TECHNIQUES FOR LABORATORY REARING OF SAND FLIES (DIPTERA: PSYCHODIDAE)[1]

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ABSTRACT. Nine species of phlebotomine sand flies in the genera *Lutzomyia, Phlebotomus* and *Sergentomyia* were maintained in closed laboratory colonies for 6 or more generations. Techniques for establishing and maintaining the sand flies are described and discussed.

INTRODUCTION

The importance of establishing productive colonies of sand flies in the laboratory was emphasized by the WHO Scientific Working Group on Leishmaniasis (Anonymous 1977). "Colonies are valuable in work on vector potential, life cycles of *Leishmania* and transmission by bite. They are indispensable in genetic studies and in controlled observations on the physiology and behavior of sand flies all of which are neglected subjects of high priority. Colonies are of particular value for screening insecticides."

Killick-Kendrick (1978) summarized recent advances in sand fly colonization, pointing out persistent problems of high larval mortality, intensive labor requirements, and death of females at oviposition. Some vector species, notably *Phlebotomus papatasi* (Scopoli) and *Lutzomyia longipalpis* (Lutz and Neiva), have adapted readily to laboratory conditions but others have not when reared under the same conditions. Fewer than 20 of the approximately 600 known phlebotomine species have been colonized in large numbers for more than 10 generations (Killick-Kendrick 1978, Young et al. 1981 and Dr. Paul Ready, personal communication).

We and our colleagues have successfully colonized 9 phlebotomine species, including 3 man-biters (Table 1), using a standard larval food and other simple procedures. Aside from differences in adult feeding behavior, each species was reared in the same manner with minimum labor and expense. Detailed results of life cycles, longevity, vector competence and other information for each species will be published elsewhere. In this report, we give general information on the procedures used for establishing and maintaining sand fly colonies.

METHODS AND MATERIALS

FIELD COLLECTIONS. Depending on the habits of the target species, sand flies were captured alive in CDC light traps (Sudia and Chamberlain 1962), from diurnal resting sites and on human or animal baits (Table 1). We used a simple tube aspirator (Fig. 1) to collect and transfer the flies, being especially careful not to injure the specimens by forceful suction or overcrowding.

Soon after capture, flies of both sexes were gently blown from the aspirator into a 120 ml specimen container modified as shown in Fig. 2. When 50 or fewer flies were inside, the container was placed upright on wet paper towels at the bottom of a rectangular expanded polystyrene (Styrofoam®) box.

After being transported to the field laboratory, all sand flies were released into a feeding cage (Fig. 4). Each blood-fed or gravid female was then gently prodded into a 7 dr plastic vial (Fig. 3) and a screened lid (18 mesh/cm) was snapped in place. Previously, the plaster of paris

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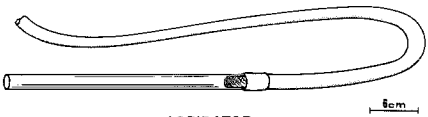
Table 1. Phlebotomine species recently colonized in laboratories at Gainesville, FL, Fort Detrick, MD, Pedro Sanchez, Dominican Republic, and Nairobi, Kenya. Beach et al. (1981) give further information on rearing *Phlebotomus martini*. An egg batch consisted of 10–50 eggs.

