*Journal of the American Mosquito Control Association,* 10(3):456–459, 1994


# NEW TECHNIQUES FOR REARING BLACK FLIES FROM PUPAE (DIPTERA: SIMULIIDAE)

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ABSTRACT. Simple techniques are described for collecting black fly pupae from streams using plastic strips and for rearing large numbers of adult black flies in inexpensive enclosures made of chicken wire and cloth mesh netting, and 2 methods are described for rearing adult black flies individually from pupae. The first method of rearing individual black flies uses 1.5-ml microcentrifuge tubules and the second uses easy-to-construct rearing chambers that provide moisture for the developing pupa and water for the adult to imbibe. Instructions for assembly are provided. Specimens obtained using these rearing chambers are of museum quality.

Black fly larvae and pupae are found in a variety of streams. Species composition and population densities can fluctuate widely throughout the season both within and among streams. Large series of reared adults are often needed for taxonomic purposes. For behavioral, physiological, or ecological studies, one might need large numbers of flies of known age and history. Rearing adults from field-collected pupae is often the simplest way to obtain such material. However, it is time consuming to search streams by hand for large numbers of pupae.

Several authors have described ways in which to rear black flies from eggs, early larval instars, or pupae through to adults, using elaborate and expensive artificial streams (Bernardo et al. 1986, see review *in* Edman and Simmons 1987). Published methods for mass rearing adults from field-collected pupae have stressed the use of flow-through chambers or aquaria (Tarshis 1968). Brief accounts also exist of rearing adults from individual pupae placed in tubes stoppered with moistened cotton (Rubtsov 1956). Wood and Davies (1966) placed pupae singly in ½-in. (1.3-cm) square compartments of a sheet of plastic overhead lighting grid, laid on top of wet filter paper in a shallow tray. Each pupa was then covered with a 2-in. (~5-cm) length of glass tubing, stoppered with cotton. Golini (1981) replaced the lengths of glass tubing with a sheet of plastic kitchen wrap. All of these previously suggested devices enclose the newly emerged adult in a small space, and require that it be transferred to a holding chamber before pinning. Invariably the inner surfaces of the chambers become wet with condensation, which tends to trap the adult, especially small specimens.

The need for large numbers of healthy, reared black fly adults for life history and taxonomic studies led to the development of the following simple and inexpensive techniques that should prove useful to other simuliid researchers.

*Collecting pupae:* The method described herein is suitable whether pupae are for mass or individual rearing. It takes advantage of the fact that black fly larvae and pupae will readily colonize artificial substrates such as plastic strips (Olejnícek 1980) that remain relatively taut in the current (Walsh et al. 1981).

Six 25 × 10-cm green garbage bag strips are tied at approximately 10 cm intervals to a length of plastic twine (Fig. 1A). At least 30 cm of twine is left free at either end for attachment to sticks that are pushed into the stream bed, thus securing the strips so that water flows over them. Depending on the type of stream, the series of strips can be anchored transversely across the flow or in line with the flow. The latter method has proven especially effective in small shallow trickles inhabited by some species of *Greniera, Stegopterna, Simulium* (*Eusimulium*) spp., and other headwater specialists. It may be necessary to partially cover the strips with sand and other fine benthic matter if collecting *Greniera* and *Stegopterna,* which typically pupate in tiny cracks and crevices beneath the substrate.

Many larvae and pupae often are found on the strips within 4–6 days. The strips are removed from the stream and transported back to the laboratory in plastic bags kept on ice. Strips are rinsed in a large basin of water to remove larvae and other aquatic insects, thereby leaving only the black fly pupae (Fig. 1B).

*Mass rearing of adults:* If species-pure samples are needed, the plastic strips can be examined under a dissecting microscope and pupae of unwanted species removed with fine forceps. The strips are then ready to be placed in rearing enclosures. If mixed species assemblages are required, strips are placed directly into enclosures after rinsing.

