Technique Notes

A system for measuring longevity and fecundity in flies.

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Introduction

Longevity and fecundity are often studied using flies, and many experimental systems have been devised for this. We report here a simple apparatus we have used for measuring longevity plus fecundity in *D. melanogaster*. Our system was designed to track these parameters in individual flies, singly or in male-female pairs, housed in units of 100 cells. The basic materials are a few plastic "egg crate" lighting diffuser panels and a light box, plus some small metal pans, insulative foam-board, and fine nylon mesh.

Design and Use

In the egg crate panels we used, the small square openings have inside dimensions of 16 mm on each side. We cut these panels into squares with 10 × 10 openings, with outside dimensions of 167 × 167 mm. We then glued these pieces together in stacks of six, with a sheet of nylon mesh between the top two pieces. This makes a box with 100 separate cells, each about 40 × 16 × 16 mm, bounded by mesh at the top, and open at the bottom (Figure 1). Each cell can hold a pair of flies. By gluing together two more squares of egg crate panel, we made a separate, matching, lower unit to combine with this, which can be pressed into a small metal pan of shallow agar medium as it solidifies, to provide the daily food and egg-laying surfaces below the flies (Figure 2). This lower unit is firmly stabilized in the agar pan by 1) a square sheet of one-eighth inch aluminum glued to the inside bottom of the pan and 2) four set screws through the sides of the pan that contact the plastic and can be adjusted by a hex key. The lower unit also has thin aluminum strips or flanges along its upper sides to provide guides so that the box containing the 100 cells, filled with flies, can be aligned exactly with the second grid of cells below, containing the 100 squares of agar. The two parts are then held together with rubber bands (Figure 3).

We replaced the agar pan each day. To accomplish this, the combined unit shown in Figure 3 containing the flies is inverted and lowered into the illuminator-anesthetizer box shown in Figure 4. This box is made of a light box (Porta-Trace, from Gagne, Inc., 41 Commercial Drive, Johnson City, NY, 13790), with four pieces of insulative foam attached to the top by an encircling band of duct tape, and four strips of aluminum on the inside of the foam to provide a supporting ledge, and finally a covering of white duct tape on the inside surfaces of foam and aluminum, to reflect the light.

The space inside the illuminator-anesthetizer can be filled with CO_2 from a port through the foam in the back. After a few minutes of CO_2 the flies fall onto the nylon mesh. (This is promoted by removing the inverted unit with the flies and dropping it onto the bench several times from a height of a few inches, during the anesthetization process.) Then the old agar pan and lower grid can be lifted off and replaced by fresh ones. If dead flies need to be removed (or replaced to maintain

sexual pairs) this can be done while the flies are anesthetized, before the new agar pan is attached. We also made a clear, sliding Plexiglas cover (not shown) to place over the box of cells while the flies are anesthetized, during the interval when the old agar pan is not attached. A small hole in this cover allows targeted access to individual cells without exposing all cells. Finally, the flies are given a few minutes to awaken fully, with the whole unit still in the inverted position, before turning it upright again. The eggs per cell on the agar surface of the old pan can be easily counted under a stereomicroscope. We numbered each cell in the agar grid with tiny digits so that the cell numbers are visible during counting. Pans can be stored in the refrigerator to stop egg development while they await counting.

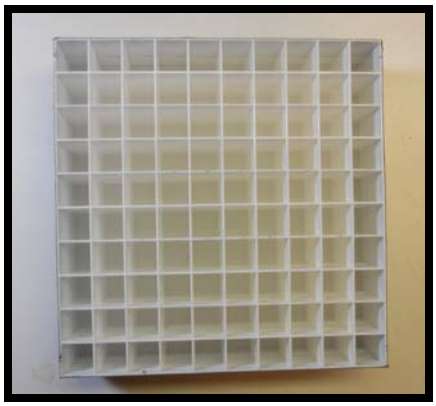

Figure 1. Fly box with 100 cells.

Figure 2. Base consisting of grid and pan.

