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## Some homemade simple tools and their use in handling of fruit fly *Drosophila melanogaster* --Manuscript Draft--

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Dear Editor,

I have carefully checked each detail of my tools and thoroughly revised the manuscript (text and figures). The major change in this version is that I describe an acrylic bottle-shaped small egg collection cage for 60 mm petri dishes. I am fully ensured that it is much more convenient than the popular egg collection cage, as one do not need anaesthetizing large amount of flies before and after egg collection.

I have tried my best to make myself clear. Please tell me if there still exist language problems in the manuscript, I will send my manuscript to American Journal Expert, and ask a native speaker to polish my English.

Sincerely,

Daxiang Yang

**TITLE:**

Simple Homemade Tools to Handle Fruit Flies—*Drosophila melanogaster*

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**KEYWORDS:**

*Drosophila*, adult transferring, cooling methods, adult killing, culture vial cleaning, egg collection

**SUMMARY:**

Described here is the use of several homemade tools to transfer, chill, and kill adult *Drosophila*, as well as to clean glass culture vials and collect eggs. These tools are easy to make and are rather efficient in handling *Drosophila*.

**ABSTRACT:**

The fruit fly, *Drosophila melanogaster*, is widely used both in biological research and biology education. Handling adult flies is common but difficult in practice, as adult flies fly. Demonstrated here is how to make some simple and cost-effective tools to address difficult issues in the handling of *Drosophila*. Holes in foam stoppers are made and pipette tips or funnels are inserted into the holes. Flies then move only in one direction into the pipette tip/funnel assemblage, allowing efficient control of the transfer of adult *Drosophila* into or out of a vial. Existing protocols have been modified for cool-anesthetizing flies by chilling in crushed ice and transferring them onto a cold, hard icepack surface. The icepack is covered with a piece of medical gauze that keeps immobilized flies from the condensed water when examined under a stereomicroscope. The flies are finally euthanized for counting and sorting or discarded by microwaving. A bottle-shaped cage has also been developed for collecting eggs, as well as a labor-saving device and accompanying protocol for cleaning glass culture vials.

**INTRODUCTION:**

The fruit fly, *Drosophila melanogaster*, is a model organism widely used in biological research and biology education to study a wide range of topics<sup>1,2</sup>. The basic problems of handling *Drosophila* are the transfer of adults from vial to vial and immobilization of the flies so they are easier to handle, as all adults (except for some mutants<sup>3,4</sup>) can fly.

Conventionally, a researcher transfers flies from one vial to another by holding two vials mouth-to-mouth, tapping the flies down or allowing flies to fly up into another vial, then separating and replugging both vials<sup>4</sup>. Obviously, this requires that the opening of two vials with

the same diameter, and it is hard to control the quantity of flies transferred. Meanwhile, this requires quick hands to get the job done, and escaping stray flies can result in problems for the laboratory or classroom. Adding extra virgin flies or male flies to an already prepared cross is another routine task in *Drosophila* experiments. Conventionally, flies must be immobilized in the cross vial before the addition of extra flies.

Adult *Drosophila* are routinely anaesthetized by ether, CO<sub>2</sub>, or chilling<sup>5</sup>. Compared to ether and CO<sub>2</sub> exposure, chilling is the most cost-efficient agent for immobilizing adult *Drosophila* and the least harmful to both the flies and researchers (especially young students)<sup>6,7</sup>. However, water that condenses continuously on the cold surface or chamber wets the flies. It is difficult to determine the phenotypes of wet flies, and they can easily become damaged during manipulation<sup>8,9</sup>. This has kept the chilling method from becoming more widely accepted.

Tools for fly transferal and a method for fly cooling have been previously described<sup>10</sup>. Herein, a modified chilling anesthesia technique is reported that is safe, reliable, and feasible for *Drosophila* experiments. Also described in this paper are 1) methods for killing adults for counting, sorting, or discarding, 2) labor-saving devices and protocols for cleaning glass culture vials, and 3) a simple cage for collecting eggs. The easily designed and cost-effective tools described here can be used to address the difficult issues of fly handling, and these methods have been tested and are proven to be robust, reliable, and easy-to-handle for experienced and novice researchers.

## PROTOCOL

### 1. Preparing tools and accessories

#### 1.1. Tip/funnel stoppers

1.1.1. Obtain two sponge plugs (the diameter of the plugs must be slightly larger than the internal diameter of the vials used to transfer flies). Make a hole in the centers of the sponge plugs with a heated electric soldering iron.

1.1.2. Obtain two 1 mL pipette tips, cut one in half transversely with a sharp knife, and discard the pointed end. Then, cut 1.5 cm of the pointed end off the second pipette tip. Glue the remains of the two pipette tips together with an all-purpose adhesive to make an elongated pipette tip (**Figure 1A**).

1.1.3. Insert a funnel and the elongated pipette tip into the sponge plugs to make a tip and funnel stopper (hereafter referred to as T- and F-stoppers) and cap the pipette tip with a 100 µL microcentrifuge tube (**Figure 1A**).

NOTE: The length of the funnel stem must be greater than the height of the plug. If it is shorter than or equal to the height of the plug, then flies will escape from the stem opening. The end of

the funnel stem should be situated at least 2 cm above the surface of the culture medium or the bottom of an empty vial. Small funnels (e.g., disk diameter <60 mm) with small internal stem opening diameters (<5 mm) are preferable. Either a glass or a plastic funnel can be used to make an F-stopper. However, plastic funnels are preferable for biology classes, as they break less readily than glass funnels.

## 1.2. Microdissecting needles

1.2.1. Obtain mechanical pencils that feel comfortable in the hand and insect pins that match the diameters (e.g., 0.5 mm, 0.7 mm) of their lead refills.

1.2.2. Cut the wide ends of the insect pins with a pair of pliers and file the cut flat. Replace the lead with the pins (**Figure 1B**). Press the click button and feed out 0.5–1 cm of a pin to conduct a dissection. Clean the pin and push it completely back into the pencil shaft after a dissection activity to make it safe for any person to handle.

NOTE: Microdissecting needles are useful not only in dissections of organs such as larval salivary glands but also in counting and sorting dead adult flies.

## 1.3. Hard icepacks

1.3.1. Obtain several refreezable hard icepacks (large-sized icepacks are preferable). **Figure 1C** shows an icepack that worked well, which measures 26.5 cm x 14.5 cm x 2.5 cm and has top and bottom sides that are completely flat.

1.3.2. Cut medical gauze (nonsterile) into pieces that are slightly smaller than the cold surfaces of the icepacks they cover. For example, a piece of medical gauze slightly smaller than 26.5 cm x 14.5 cm is preferable to cover an icepack shown in **Figure 1C**.

