting new stocks

1. Prepare and label new food vials for the stocks.
2. Knock flies from the original stock vial into a chilled empty vial (Fig. 1).
3. Taking the chilled vial out of the ice, pour the flies into the new food vials, and plug the vials. Flies will regain consciousness shortly thereafter. *Flies are so lightly knocked out that they do not get stuck in the food; it is not necessary to sweep them onto the side of the vial for starting new stock vials.*

How to examine flies for set-up of crosses, sexing, and counting

1. Place a metal outlet plate on ice in a bowl (or plate) and allow it to chill for a minute (Fig. 2). Push the plate down so there is no air pocket between the plate and the ice. Wipe the plate with a Kimwipe if any ice has gotten onto its surface. *Some dissecting microscopes cannot be focused with a tall bowl, so check your microscope to see whether you need to use a plate instead.*
2. Knock the flies into a chilled empty vial (Fig. 1).
3. Pour the flies out onto the metal plate (Fig. 2).

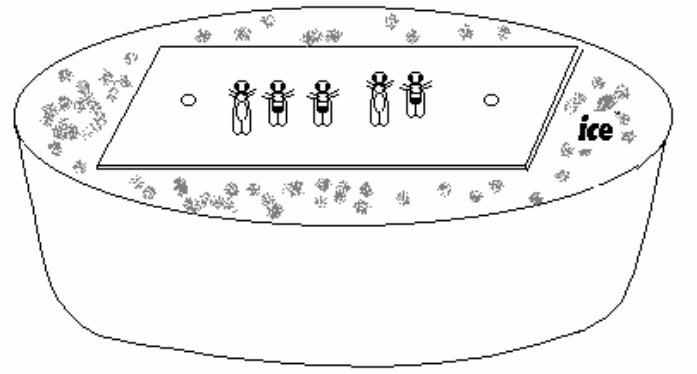

Figure 2. Use of metal outlet plate on ice for keeping flies immobilized during sexing and counting.

4. Using a paintbrush, move the flies around as needed for sexing and counting. *Be careful to be gentle with the flies. Condensation of water on the plate may wet the flies and rough handling of wet flies may damage their wings. Use a Kimwipe to dry sections of the plate if needed.*
5. To transfer flies into vials for crosses, sweep flies onto half-length index cards and then sweep the flies into the vials. Keep the vial on its side until the flies have warmed and regain normal posture (this will occur in only a few minutes).
6. If the ice melts, the metal plate with flies on it can be transferred to a new bowl or plate with fresh ice.

Some tips for counting large batches of offspring

Transfer small batches onto the metal plate. Hold the remainder of flies in the vial on ice. When you have finished counting the flies on the plate, discard them by sweeping them into the morgue. Then wipe the plate with a Kimwipe before placing the new batch of flies on the plate. This will reduce the amount of condensation on the plate and helps avoid having the flies become wet.

Some tips for using this method to set up reciprocal crosses

If setting up a reciprocal cross, have one team member transfer virgin wild-type flies to one chilled vial to then sort and sex those flies. Have a second team member immobilize the virgin mutants in a separate chilled vial to sort and sex on a second chilled plate. In this manner, when both wild and mutant flies have been sexed, the flies can be transferred at the same time to the new cross vial. Use the half-length index card to sweep the desired males and females together and sweep them into the cross vial.

Student-devised procedure modifications ("tricks")

Students provided some variations on the procedures. Some modifications helped them to keep flies on the plate (Fig. 3) or rescue flies that fell off of the plate (Fig. 4). Another technique allowed the addition of an extra fly to an already-prepared cross (Fig. 5).

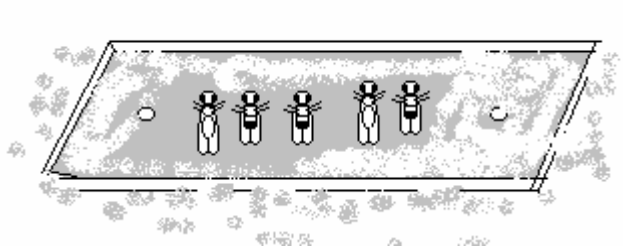

Figure 3. Flipping the metal outlet plate over to use the edges as "walls" to keep flies from falling off. Some students like this approach. The disadvantages of this plate orientation are (a) the back of the outlet plate is not uniformly white and (b) edges may interfere with sweeping flies off onto the half-length index card when transferring flies. However, there is no harm in letting the students choose their own preferred way.

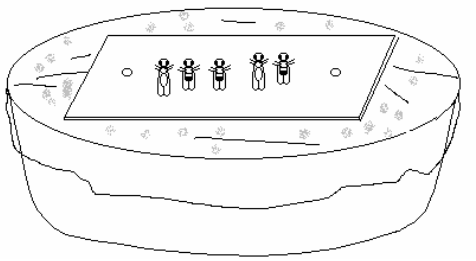

Figure 4. Use of saran wrap over the top of the ice to allow rescue of flies that fall off the metal plate. This method requires that the plate is pushed down well into the ice so that no air pocket forms between the saran wrap and ice or saran wrap and plate. If there is any space, the plate does warm and flies start to walk around.