An inexpensive rearing enclosure can be constructed by bending a 120 × 60-cm sheet of

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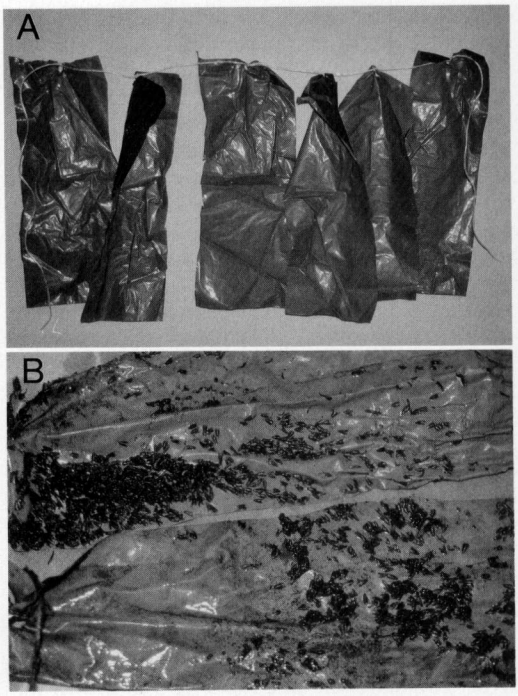


Fig. 1. Plastic strips used for collecting pupae from streams. A. Strips before use. B. Pupae-covered strips removed from streams after 4–6 days.

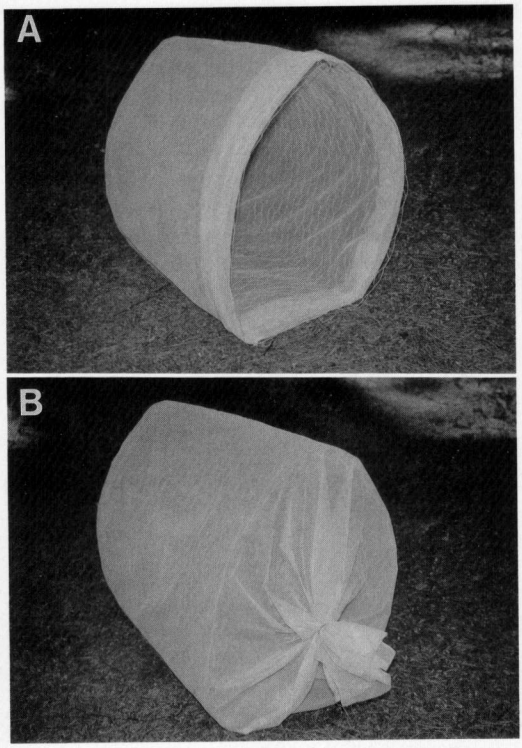


Fig. 2. Rearing enclosure made from chicken wire and cloth mesh netting. A. Rearing enclosure fully opened at one end to show chicken wire frame. B. Rearing enclosure tied off at both ends.

chicken wire into a triangular "A-frame" (Fig. 2A). The frame is covered with an open-ended rectangular bag made of fine cloth mesh (e.g., Vista® curtain sheer material).

Four sugar-water containers (20-ml glass scintillation vials with a folded dental wick and an artificial nectar or artificial honeydew solution) are suspended from the peaked roof of each enclosure. Artificial nectar consists of 3.5 g glucose, 2.5 g fructose, and 4.0 g sucrose in 100 ml distilled water; artificial honeydew consists of 1.0 g glucose, 1.5 g fructose, 3.5 g sucrose, and 4.0 g melezitose in 50 ml distilled water.

One or 2 series of pupae-covered strips are hung from the roof of the enclosure (using paper clips hooked into the loops of the chicken wire frame). The mesh bag is gathered and tied off at both ends using string or elastic bands (Fig. 2B) and the enclosure is left until flies begin to emerge. It is not necessary to mist the strips with water.