NOTE: The necessary accessories for these chilling tools include: an ice box (we used a 25 cm x 15 cm x 15 cm foam box for one person and 37 cm x 28 cm x 20 cm box for more than one person), which is used to store crushed ice; a pair of fine-point tweezers, which are used to grab chilled flies by their wings and transfer them to a vial; a pair of protective work gloves, which are used to take chilled icepacks out of a -20 °C freezer; and plastic film, which is used to cover the stage of a stereomicroscope.

## 1.4. *Drosophila* egg collection cage

NOTE: Ready-made *Drosophila* egg collection cages are available from many biotechnology companies<sup>11</sup>. Described here is a small acrylic bottle-shaped egg collection cage for 60 mm Petri dishes (**Figure 1D** left; the cage design is shown in the middle). It can be adapted for other Petri dish sizes (e.g., 100 mm, 35 mm). This allows the transfer of flies into or out of the cage with ease. A simple cage can be prepared as follows.

1.4.1. Use a snap cutter to cut a soft plastic drink bottle (500 mL, internal diameter ca. 65 mm) into an approximate 2:1 (pointed end:blunt end) ratio and discard the blunt end.

1.4.2. Wrap a strip of card paper around an apple juice plate (internal diameter 60 mm) with adhesive tape [the apple juice plate is used to collect eggs (**Figure 1E**, right)].

## 1.5. Cordless tube brush driver

1.5.1. Obtain a cordless drill driver (max speed = 500 rpm).

1.5.2. Obtain a tube brush that has bristles along its sides as well as its front. Ideally, the diameter of the brush should be slightly larger than the diameter of the culture vials that need to be cleaned. Cut the end of its handle so it can be inserted into the drill chuck (**Figure 1D**).

NOTE: The necessary accessories for these cleaning tools include stainless steel sponges and long cuff rubber gloves.

## 2. Transferring adult flies from vial A to vial B

NOTE: Transferring adult flies from one vial to another is the most common practice conducted in *Drosophila* experiments [e.g., transferring flies from old culture (A) to fresh culture (B) or from a cross vial (A) to empty vial (B)] for anesthetizing. The protocol described here can be used for any adult fly transferring activities. Unless otherwise stated, this protocol is used to transfer flies from vial A to vial B throughout this paper.

2.1. Check the stem of the funnel of an F-stopper and the pipette tip of a T-stopper carefully, then clear any flies that remain in the stoppers with a rubber air blower. This step is of paramount importance, especially when one set of T- and F-stoppers is used for the continuous transferring of different *Drosophila* lines.

2.2. Tap down the flies in vial A and replace its plug with a T-stopper, then plug vial B with an F-stopper.

2.3. Invert vial A over vial B, insert the pipette tip end of the T-stopper into the funnel opening of the F-stopper, knock the edge of inverted vial A to allow flies to slip out of the pipette tip and through the stem of the funnel, and drop into vial B. If any old food in vial A becomes less compact, it may drop when vial A is inverted and knocked. In such a situation, invert vial B over vial A and allow the flies to crawl up into vial B.

2.4. Separate the T-stopper from the F-stopper. Cap the pipette tip end of the T-stopper with a 200  $\mu$ L microcentrifuge tube if the remaining flies in vial A need to be transferred to other vials momentarily; otherwise, remove the T-stopper and replug vial A. Remove the F-stopper and replug vial B.

### 3. Immobilizing flies by chilling

3.1. Keep hard refreezable icepacks in a -20 °C freezer for at least 24 h before use.

3.2. Place a chilled, hard icepack at room temperature (RT) for 20 min. Slightly moisten a piece of non-aseptic medical gauze with some running water and allow it to closely cling to the surface of the icepack. The medical gauze can be reused in the next fly chilling. At the same time, chill an empty vial in crushed ice.

3.3. Transfer adult flies that need to be immobilized into the chilled empty vial (CEV). When the two transfer vials are separated, cover the CEV with a Petri dish or a plug and knock the CEV against the crushed ice to tap all the flies in the CEV down to the bottom. Repeat this process several times until all flies are immobilized. The flies will be immobilized within 30 s. Next, place the CEV in the ice for 1 min. It is not advisable to transfer too many flies at one time for anesthetizing.

3.4. Pour the chilled flies out onto the medical gauze that covers the ice pack. Spread out the overlapping flies with a paintbrush and make sure that each fly can be chilled by the cold surface of the icepack. If a chilled hard icepack swells slightly, place it on a towel and work on its flat side.

3.5. Remove the stage clips from the stereomicroscope, cover the stage with a piece of plastic film, and put the icepack onto the stage. Turn on the top light (a cold light source is desirable), focus the stereomicroscope and move the icepack until the chilled flies can be seen clearly.

### 4. Killing adult flies for counting, sorting or discarding

4.1. Transfer adult flies into an empty vial and cover it with a Petri dish.

4.2. Invert the vial, heat it for 1 min + 20 s in a microwave oven, and allow the dead flies drop into the Petri dish.

4.3. Put on protective work gloves and take the vial out of the microwave. Pour the dead flies onto a white paper card, count or examine the flies with a microdissecting needle under a stereomicroscope, and dispose of the fly bodies in a garbage can after observation.

4.4. To kill unwanted flies, heat the flies for 2–3 min in a microwave oven, then tap the carcasses into a garbage can.

NOTE: It is not advisable to kill some wing mutant strains (e.g., wing length mutants) for examination, as it is difficult to judge from the carcasses if the wings extend beyond the tip of the abdomen, which is seen in wild-type flies.

## 5. Transferring flies in/out of bottle-shaped egg collection cage

NOTE: As mentioned above, T- and F-stoppers are used to transfer flies into and out of the egg collection cage. Flies do not need to be anesthetized throughout this process. Other details, such as preparing the apple juice medium, egg collection, and dechorionization, can be found in the literature<sup>12</sup>.

5.1. Insert the egg collection cage into the apple juice plate or mount the apple juice plate to the cage made of a soft drink bottle. Seal the joint around the two components with a strip of paraffin film.

5.2. Place as many flies as possible into the cage and replug the cage with a foam stopper after transferring the flies.

5.3. To change the food for flies in the cage, transfer the flies in the cage to an empty vial.

5.4. Replace the apple juice plate and reseal it, then transfer the flies from the vial back to the cage.

5.5. When egg collection ends, transfer the flies into an empty vial and transfer them into culture vials.

## 6. Cleaning glass culture vials

NOTE: Generally, an old culture vial contains live flies. In the protocol described here, these flies DO NOT need to be killed before cleaning unless they are transgenic flies.