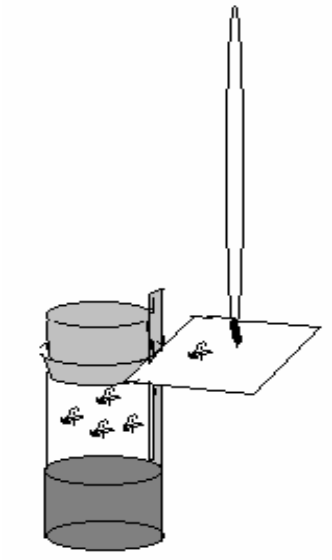

Figure 5. Adding an extra fly to a prepared cross using a spatula. Because chilled flies are only lightly knocked out by our methods, a fly dropped into a food vial will not get stuck in the food (it will stand up within minutes). Some students used a curved spatula as a tunnel to slip an extra fly from a half-length index card into a cross vial with other conscious flies. The person adding needs to watch the other flies to avoid escape or squashing with the plug, but this method does work.

Results and Discussion

Student Response and Benefits

Students adapted to this technique quickly. In our spring 2002 labs, 34 student teams, supervised by three different instructors, used the techniques illustrated in this workshop to prepare and count offspring for reciprocal wild x mutant crosses for both F1 and F2 generation results. There were few complaints and the students made positive comments about this method in comparison with the methods they used in high school. Overall, most students report that they used ether or flynap™ (Carolina Biological, Burlington NC) in high school. A few students used CO_2. Only 2 of my 54 students reported using ice techniques. Certainly, those labs with CO_2 equipment may have no reasons to switch to ice methods. However, those using chemical anesthetization methods may consider switching to ice methods and this switch is not expensive.

Only 45 min was needed for a class to set up their parental crosses. In all my lab sections, all student teams finished in less than the allotted 45 min. In earlier years using etherization, an entire 3-hour lab needed to be devoted to this because when flies were killed or the sexed flies flew away, the team needed to start again. However, those cross difficulties were eliminated with the new ice techniques.

Concerns about Damage to Flies: Effects of Cooling

Overall, the cooling of flies using ice does not impair the flies that will be used for crosses. Research into the effect of cooling and cooling rates on survival and early fecundity in *Drosophila melanogaster* support this assertion. Kelty and Lee (1999) monitored egg production of female flies that were mated after a direct transfer from 23ºC to -1ºC and held at -1ºC for 3 hr and found no

reduction in egg production compared to control (23ºC) females. There was also no effect at -3ºC. It was lower sub-zero temperatures (-6ºC to -9ºC) that resulted in deaths of flies in this and earlier studies. It appears that using ice for cooling vials and metal plates to temperatures close to 0ºC, rather than using items chilled in a -20ºC freezer or placing the flies in the freezer themselves, would be appropriate for handling flies that will be used for crosses. In practice in the classroom, there were no apparent difficulties with obtaining large numbers of offspring.

Concerns about Damage to Flies: Wetting of Wings

The difficulty that does arise with this and other ice methods is that the wings of the flies may become wet as condensation accumulates on the cold surface used. Wiping with a Kimwipe before transfer of flies to the surface does help reduce the problem of wetting. Fortunately, most flies that are placed in vials with wet wings do dry to have normal wing extension and wing posture. In practice, the wings of some of the flies manipulated on a wet surface do however become damaged. This is a concern because male flies need to use their wings to sing a courtship song before being able to mate with a female (for a summary of courtship behavior students can read, see Greenspan, 1995). In classroom practice, placing several males in each cross vial reduces the risk of not having a successful mating occur in the vial. It is recommended that at least two (3-4 even better) males be placed with 2-4 females for each parental cross.

One concern was that wetting could result in difficulty determining phenotype when using wing mutants. In spring 2002, our classes used the wing mutant *miniature* as one of the mutant strains. In *miniature* flies, the wings extend only to the end of the abdomen, not beyond the tip of the abdomen as seen in wild-type flies. Students working with this mutant did not report difficulties when counting the flies; this seemed to be a non-issue. In some instances, wet flies that were difficult to determine the phenotype of were placed back in the vial to dry and to be counted later. This procedure was also followed for newly-emerged flies whose wings had not yet fully expanded. We will test other wing mutants in later years. It might be suggested that if conditions are humid in your area, it would be wise to avoid a mutant such as *scalloped* when working with ice techniques because this wing mutant is identified by the rough border along the wing. Try other mutants until you have determined whether problems with wing wetting occur in your classroom.

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