

## A TECHNIQUE FOR MEASURING AND MARKING LIVE FLIES

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### Abstract

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We designed a simple device for physically restraining live Diptera for measurement and marking. This new technique does not involve powders, anaesthetics, or handling with fingers or forceps, and can be adapted for use with flies of any size. We tested it on acalyprate flies of 1.5–3.0 mm body length, and were able to sex, measure, and mark them with individual codes (enamel paint applied to the thoracic notum) in 5–7 min per fly, with a 96% success rate. This technique may be applicable to a range of behavioural and ecological research.

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### Résumé

Nous avons mis au point une technique simple destinée à immobiliser physiquement les diptères vivants pour les mesurer et les marquer. Cette nouvelle technique n'utilise aucune poudre ou substance anesthésiante et ne suppose aucune manipulation avec les mains ou avec des pinces; elle peut être adaptée à des mouches de toutes les tailles. La technique a été testée sur des acalypères de longueur totale de 1,5 à 3,0 mm et nous avons pu déterminer le sexe chez chacune des mouches, les mesurer et les marquer de codes individuels (touche d'émail sur le notum thoracique), tout cela en 5–7 minutes par mouche, avec un taux de succès de 96%. Cette technique est utilisable dans une grande variété de types de recherche comportementale et écologique.

[Traduit par la Rédaction]

### Introduction

Behavioural research on Diptera frequently involves sexing, measuring, and marking of live flies (e.g. Dow and von Schilcher 1975; Wilkinson 1987; Droney 1992; Jarvis and Rutledge 1992). Dusting with fluorescent powders (e.g. Partridge et al. 1987) is effective for marking hundreds or thousands of individuals by cohort. Flies large enough to be handled easily (e.g. 1 cm body length) can be marked by gluing "Bee tags" (Graze Bienenzucht Geräte, 71384 Weinstadt, Germany) to their thoracic nota. However, it is often necessary to apply individual codes to much smaller flies (e.g. *Drosophila melanogaster* Linné). In these cases, anaesthetics are usually employed (e.g. Partridge and Farquhar 1983), and insects are handled with fingers or forceps. Mortality and injury rates, although rarely reported, must usually be high, especially among smaller individuals in the sample.

Here we describe a new technique for measurement and marking of live flies by means of a physical restraining device. The technique is especially useful for small acalyprate flies that must be marked with an individual code, but is also effective with large calyprate Diptera and bulk marking of moderate numbers of flies. Although we have only tested the technique on Diptera, it is probably applicable to other insect orders.

### Materials and Methods

**Restraining Device.** The device consists of two cylinders, one for measuring and one for marking (Fig. 1), and a piston that fits both cylinders. The cylinders are open at one end and covered at the other end with either clear plastic (measuring cylinder) or rigid screen (marking cylinder).

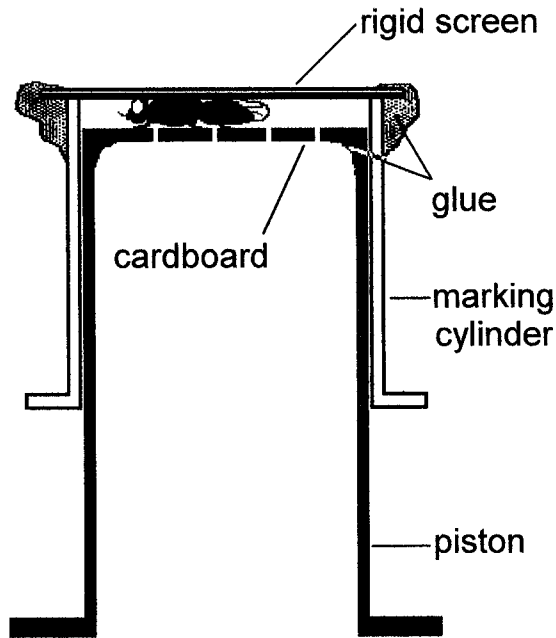


FIG. 1. Longitudinal section through the marking device, showing a restrained fly in the cylinder.