6.1. Remove any permanent marker ink from the glass culture vials with wet, stainless steel sponges.

6.2. Soak the culture vials in running water.

6.2.1. Fill a laboratory sink with water, add liquid dishwashing soap into the water, and mix.

6.2.2. Immerse the culture vials into the water, then remove the plug, allowing the water to run into the vial. The dish detergent in the water will make any remaining adult flies sink to the bottom and drown in the water.

6.2.3. Soak the old culture vials in water for at least 30 min.

6.3. Loosen the chuck of the drill, insert the test tube brush and retighten the chuck. Check the direction of the rotation selector and ensure that the drill rotates clockwise. Adjust the speed



trigger and ensure that the maximum speed is less than 500 rpm.

6.4. Clean the culture vials.

6.4.1. Clean the culture vials roughly.

6.4.1.1. Put a long cuff rubber glove on the non-dominant hand and hold the vial in the water.

6.4.1.2. Hold the cordless tube brush driver with the bare dominant hand, squeeze the brush into the culture vial, and squeeze the trigger.

NOTE: Do not dip the battery into the water. The rotating brush will break up old food, pupa, etc., and remove more than 95% of the waste.

6.4.1.3. Dump the waste into a separate garbage can. Repeat this process until most of the waste in each vial has been cleaned.

6.4.2. Clean the culture vials thoroughly.

6.4.2.1. Clean the tube brush, drain and clean the sink, and refill it with clean water.

6.4.2.2. Remove the remaining waste from each culture vial as described in section 6.4.1.

#### **REPRESENTATIVE RESULTS:**

The T- and F-stoppers were developed as a set of simple tools that can be adapted and used in any fly transferring activities. Transferring flies from an old culture into several fresh cultures involves removing the plugs of the fresh vials, replacing them with F-stoppers, then tapping down the flies in the old vial, quickly removing its plug, and replacing it with a T-stopper. If the old food is compact, then it is important to flip the old vial and insert the tip of T-stopper into the opening of an F-stopper, then tap the flies down into the fresh vial. Then, replacing the T- and F-stoppers and replugging the vials is performed. If the old food becomes less compact, it is advised to flip the fresh vial over, mount the F-stopper to the T-stopper, and allow the flies to crawl up into the fresh vial.

To add extra flies to an already prepared cross, it is important to tap the flies in the cross vial down and replace its plug with an F-stopper. Then, the experimenter must examine the chilled flies under a stereomicroscope, pick up a desired fly by its wing using a pair of pointed tweezers, and allow it to slip into the cross vial through the stem of the funnel. If a fly is trapped in the stem of a funnel, it is advised to gently blow on it with an air blower and let it slip into the vial. Replacing the F-stopper and replugging the vial when enough flies for a cross have been collected is then required. T- and F-stoppers were introduced in 2010<sup>13,14</sup>; thus far, more than 1,200 students have benefitted from these fly transferring devices. T- and F-stoppers have also been introduced to instructors and researchers through a laboratory guide<sup>15</sup>, which has been adopted for use in teaching and research labs.

Existing chill anesthetizing methods have been modified for use in this study. Crushed ice or ice-water mixtures are used to chill the adult flies then transfer the immobilized flies onto the cold surface of an icepack covered with a piece of nonsterile medical gauze. The gauze fibers soak up the condensed water and keep the flies dry when they are examined. At the same time, the tiny holes between the warp/weft threads allow flies to touch the cold surface of the icepack and keep them immobile (**Figure 2**). At a room temperature of 25 °C, the temperature of the surface of a chilled, hard icepack increases dramatically from -19 °C to -2 °C within 20 min and reaches a plateau that is safe for both old and newly hatched flies (**Figure 3**). An icepack works quite well within the plateau, and the chilled flies regain consciousness at room temperature within 30 s. Because a hard icepack is thin, it can then be placed under a stereomicroscope to examine the flies. The hard icepack described here costs less than \$2; furthermore, 60 hard icepacks for a class of 100–150 students each semester have been used, and they are reusable for many years. This modified version of the chilling anesthesia technique was introduced to a specific genetics class three years ago, and its robustness has been tested by more than 300 students and those in other universities.

It has been found that microwave dielectric heating is a faster, more convenient agent to kill adult flies (if they are no longer needed after observation) compared to agents such as overetherizing or deep freezing (**Table 1**). Microwave dielectric heating requires a far shorter time to kill flies than overetherizing or deep freezing. All flies die within 80 s, so counting and sorting a large batch of flies within a short timeframe is feasible<sup>16</sup>. Assuming that the experimenter needs to kill flies 20x to count and sort batches for an experiment, it will take 3 h + 20 min and 5 h to kill the flies by overetherizing and deep freezing, respectively; however, only 27 min are needed using a microwave.

Similar to overetherized flies, microwaved flies extend the wings at right angles from the bodies. Generally, fly carcasses killed by microwaving were significantly lighter than those killed by ether or chilling, but the heat does not distort the body shape, and the carcasses do not become crisp or turgid. Characteristics (e.g., body color, eye color, and wing shape) of microwaved flies are similar to those killed by ether or freezing (**Figure 4**), and there are no significant differences in wing sizes (area, length, width) of flies killed by the three agents (**Table 1**). Therefore, carcasses of flies killed by microwaving can be used for counting, sorting, and measuring of some traits, such as wing size. Microwave heating is also a good method to kill unwanted flies and dispose of them in a timely manner. Additionally, fly morgues (bottles containing flammable ethanol, methanol, or soap solutions), which are used to store dead or discarded flies, are no longer needed in fly labs or biology classes<sup>3</sup>.

A small, bottle-shaped egg collection cage was designed for this protocol. Using T- and F-stoppers, a large number of flies can be transferred into or out of the cage, and the apple juice medium plates can be changed with greater ease. Finally, the flies do not need anesthetizing before and after egg collection.

A cordless tube brush driver and protocol for use of this equipment to clean culture vials was

also developed for the protocol. This battery-powered tube brush can easily break down old food and pupa attached to a glass culture vial, a vial can be cleaned within 30 s, and the efficiency of cleaning is increased greatly; therefore, cleaning large amounts of glass culture vials is no longer a tedious task.

## FIGURE AND TABLE LEGENDS:

**Figure 1: Tools used in handling *Drosophila*.** (A) Shown are fly-transferring tools and the necessary accessories. They are (from left to right) an air blower (used to blow out adult flies remaining in the funnel stem); T- and F-stoppers (inserted into vials); and an empty vial covered with a Petri dish (36 mm, the bottom half of a 40 mm Petri dish). The foam stoppers are larger than the openings of the vials so they can be used with vials of variable opening sizes. The size described here can be changed if necessary. (B) Shown are materials needed for the micro-dissecting needles. (C) Shown is the hard icepack used to chill flies. (D) Shown is the bottle-shaped egg collection cage (left), its design plan (middle), and a simple egg collection cage made of a soft drink bottle (right) (E) Shown are the materials needed for the cordless tube brush driver. The white-colored round brush that can be mounted to the drill driver is used to clean Petri dishes.