If flies of known age are required, strips can be transferred to new enclosures at variable intervals (24, 48, 72, 96 h) postcollection. Maximum adult emergence generally occurs on the 2nd or 3rd day; by the 6th day no further emergence is expected. Strips checked on the 6th day showed an emergence success rate as high as 85.7% (representing a total of 4,134 emerged adults from 4,822 pupae, primarily of the *S. venustum/verecundum* complex; average no. pupae per strip = 338.1 ± 113.9, $n$ = 12 strips).

The emergence success rate reported here is much higher than the 67.7% reported by Tarshis (1968) who, using gauze strips that were 2.4–4.5 m in length, reared 20,943 adult flies from 30,914 pupae. However, Tarshis exposed the gauze strips for only 24 h in the streams. As a result, black flies were brought into the lab as larvae and then placed in an artificial stream. Pupation success was less than 30% (30,914 pupae from >104,000 larvae) and it is likely that many of those that did pupate did so under stressed conditions. In contrast, our small plastic strips were left in the streams for 4–6 days and were removed only after large numbers of simuliids had pupated on them. Our higher emergence success rates likely resulted from our starting with healthier, older pupae.

*Individually reared flies:* For taxonomic purposes it is often necessary to have individually reared adults with associated cocoons and pupal exuviae. Two methods of individual rearing are

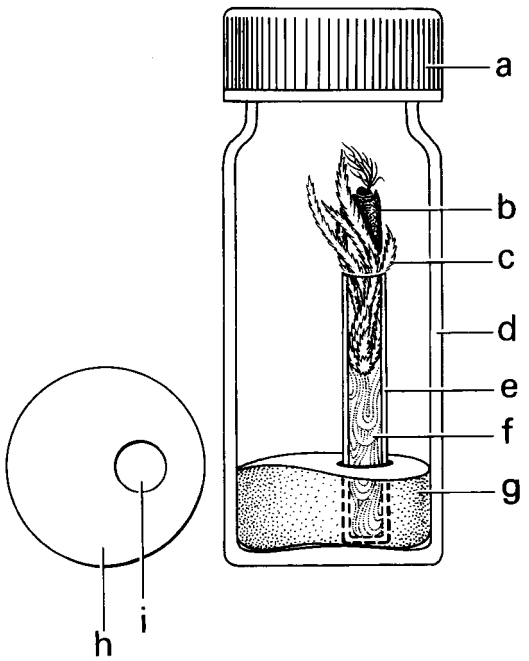


Fig. 3. Container for rearing individual flies of museum quality. a. screw cap (loose, to avoid condensation), b. simuliid pupa, c. sphagnum moss support for pupa, d. glass vial, e. shell vial, f. cotton (wet), g. polyethylene (Plastazote®) foam disc, h. foam disc as seen from above, disc cut with sharp edge of plastic vial, i. support for shell vial, hole made with cork borer.

described below; one for museum-quality specimens and one for checking species identity when adult structures are needed for identification.

*Technique for rearing flies individually for rapid identification:* A simple, fast, and inexpensive method of obtaining reared adults is to cut around individual pupae that are attached to the plastic strips. Each pupa is then set on a moistened piece of filter paper in a 1.5-ml Eppendorf® microcentrifuge tubule. The lid of the tubule is then snapped shut. All specimens from a single site are stored together in a labeled Ziploc® bag. Tubules are checked twice daily for emerged adults, which are visible through the opaque plastic. Adults are left to harden in the dark (to restrict activity) for 24 h so that coloration patterns are well defined.

The hardened fly can then be tapped to the bottom of the tubule, the lid opened, and 70% alcohol introduced from a squeeze bottle. We have found that specimens stored in this manner are still usable even after 6 years, provided that the microcentrifuge tubules were tightly capped. For long-term storage, we recommend that the tubules be kept in an alcohol-filled sealer jar. Unfortunately, coloration patterns tend to fade in alcohol-preserved specimens. Alternatively, therefore, tubules with reared flies can be placed directly into a freezer (−20°C) and the frozen flies mounted as described below.

*Technique for rearing flies individually for museum-quality specimens:* The device described here (Fig. 3) overcomes many of the disadvantages of devices described by other authors. It provides a moist surface for the pupa, and for the adult to drink from, in an otherwise dry environment.