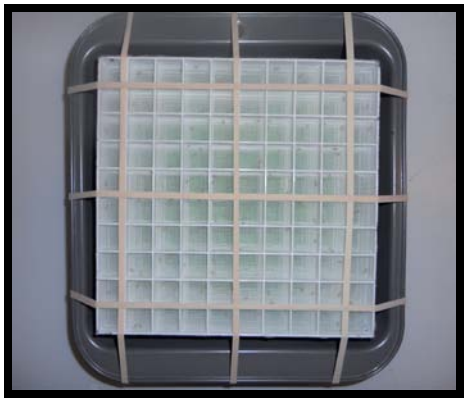

Figure 3. Combination of fly box and base.

Figure 4. Illuminator-anesthetizer.

At each agar transfer, care must be taken to minimize CO_2 exposure. When the experiment is initiated and the inverted box of cells is filled with flies for the first time, the box should not be kept on the anesthetizer at all, because it takes so long to fill all 100 cells the first time that the first flies added would receive much more CO_2 than the last. Instead the inverted box is placed on the benchtop, and as each row of 10 cells is filled with freshly and lightly anesthetized flies, the cells of that row are immediately covered with a strip of wide scotch tape. After all 100 cells are filled, the box is quickly gassed again on the anesthetizer, the whole covering of scotch tape is ripped off at once, and the agar pan is then attached. (The flies do not get stuck on the tape.)

Special care must be taken during construction of this apparatus to make sure all parts fit together tightly and exactly, taking into account any small irregularities in the egg crate material. In an experiment involving four simultaneous sets of (initially) 200 flies, no flies ever escaped or moved accidentally between cells in over 60 consecutive daily transfers.

Fluorescein dye as a tagging agent for *Drosophila* dispersal studies.

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Dispersal, or the unidirectional movement of organisms in search of improved conditions, is a commonly investigated and ecologically important aspect of plants, invertebrates, and vertebrates (Howe and Smallwood, 1982; Johnson and Gaines, 1990; Levin, 2006). This action is particularly important for organisms that are born into habitats with high population densities such as *Drosophila*. For these animals, rotting vegetation provides a nutritional resource for adults and larvae, as well as a location to meet mates and lay eggs. And while these conditions may support large populations, *Drosophila* are known to disperse (see Dobzhansky, 1973; Johnston and Heed, 1975; Grossfield, 1978). Of the studies that have empirically investigated dispersal outside the laboratory, many have relied on dusting flies with fluorescent microparticles to delineate the released population from wild individuals (see Crumpacker, 1974). This dusting can be performed actively in a "shake and bake" methodology, or through passive contact as the larvae emerge from their pupal stage when their media is doped with the particles. While dusting is a useful technique for examining dispersal, its possible influence on such behavior has been questioned (Turelli *et al*., 1985).

As part of a larger project to determine the dispersal and feeding range of the invasive fruitivorous *D. suzukii*, we investigated an alternative to dusting, namely the use of the fluorescent marker fluorescein sodium (C_{20}-H_{10}-O_5-Na_2). This common, relatively non-toxic water-soluble laboratory chemical is used in a number of applications including ophthalmological examinations of the cornea and as a tracer in ecohydrodynamic experiments (Maurice, 1967; Zimmer-Faust *et al*., 1995; Pesticide Action Network). Its ability to fluoresce green under UV or blue light makes it easy to visualize, while its safety and inexpensive nature make it a useful product for *Drosophila* work.

Methods and Results

Fluorescent techniques

Four application techniques were tested: dusting adults with fluorescein powder, spraying adults with a concentrated fluorescein solution (30 g/l), feeding adults media containing concentrated fluorescein solution (8 ml solution/vial), and feeding larvae the same media containing concentrated fluorescein solution. For the first two treatments (dusting and spraying), juvenile (<24 hours post-eclosion) flies from a laboratory population (Canton-S) of *Drosophila melanogaster* were collected and treated under CO_2 anesthesia and then placed (n = 15) in 5 replicate vials. Flourescein-fed-adults were collected after spending either 1 or 7 days on doped media, and flies from the flourescein-fed larval treatment were collected as emerging adults after spending their larval period in doped media. Pre-trial results determined that propionic acid, sometimes used when making the medium (Formula 4-24 Instant Media – Carolina Biological) to combat fungal infections, interacted with the dye decreasing or eliminating its fluorescence. Therefore, no propionic acid was added to the rearing media.