**Figure 2: Chilled flies on the cold surface of an icepack.** The condensing water is absorbed by the medical gauze, and the chilled flies are kept dry.

**Figure 3: Variation of the temperature of the icepack surface with time.** The data were collected from five hard icepacks, and the temperatures were measured in two locations in the center of an icepack with an infrared thermometer at a RT of 25 °C and relative humidity of 29%. The freezer temperature was -24.5 °C.

**Figure 4: Comparison of fly carcasses killed by microwaving to those killed by ethyl ether and deep freezing.** When the fly carcasses killed by microwaving were examined under a stereomicroscope, no scorches or distortions were found on the bodies, and no noticeable differences were found in body color, eye color, and wing shape.

**Table 1:** The effects of the three killing agents on the carcass weights and wing sizes of adult *Drosophila*.

## DISCUSSION:

Some homemade tools for handling basic activities involved in *Drosophila* rearing and experimentation are described in this paper. These tools are simple but rather effective. Virtually, any lab can make these tools with ease, and a research or a teaching laboratory does not need to find a ready-made alternative that is perhaps not available locally.

Fly transferring is the most common practice and a difficult task in *Drosophila* experiments. Unfortunately, until now, there have been no described transferring tools<sup>3,4,12,17</sup> Here, T- and F-stoppers are described. These simple tools make transferring the flies much easier and more

controllable, and fewer flies escape during transfer, as evidenced by the fact that few stray flies have been found in the genetics classes in recent years. As the sponge plug is elastic, it does not require that the opening of the vials have the same inside diameter. Additionally, only one fly is allowed to pass through the opening of the pipette tip at a time; therefore, T-stoppers prevent flies from surging into a vial, and the experimenter can easily stop the process and control the number of flies transferred. T-stoppers can also prevent old food from dropping into a fresh vial. T- and F-stoppers are easy to make and use, and even an inexperienced handler can complete fly transfers quickly and easily.

F-stoppers are used to guide flies into a new vial. The adult flies tend to associate under the stopper and do not escape from the stem of the funnel. This makes some work easier and more controllable (e.g., transferring flies from one vial to another or adding extra virgin flies or male flies to a prepared cross). It has been found that when a vial is placed in the laboratory for a fairly long time (e.g., 1 h), only very few flies will escape from the stem of the funnel.

In this paper, a feasible chilling method for immobilizing flies is described. This method is a great alternative to ether and CO<sub>2</sub> and can be used in both research and teaching labs. This method is particularly friendly to a teaching lab, as an instructor does not need to be as concerned about potential health risks to students or make great efforts to construct an expensive staging area in a crowded teaching lab. This method is cost-effective, as the icepacks are inexpensive and reusable. A researcher or student can chill and inspect flies anywhere, as this “cold pad” does not connect to any pipe. This method is not only safe for people but also to flies, as the system works at temperatures higher than -2 °C. Flies are lightly knocked out and remain immobile as long as they remain on the cold surface and are not killed. The flies regain consciousness quickly once they return to room temperature. Those who apply this method does not require a training period, and there are no concerns with excessive or inadequate anesthesia concentrations. However, experimenters should pay close attention to the size of the icepack, as small-sized icepacks (e.g., 400–500 mL, ca. 19 cm x 11 cm x 2.5 cm) are not desirable for fly chilling since they swell when they are frozen and it becomes awkward to work on the surfaces.

A bottle-shaped egg collection cage was also developed for the protocol. Taking advantage of the T- and F-stoppers, large amounts of flies to the cage can be added or transferred without requiring immobilization of the flies beforehand. It has been found that microwave heating is an efficient way to kill flies for inspection or discarding. A mechanical pencil-based microdissection needle and drill-based a cleaning tool were also utilized. All of these tools are simple and work well.

#### **DISCLOSURES:**

The author have nothing to disclose.

