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A SIMPLE TECHNIQUE FOR MASS REARING *LUTZOMYIA LONGIPALPIS* AND *PHLEBOTOMUS PAPATASI* (DIPTERA: PSYCHODIDAE) IN THE LABORATORY[1]

Abstract. A simple technique for mass rearing phlebotomine sand flies (*Phlebotomus papatasi* and *Lutzomyia longipalpis*) in the laboratory is described. Using this method, 4000–5000 adults can be produced weekly with minimal labor (2–3 h of care per day). This is the first report of mass production of sand flies, a group of insects that heretofore has been difficult to colonize and maintain under laboratory conditions.

Since the first successful colonization of phlebotomine sand flies in 1907 by Grassi (1907, Mem. Soc. Ital. Sci. Ser. 3, **14:** 353–94), a variety of techniques have been described for rearing these insects under laboratory conditions (Hertig & Johnson, 1961, Ann. Entomol. Soc. Am. **54:** 753–54; Schmidt, 1964, Bull. WHO **31:** 577–78; Gemetchu, 1971, Trans. R. Soc. Trop. Med. Hyg. **65:** 682–83; Christensen, 1972, Ann. Entomol. Soc. Am. **65:** 683–86; Sherlock & Sherlock, 1972, Rev. Brazil. Biol. **32:** 209–17; Chaniotis, 1975, J. Med. Entomol. **12:** 183–88; Killick-Kendrick, Leaney & Ready, 1977, J. Med. Entomol. **13:** 429–40; Ward, 1977, J. Med. Entomol. **14:** 469–76; Young, Perkins & Endris, 1981, J. Med. Entomol. **18:** 446). Regardless of the method used, laboratory colonization of sand flies is difficult and extremely labor intensive. Of ca. 600 known sand fly species, none has been mass reared and only 6 or 7 have been reared in large numbers for more than 10 consecutive generations (Killick-Kendrick, 1978, Acta Trop. **35:** 297–313). The difficulties and tedious work required to maintain laboratory colonies of these insects have discouraged many scientists from working with them. During the past 2 years, we have been involved in studies on the vector competence of *Phlebotomus papatasi* (Scopoli) and *Lutzomyia longipalpis* (Lutz & Neiva) for various arboviruses. This work has required a regular supply of female sand flies. After trying a number of rearing methods, we developed a relatively simple technique for producing several thousand adults of each species per week. This communication reports the details of the technique.

In developing this technique, our primary objective was to produce the maximum number of healthy flies with the minimum amount of work.

Rearing containers. Immature stages, as well as gravid females for oviposition, are held in 500-ml polymethylpentene jars (Cat. No. 2117-0500, Nalge Co., Rochester, NY 14602). Prior to use, 30 to 35 holes (7.0 mm in diam) are drilled in the bottom of each jar. A layer of plaster of Paris (2 cm thick) is then poured into the upright container. The plaster surface is allowed to dry for 3–4 days. Just before use, the jar is soaked in water until the plaster is saturated. The excess water is poured off and the interior of the container is blotted dry with a clean, absorbent cloth. If the plaster is too wet, gravid females become entrapped on the moist surface.

Approximately 200 to 250 gravid females are confined in each container for oviposition. The mouth of the jar is covered with fine nylon organdy, which is fastened at the sides by rubber bands (Fig. 1). A hole large enough to admit an aspirator is made in the organdy covering the container to allow introduction or removal of adult flies. When not in use, the hole is plugged with a piece of cotton to prevent newly emerging adults from escaping (Fig. 1). A small piece of cotton wool, soaked in 30% fructose solution, is also placed on top of the organdy and provides a source of nourishment for the adult insects. Rearing jars are kept in tightly covered plastic boxes (20 × 28 × 10 cm). A wet sponge is placed inside each box to maintain high humidity. Plastic boxes are held in an illuminated incubator maintained at 28 °C, 60–70% relative humidity, and a 14:10 (L:D) photoperiod.

After 5 or 6 days of confinement in the rearing jar, most of the gravid females oviposit and die. At this time dead females are removed from the container with fine forceps. Most of the eggs (usually several thousand) are

FIG. 1. Rearing container used for the immature stages and gravid ♀. Note layer of plaster of Paris on bottom of the container, which is covered with a layer of the larval food. Recently emerged adults can be seen resting on the nylon organdy within the rearing container. The entire life cycle of the sand flies takes place in this container.