After removal from the streams, pupae are kept in petri dishes on wet sphagnum, cotton, or paper, until pharate adults appear ready to emerge. Then each pupa, either free, or still attached to a small piece of substrate, is placed among strands of sphagnum moss protruding from a shell vial containing wet absorbent cotton inside a larger screw-cap vial. Vials are labeled either by grouping them in a larger labeled container, or inserting individual pre-prepared labels with the pupae. Each shell vial is "topped up" daily, with water from a dropper, so that the amount of moisture surrounding the pupa remains relatively constant (the pupa in Fig. 3 is shown farther away from the top of the vial than usually placed). Any water spilled inside the screw-cap vial should be immediately removed with cotton. We have used both distilled water and water from the same source as the pupae. Tap water is not considered suitable because of added chlorine or similar antibacterial compounds, and perhaps because of excessive copper ions (as discussed by Wood and Davies 1966).

To support the pupa we routinely use sphagnum moss, harvested from living sphagnum mats, because it holds water better than cotton and is more resistant to fungal attack. However, we have also used absorbent cotton alone, changed between each successive rearing. We have not tried commercial peat moss, other types of mosses, or artificial sponges, although these could perhaps be used if not contaminated with wetting agents.

The inner shell vial is filled with water, the cotton wick and sphagnum support inserted, and the outside of the vial dried, before it is positioned inside the larger screw-cap vial. The shell vial is held upright by a disc of polyethylene cut from a sheet of foam used as the pinning bottom for unit trays (Plastazote®). This disc is cut using the sharpened upper rim of a plastic vial of the same diameter as the inside of the screw-cap vial. The small hole to support the shell vial, bored with a cork-borer, is off-center to allow for better access to the interior of the screw-cap vial with forceps or an aspirator.

Only pupae containing pharate adults close to emergence are placed in the screw-cap vials to

minimize desiccation of pupal gills. The screw caps are always kept loosely closed to prevent moisture from forming inside the screw-cap vials. Temperature shifts are avoided as much as possible to avoid condensation. Vials are checked twice daily and those containing emerged adults are set aside in the dark for 24 h to harden and darken. During this time adults often come to the edge of the sphagnum to imbibe water. Mortality is higher during this period in the vials that have dried up; some adults even force their way into the sphagnum in dried up vials, presumably in search of water. We have not attempted to provide a sugar meal, although this might improve their longevity still further, provided they do not become stuck in the sugar source.

After adults have remained 24 h in the dark, at room temperature, they are transferred into a deep freezer. All but the most hardy species are killed within a few hours; some early spring species require longer. This method of killing requires no handling of living adults and, therefore, no opportunities of escape or damage during handling. The only disadvantage is that the water-filled inner shell vial will sometimes burst.

Flies frozen in their vials can be left for up to a week before being pinned. They still retain their flexibility, but any disturbance should be avoided to prevent damage to the frozen specimens. Each adult is attached by the right side of its thorax to a small drop of shellac gel on a no. 1 or 0 insect pin, 1 cm from the head of the pin (Shewell *in* Martin 1977). Pinned flies are immediately returned to the freezer for 4–6 months, or until completely freeze-dried. The associated pupal exuviae and cocoon may either be stored in glycerol in a genitalia vial pinned beneath the fly, or glued when dry to the side of the pin beneath the fly.

Between rearings, cotton and sphagnum are removed from the shell vials and discarded. The screw-cap vials are wiped out to remove traces of moisture and meconium and the shell vials are cleaned, reloaded with wet cotton, their outer surfaces dried, and replaced in the screw-cap vials. Between seasons, each component is thoroughly washed and dried.

We thank the Ontario Ministry of Natural Resources for allowing this work to be done at the Wildlife Research Station, Algonquin Park. Figure 3 was drawn by Ralph Idema. Funding was provided, in part, by Operating Grants from the Natural Sciences and Engineering Research Council (Canada) to F. Hunter and M. Wood.

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