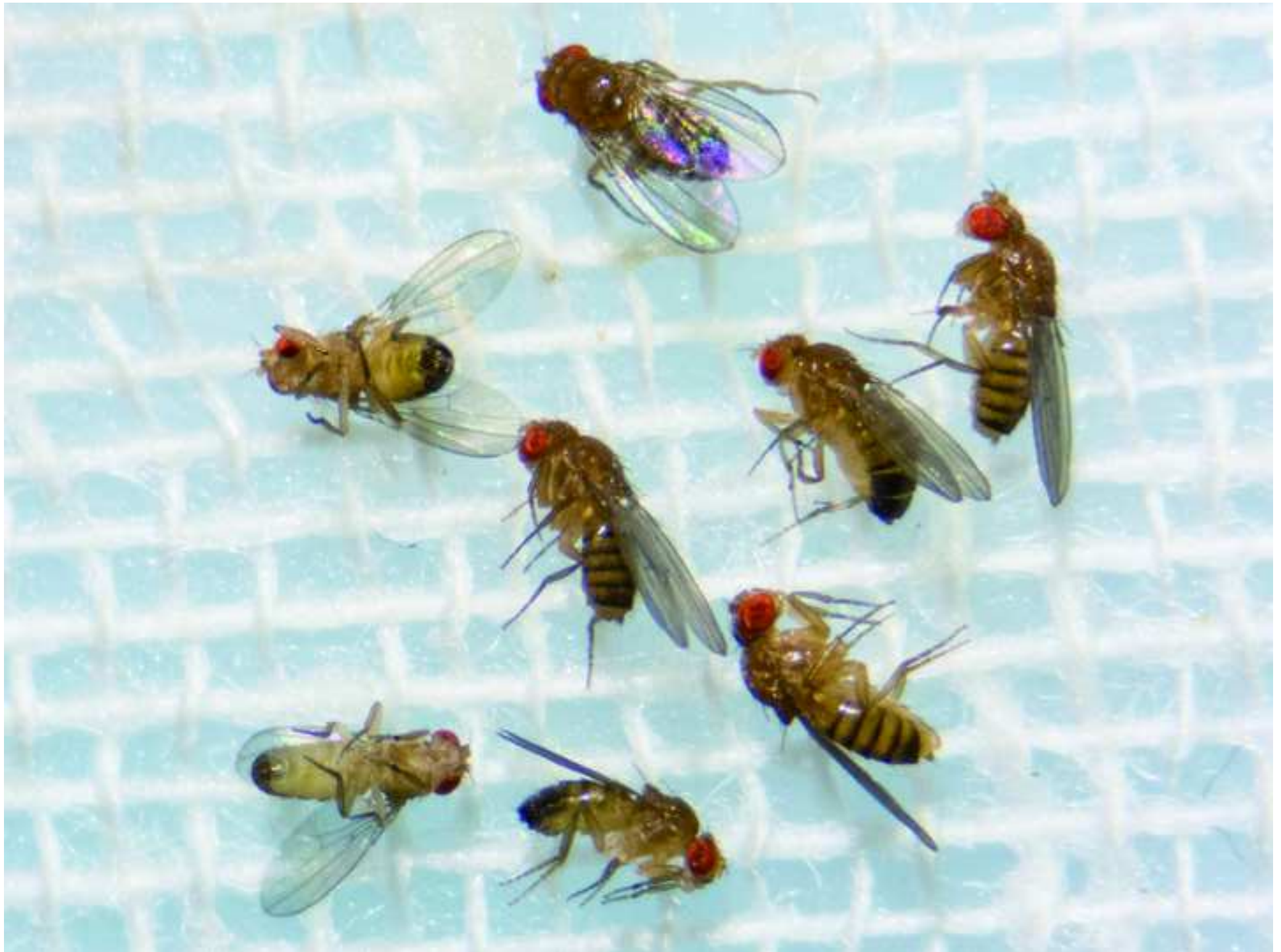
#### **REFERENCES:**

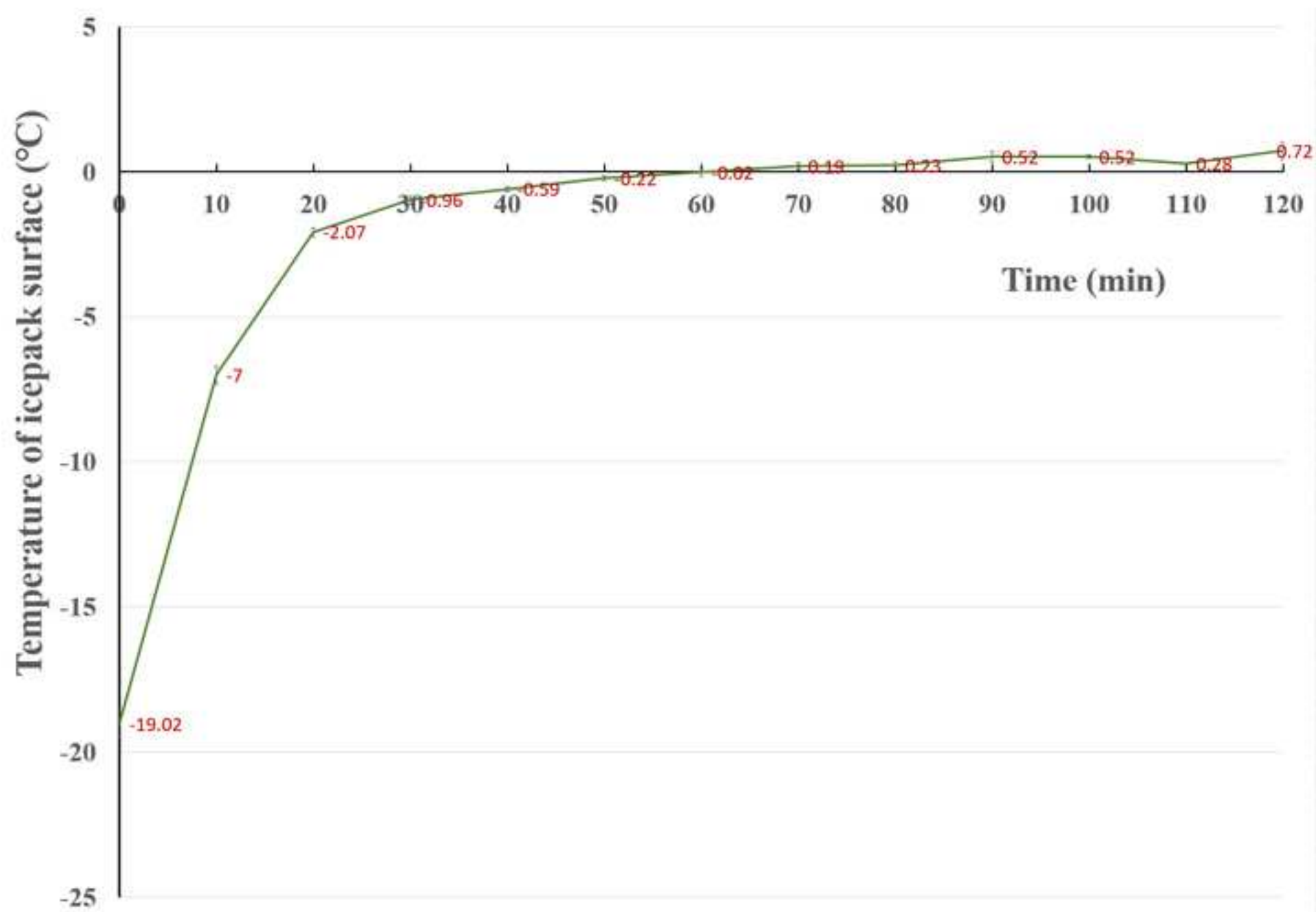
- 1 Jennings, B. H. *Drosophila – a versatile model in biology & medicine. Materials Today.* **14** (5), 190-195 (2011).