The cylinders can be constructed from 20-g, 1.5-in vacutainers (Becton Dickinson & Co., Rutherford, N.J., reorder number 4889). The needle is removed and an approximately 3 cm long section of the tube (from the flat base) is cut off and the cut end is flattened and smoothed. To make the measuring cylinder, the cut end is covered with a small piece of plastic clingwrap, which is stretched over the opening and affixed to the outside of the cylinder with scotch tape. To make the marking cylinder, a piece of rigid insect screen, of slightly larger diameter than the vacutainer, is attached to the cut end of the tube by means of a glue gun. For small acalyptate flies (1.5–3 mm body length), we found that a mesh size of 22 (22 openings per linear inch, each opening approx.  $0.9 \times 0.9$  mm in size) was optimal, but standard metal mosquito screen (mesh size 18) can also be used. For flies of 3–5 mm body length, a mesh size of 16 or 18 is effective, whereas for flies of body length  $>5$  mm, a mesh size of 6 can be used.

The piston can be constructed from a 10-mL syringe (“Luer Lok”, Becton Dickinson & Co., reorder number 9604). An approximately 5 cm long section of the plastic syringe (from the flat base) is cut off and the cut end is flattened and smoothed with a razor blade. This end of the tube is then closed by gluing on a round piece of thin cardboard, which should be perforated several times with a pin. The final step is to adjust the piston diameter to fit loosely but snugly into the cylinders by wrapping several layers of textured masking tape (e.g. “Mastercraft General Purpose Masking Tape”, Canadian Tire Corp. Ltd., Toronto) around the syringe tube.

**Sexing and Measuring Technique.** The fly is released into the measuring cylinder and the piston is quickly inserted and adjusted so as to immobilize the fly, with either its dorsal or ventral surface against the transparent membrane. Care should be taken to avoid exceeding the very slight pressure required to immobilize the animal. The device is then placed on the stage of a dissecting microscope to examine sex, and measure the fly by means of a micrometer or calipers. Most dissecting microscopes can be adjusted to focus on the top of the device, approximately 5 cm above the stage.



FIG. 2. Marked specimen of *Protopiophila litigata*.

**Marking Technique.** The specimen can be transferred into the marking cylinder by taking advantage of the fly's negative geotaxis or positive phototaxis. For very small flies, it may be necessary to wrap the screen end of the cylinder in clingwrap to prevent escape during transfer. Once the fly is in the marking cylinder, the piston is inserted and adjusted so as to immobilize the fly with its notum protruding through an opening in the screen (taking care not to squash the fly). The device is placed under a dissecting microscope and enamel paint ("Armor Coat", Canadian Tire Corp. Ltd.) is applied to the fly's notum by means of a finely pointed strip of thick paper. Because the quantities of paint are minute, a small droplet may be transferred to the edge of the cylinder to minimize subsequent "brush" movement and drying of paint. While the paint is wet, unwanted marks can be removed with a pointed sliver of wood.

### Results and Discussion

**Success Rate.** We tested our technique on *Protopiophila litigata* Bonduriansky 1995 (Diptera: Piophilidae), which has strong site fidelity and is quite tolerant of close observation. Our marking success rate varied from approximately 90% in 1993 to 96% in 1995. Unsuccessful attempts included squashed flies and escapees. Successfully marked flies were vigorous immediately after marking, and several males were observed in copula 15 min after marking and release. Codes applied by this technique could be read with the unaided eye from a distance of 20 cm on the nota of flies as small as 1.5 mm body length (Fig. 2). Of 614 males successfully marked and released in 1994, 534 (88%) were subsequently observed at least once in mating aggregations, and <1% of these lost paint over their lifetimes. Some marked individuals were observed >30 days after release. The time required to sex, measure, and mark a fly ranged from approximately 10 min in 1993 to 5–7 min in 1995.

### Conclusions

With practice, our technique is relatively easy to use and enables a researcher to examine and apply an individual code to small acalyptate flies without anaesthetics, which may poison the specimen, or handling with fingers or forceps, which may cause physical

injury. Moreover, we found that marking the notum, in contrast to marking the wings, had no discernable effect on the fly's behaviour. This technique may facilitate research on a wide variety of Diptera.

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