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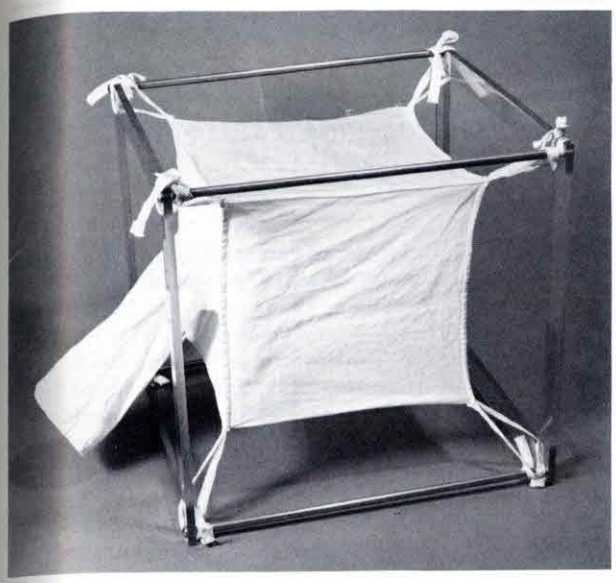

FIG. 2. Holding cage for adult sand flies. To maintain high humidity, the cage is placed inside a large plastic bag containing a wet sponge.

deposited on the damp plaster surface. Any eggs laid on the side of the jar are removed and placed on the plaster with a fine camel hair brush. A small quantity of larval food (described below) is then sprinkled over the eggs. Development of the eggs, larvae, and pupae takes place within the same container. Rearing jars are checked 2 or 3 times each week; additional food and water are added as needed. When the adult sand flies emerge in the jar (5 or 6 weeks later), they are transferred by aspirator to adult holding cages. After all adults have emerged, the rearing jar is autoclaved, washed, and reused.

Adult holding cages. The holding cage for adult flies consists of an 18-cm³ bag with sides made of white cotton cloth and nylon organdy (Fig. 2). The bag is suspended within a 25-cm³ steel and plastic frame by strings attached to its 8 corners. About 300 to 500 adult sand flies are released in each cage. The insects mate and feed within this container. For feeding, a hamster anesthetized with ketamine hydrochloride is placed directly in the holding cage (through the sleeve). Cotton wool soaked in 30% fructose is placed on top of the cage to provide a continuous source of water and sugar. Since the adult flies require constant high humidity (80–100%), the holding cage is placed inside a large plastic bag containing a wet sponge. Adult sand flies are kept at 25 °C. The insects can be maintained for as long as 3 weeks in this container, provided they have access to sugar water.

Larval food. The most important factor in maintaining a vigorous sand fly colony is the larval diet. We use a modification of a diet described by Young et al. (1981, loc. cit.). Basically, it consists of a mixture of dried rabbit feces, rabbit chow (Purina Rabbit Chow Complete Diet 5315, Ralston Purina Co., St Louis, MO 63188), and beef liver powder. Equal amounts of rabbit feces and chow are ground together, and the resulting powder is spread in a layer 3–4 cm deep in a large plastic box. This mixture is thoroughly wetted with tap water, tightly covered to prevent drying, and aged for 4–6 weeks at ambient temperature. The aging mixture is stirred 2–3 times weekly to disrupt fungus. Initially there is a prolific growth of fungi on the mixture; however, after a month the fungi disappear and the larval food resembles rich humus. At this time several tablespoons of beef liver powder are stirred into the mixture and it is allowed to age for another 2 weeks. The addition of liver powder to the diet enhances larval development (Gemetchu 1971, loc. cit.). After aging, the larval food is ready for use. It is allowed to dry and is stored at room temperature. When added to the rearing jar, it absorbs moisture and becomes soft and damp. Sand fly larvae can be observed feeding on the mixture and frequently burrow under it. As the food is consumed, it is replaced by larval feces. Additional food is added as needed.

Using the aforementioned technique, we currently produce 4000–5000 adults of both *P. papatasi* and *L. longipalpis* per week. In fact, our current sand fly production far exceeds our experimental needs. By rearing large numbers of larvae and pupae together and by naturally aging the larval food instead of sterilizing it, the sand fly colonies require a minimum amount of care (about 2–3 h per day). This investment of time is not much different from that required to maintain other dipteran colonies.

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ADDENDUM

Since submission of this manuscript, we have also used this technique successfully for mass rearing *Lutzomyia anthophora* (Addis) and *Phlebotomus perniciosus* (Newstead).