2 JoVE Science Education Database. Biology I: yeast, *Drosophila* and *C. elegans*. An  
 Introduction to *Drosophila melanogaster*. JoVE, Cambridge, MA. (2018).  
 3 Ashburner, M., Roote, J. Laboratory Culture of *Drosophila*, in *Drosophila Protocols*. (eds  
 William Sullivan, Michael Ashburner, & R. Scott Hawley) Ch. 585-599, Cold Spring Harbor  
 Laboratory Press. (2000).  
 4 Greenspan, R. J. Fly pushing: The theory and practice of *Drosophila* genetics. *Cold Spring*  
*Harbor Laboratory Press*. (2004).  
 5 Ashburner, M., Thompson, J. The laboratory culture of *Drosophila*, in *The genetics and*  
*biology of Drosophila* Vol. 2a (eds M. Ashburner & T. R. F. Wright) 1-109, Academic Press.  
 (1978).  
 6 Ratterman, D. M. Eliminating ether by using ice for *Drosophila* labs, in *Tested Studies For*  
*Laboratory Teaching*. (ed M. A. O'Donnell) 259-265.  
 7 *Culturing techniques for Drosophila* <[https://www.ptbeach.com/cms/lib/NJ01000839/](https://www.ptbeach.com/cms/lib/NJ01000839/Centricity/Domain/113/ap%20biology%20Labs/Culturing%20techniques%20for%20Drosophila.pdf)  
 Centricity/Domain/113/ap%20biology%20Labs/Culturing%20techniques%20for%20Drosophila.  
 pdf>.  
 8 Markow, T. A., O'Grady, P. M. *Drosophila: A Guide to Species Identification and Use*.  
 Academic Press. (2006).  
 9 Artiss, T., Hughes, B. Taking the Headaches Out of Anesthetizing *Drosophila*: A Cheap &  
 Easy Method of Constructing Carbon Dioxide Staging. *The American Biology Teacher*. **69** (8),  
 e77-e80 (2007).  
 10 Qu, W. -H., Zhu, T. -B., Yang, D. -X. A Modified Cooling Method and its Application in  
*Drosophila* Experiments. *Journal Of Biological Education*. **49** (3), 302-308 (2015).  
 11 *Egg-laying cages for drosophila* <[https://www.kisker-biotech.com/frontoffice/product?](https://www.kisker-biotech.com/frontoffice/product?produitId=0H-19-17)  
 produitId=0H-19-17>. (2018)  
 12 Roberts, D. B. *Drosophila: a practical approach*. 2nd edn, Oxford University Press. (1998).  
 13 Tang, M., Peng, Q. -F., Yang, D. Two devices for *Drosophila* experiments (in Chinese).  
*Bulletin of Biology*. **45** (11), 49-50 (2010).  
 14 Zhou, T.-y., Gan, J., Yang, D. Preparation of sponge plug and sponge plug based fly  
 transferring device for *Drosophila* experiments (in Chinese). *Bulletin of Biology*. **46** (6), 49-50  
 (2011).  
 15 Yang, D. *Genetics laboratory investigation*. 3rd edn, Science Press. (2016).  
 16 Yang, D. Carnivory in the larvae of *Drosophila melanogaster* and other *Drosophila* species.  
*Scientific Reports*. **8**, (2018).  
 17 Stocker, H., Gallant, P. Getting Started: An Overview on Raising and Handling *Drosophila*, in  
*Drosophila: Methods and Protocols*. (ed Christian Dahmann) Humana Press (2008).











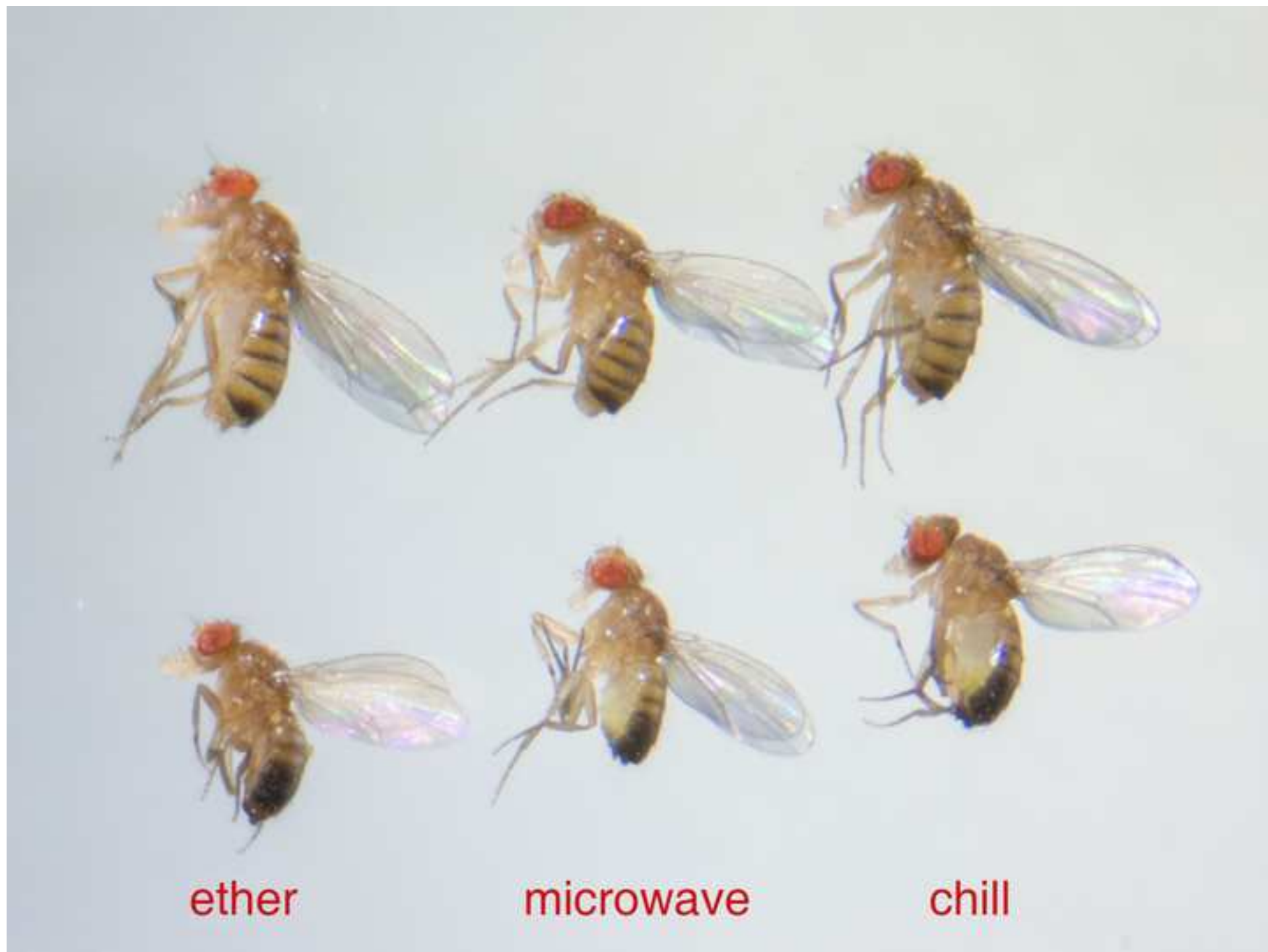


Table 1 Effect of three killing agents on the carcass weight and wing

Killing agent	Time used to kill flies	Weight (mg/30 flies)		Wing (mm)		
		Female	Male	Female		
				Area <sup>ns</sup>	Length <sup>ns</sup>	Width <sup>ns</sup>
Heat	1 min 20 s	36.60±0.00 a A	22.65±0.95 a A	1.51±0.16	2.30±0.12	0.92±0.05
Chill	15 min	41.20±0.10 b B	25.70±1.00 ab A	1.57±0.15	2.37±0.12	0.94±0.05
Ether	10 min	43.35±0.85 b B	26.9±0.70 b A	1.57±0.16	2.36±0.11	0.94±0.05

<sup>a</sup> The adult *Drosophila* are wild type *Drosophila melanogaster*. They are captured in Beijing,

<sup>b</sup> The equipments used are heat: 1300 watt microwave oven; chill: fridge (-30°C); ether: 2 ml

<sup>c</sup> Within each column, means followed by the same letter are not significantly different by the

<sup>d</sup> Flies are selected randomly from the same culture vial. Twenty right wings of the same genotype and two replications were maintained. Digital photographs of each wings were taken and wing area

<sup>ns</sup>: Non-significant at p=0.05

size of adult *Drosophila*<sup>a</sup>  
 or mm<sup>2</sup>)<sup>d</sup>

Male		
Area <sup>ns</sup>	Length <sup>ns</sup>	Width <sup>ns</sup>
1.20±0.09	2.06±0.08	0.83±0.03
1.23±0.12	2.07±0.10	0.84±0.05
1.18±0.10	2.05±0.10	0.83±0.04

China and kept in my lab for more than 5 years, and maintained at 25°C in cornmeal medium ether, and the inner size of the etherizer is 170 ml.

ie Duncan’ s Multiple Range Test, lower/upper case letters indicate p=0.05/0.01

ler were collected from the flies killed by the same agent  
 size was measured using ImagePro Plus software

III.

Name of Material/ Equipment	Company	Catalog Number	Comments/Description
a pair of pliers			
cordless drill driver			max speed: 500 rpm
electric soldering iron			
file			
funnel			diameter of disk<60mm
ice box			
insect pins			
infrared thermometer		HCIYET HT-830	
long cuff rubber gloves			
mechanical pencils			
medical gauze			
microcentrifuge tube		100 ul	
microwave oven			
Parafilm			
peri dish			internal diameter 60 mm
pipette tips		1 ml	
plastic film			
plastic peri dish		Φ36 mm	used to cover the empty vial
point tweezers			
protective work gloves			
re-freezable hard icepacks		26.5×14.5×2.5 cm	or larger
rubber air blower			
snap cutter			
soft drink bottle		500 ml,	internal diameter c.a. 65 mm
sponge stopper			
stainless steel sponges			
tube brush			
vial		Φ 34 mm × 90 mm	

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Author(s):	Daxiang Yang

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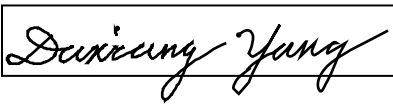

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Signature:		Date: 

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## Revision Checklist & Response to reviewers

- **Revision Checklist**

- Please submit each figure as a vector image file to ensure high resolution throughout production: (.svg, .eps, .ai). If submitting as a .tif or .psd, please ensure that the image is 1920 x 1080 pixels or 300 dpi.

I have converted the images into tif format, 300 dpi.

- Please take this opportunity to thoroughly proofread the manuscript to ensure that there are no spelling or grammatical errors.

OK

- Protocol Language:

- 1) Avoid long notes such as on Line 76-87, 97-103, etc.

Notes on line 76-87 have been shortened. But other notes have not, as I considered they are necessary illustrations to readers.

- 2) Avoid the use of pronoun “you”

I have rewritten the sentence contain “you”

- Protocol Numbering: There must be a one-line space between each protocol step.

I am afraid that I do not quite understand this requirement.

- Protocol Highlight: After you have made all of the recommended changes to your protocol (listed above), please re-evaluate the length of your protocol section. Please highlight ~2.5 pages or less of text (which includes headings and spaces) in yellow, to identify which steps should be visualized to tell the most cohesive story of your protocol steps. Please see JoVE's instructions for authors for more clarification. Remember that the non-highlighted protocol steps will remain in the manuscript and therefore will still be available to the reader.

1) The highlighting must include all relevant details that are required to perform the step. For example, if step 2.5 is highlighted for filming and the details of how to perform the step are given in steps 2.5.1 and 2.5.2, then the sub-steps where the details are provided must be included in the highlighting.

2) The highlighted steps should form a cohesive narrative, that is, there must be a logical flow from one highlighted step to the next.

3) Notes cannot be filmed and should be excluded from highlighting.

The protocol steps for film have been highlighted. The supposed logical flow of the film is in the first part of the film, I demonstrate how to make the tools, and in the second part of the film, I demonstrate how to use these tools.

- Results: We require at least some results (figures/tables) that demonstrate the success of your technique, this can be an application of your method to a specific study or general

results that validate the technique and compare it with other methods. These must be fully discussed in the Representative results. The current results do not sufficiently support and validate the technique you present. You may discuss applications of the approach to specific experiments as well.

I have tested the change of surface temperature of icepack with the time, and have compared the effects of three killing agents on the adult flies and rewrote the corresponding text. The results were shown in Table 1 and Figure 3 & 4.

- Discussion: JoVE articles are focused on the methods and the protocol, thus the discussion should be similarly focused. Please ensure that the discussion covers the following in detail and in paragraph form (3-6 paragraphs): 1) modifications and troubleshooting, 2) limitations of the technique, 3) significance with respect to existing methods, 4) future applications and 5) critical steps within the protocol.

I have revised Discussion.

- Figures: Please expand the legends to adequately describe the figures/tables. Each figure or table must have an accompanying legend including a short title, followed by a short description of each panel and/or a general description.

Figure 3 were replaced and its legend was completely rewrite. The legends of figure 1 and 2 were revised as required.

- Table of Materials: Please revise the table of the essential supplies, reagents, and

equipment. Remove any images from the table and list items in alphabetical order sort by the name.

Ok

- Please define all abbreviations at first use.

I have defined the abbreviation “T - /F – stopper” at their first use.

- Please use standard abbreviations and symbols for SI Units such as  $\mu\text{L}$ , mL, L, etc., and abbreviations for non-SI units such as h, min, s for time units. Please use a single space between the numerical value and unit.

I have checked the manuscript and corrected the issues.

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from [citation]."

My figures and tables are original and not published previously

- **Response to reviewers**

Reviewer #1:

Manuscript Summary:

This article describes several tools that can be used for simple manipulation of *Drosophila*.

Many of the suggestions are creative, cheap, and simple.

Major Concerns:

I think there is useful information in this submission, but that it is not an appropriate contribution for JoVE. Most tools in the article may be useful for teaching labs, if those are not already set up for *Drosophila*, particularly for their simplicity and low cost. However, research labs already have procedures and tools for all of the situations in the submission, some of which work better than what is described in this submission (particularly in

situations with many fly stocks and mutants to manipulate). I have seen labs that use drill + brush for vial (or bottle) cleaning, and ones that use a variation of the needle mounting (though not in a mechanical pencil, which is a cute idea). My lab is among many that anesthetizes with ice and uses chilled sorting pads (though ours do not use gauze, which is a clever variation). In terms of suitability for JoVE, the article does not always set the context well (for example, ice is just one of many ways to anesthetize flies), and it concerns multiple independent but unrelated tools, rather than a single full-protocol.

Given the utility of these tools for a teaching lab, it would be nice for the community to see them somehow, though JoVE is not the appropriate way. I encourage this article's publication but in the same educational journal where the authors previously published (ref. 10) or, perhaps alternatively, in the journal FLY since the article is very *Drosophila* specific. Or perhaps online, as a service to the community. The fly cage is particularly novel and the most different from standard tools in fly labs.

I think the F/T transfer system is not as useful for research labs that handle many vials and stocks and often need to sort flies for particular phenotypes before putting only the right ones into a vial. Those labs are also adept at transferring and controlling animal numbers per vial or moving single animals to a new vial (using suction tubes), with speed and lack of contamination. Some methods in this article raise concerns for research labs, such as effects of sorting on a -20 surface on quantitative or sensitive traits such as behavior or viability or whether microwaving flies affects eye colors (most research labs just freeze flies

to kill them).

I think that JoVE's research audience would not need or read this report. But I think that the teaching-lab audience could benefit from these the simplicity and low cost of these methods. Hence, I encourage submission to an education journal.

Minor Concerns:

The text needs some editing for language.

Line 251-252: water is not lethal to flies.

In the figure, it would help if you could label the F and T stoppers as such.

Dear Reviewer,

Thank you for your careful reading and critisizing my manuscript. Just as you see in this revised manuscript, I have carefully considered your constructive advices, add some new data to support my opinion, and corrected the errors you kindly pointed out in the comments.

I remember that in 1950s, my fellow countryman, Dr. T.C.Hsu, invented a method for animal chromosome preparing. He added some hypotonic solution to the cultured animal cells ! This invention is so ridiculously simple and I don't think a science journal would like to publish the method. However, this "low technology" completely changed the animal and human cytogenetics.

This story made me believe that there exists simple solutions for complex issues. When I finally figured out how to absorb the condensing water in the cold surface, and corrected the deadly flaw of the traditional chilling method by a piece of medical gauze, and when I see how effective my method works, I recall this story. When I see in my genetics class that any inexperienced student can immobilize adult flies easily with this method, I firmly believe my method is a **truly feasible** one, and it is a great substitute for ethyl ether, CO<sub>2</sub> or current version of chilling method, I don't know why a fly pusher would reject this invention.

I have no idea what things like in your country, but I am afraid most of the tools you described such as **suction tubes, chilled sorting pads**, are not available in China, or at least an instructor or Drosophilist should take great pains to find them. I know this from my personal communication with some Drosophilist, and from the popularity of these tools among instructors and researchers. I still remember that a Drosophilist in Guizhou province asked me to show him how to make F/T stoppers, as his lab badly need such tools; and one of my close friends, a fly pusher, who received good training in the United Kingdom (UK), borrowed microdissecting needles from me (but never return them back!), and my egg collection cage is a much more reasonable, convenient design than the ones the guy show me.....All these things make me believe that both students and Drosophilists will benefit from my little inventions, as the materials required for the making of these tools are virtually available anywhere, and the making of the tools are very easy. All these explain why I take



time to invent these low technologies and dare to submit a manuscript about these basic simple tools to *JoVE*.

In your comments, you raise a question: "Some methods in this article raise concerns for research labs, such as effects of sorting on a -20 surface on quantitative or sensitive traits such as behavior or viability or whether microwaving flies affects eye colors (most research labs just freeze flies to kill them)." I must apologize for my bad expression. I hope I have made myself clear in this revised edition. In fact, we work on a safe temperature for the flies, the ice pack maybe somewhat like the chilled sorting pads in your lab.

Reviewer #2:

Manuscript Summary:

The manuscript describes a set of very basic methods to 1) prepare tools to collect *Drosophila*, 2) collect *Drosophila* adults by chilling, 3) collect eggs, 4) transfer *Drosophila* from one tube to another, 5) kill flies by microwaving and 6) cleaning vials.

Major Concerns:

It is unclear to me for whom these protocols are intended. I doubt *Drosophila* research scientists would find these protocols useful as they are neither fast, nor thorough and we have quicker, easier and only marginally more expensive methods to do what is described

here. For university and high school students, I doubt this would be an advance as the basics of the methods shown here are already well known or are not suitable for all type of experimentation. Indeed, the efficacy of these techniques is not quantified. For instance, as someone who teaches a *Drosophila* genetics lab for undergraduates, I would not use these techniques to test flies behaviour as chilling has long term consequences on behavioural performance. We might also want to use killing flies to perform morphometric measurement, of wing size for instance, but there is no quantification of what damage the microwaving does to the integrity of the fly structure. The "representative results" section would have been a place to present such data. Finally, even though the protocols are already simple, they are incomplete. There is no indication of perhaps one of the most important step in growing flies, what food you use.

My impression is that these protocols might be useful for people wishing to do *Drosophila* work at home, but for this they would first have to invest in a stereomicroscope costing in the neighbourhood of a 1000\$. They also would not have access to articles published in JOVE.

I find the author's effort to make work with *Drosophila* more democratic very laudable. I however struggle to find how the JOVE audience would benefit from these protocols.

Minor Concerns:

Some Figures are wrongly annotated (See Figure 1 panels that do not match the text). The

English should be checked for grammatical error. Finally, a lot of what is described in this proposed protocol for JOVE is found in Qu, W.-h., Zhu, T.-b. & Yang, D.-X. A Modified Cooling Method and its Application in Drosophila 338 Experiments. Journal Of Biological Education. 49 (3), 302-308, (2015).

Dear Reviewer,

Thank you for your careful reading and critisizing my manuscript. I have carefully you're your comments and corrected the errors you kindly pointed out, and added some new data to support my opinion.

I am afraid those tools like suction tubes, chilled sorting pads that are described in many Drosophila handbooks are not available in China, at least an instructor or a researcher should take great pains to find ones. So that's why some of the tools described here, such as T/F stopper, microdissecting needles are so warmly welcomed by high school/university students, biology instructors and drosophilist as well, as they badly need these tools and they can make them themselves. I am fully ensured that students and researchers in other countries will benefit from these easy-to-make but rather effective tools too, this is the reseason I submitted this manuscript to JoVE.

The chilling method described here is a completely revised version of that we reported previously. This version is more effective and is a **truly feasible one**. The effectiveness and

feasibility have been test by serveral hundred inexperience students in my genetics class and in genetics class of other universities. These results make me believe my chilling method is a great substitute for ethyl ether, CO<sub>2</sub> or current version of chilling method. According to Barron,A.B (2000), the detrimental effect of anaesthesia on the mating behaviour by chilling is not greater than commonly used CO<sub>2</sub>, and the chilling method is much more healthy than any other methods to the person who work with adult Drosophila. I don't know why a drosophilist will reject these methods ?