Species	Origin	Site of collection	No. egg batches initiating colony	Laboratory hosts	Current no. lab generations
1. *Lutzomyia anthophora* (Addis)	Brownsville, TX, USA	*Neotoma* woodrat dens	9	Hamster, mouse, squirrel (*Sciurus*), calf, rabbit, opossum (*Didelphis*), domestic pig	15
2. *L. cayennensis hispaniolae* Fairchild & Trapido	Pedro Sanchez, Dominican Republic	Tree trunks	50–75	Lizard (*Anolis*)	5
3. *L. diabolica* (Hall)	Near Uvalde, TX. USA	Walls of latrine, human bait	12	Hamster, mouse, squirrel (*Sciurus*), calf, horse, human, dog, opossum (*Didelphis*)	6
4. *L. cruciata* (Coq.)	Gainesville, FL, USA	Tree trunk	1	None, autogenous	24
5. *L. shannoni* (Dyar)	Gainesville, FL, USA	Tree trunks, light traps	40	Same as *L. diabolica*, also pig	11
6. *L. vexator* (Coq.)	Gulf Hammock, Levy Co., FL, USA	Under loose bark of dead standing trees	15	Lizard (*Anolis*), snake (*Elaphe*), toad (*Bufo*), tree frog (*Hyla*)	6
7. *Phlebotomus martini* Parrot	Marigat, Baringo Dist., Kenya	Termite hills, human and calf bait	12	Hamster, human	6
8. *Sergentomyia africana* (Newstead)	Marigat, Baringo Dist., Kenya	Termite hills	About 15	Lizard, (Geckoniidae)	6
9. *S. schwetzi* (Adler, Theodor & Parrot)	Marigat, Baringo Dist., Kenya	Termite hills	About 15	Lizard, (Geckoniidae), hamster	7



ASPIRATOR

Fig. 1. Simple aspirator. A suction tube made of Pyrex®glass (inside diam = 1 cm) and flexible rubber tubing. One of the open ends of a smaller plastic tube, covered with fine mesh nylon screen, is inserted into the base of the glass tube to prevent inhalation of sand flies.

bottom was moistened with several drops of tap water. No standing water was allowed to remain on the surface or sides of the vial. A drop of corn syrup (Karo®)[3] or honey mixed with water (1:1 mixture by volume) was placed on the top of each screen lid. The vials containing the flies, 1 per vial, were kept in the closed styrofoam box, the towels at the bottom remoistened, if necessary, to maintain high relative humidity.

Because the primary objective was to obtain as many eggs as possible, the remaining unfed flies in the feeding cage were given an opportunity to feed on an adult hamster and/or small lizard (*Anolis* sp. or gecko). Both hosts were placed inside the feeding cage when the feeding preference of the flies was unknown. We restrained the hosts by guiding them through the open end of a cylindrical

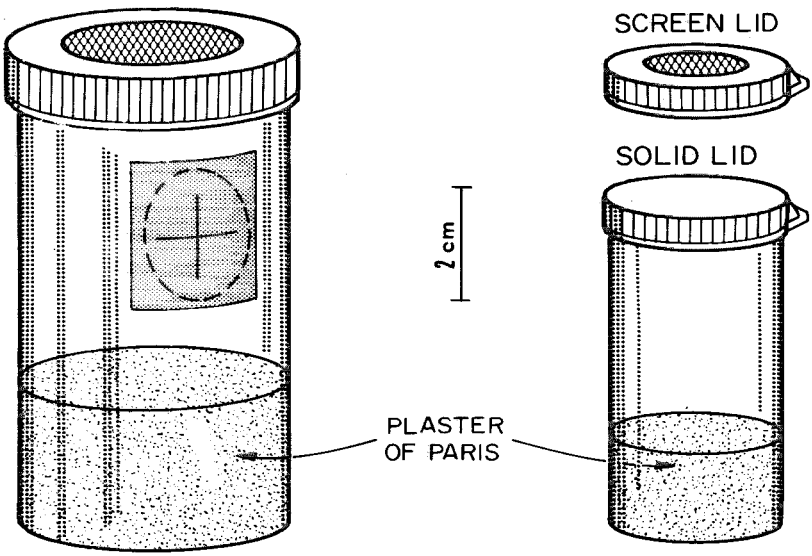


Fig. 2 (left). 120 ml specimen container. A rearing and adult sand fly holding cage with screen lid and showing "+ shaped" cuts in pieces of latex rubber glued over aspirator entrance hole; Fig. 3 (right). A cage for rearing 50 or fewer larvae and for holding 1–2 adult sand flies.

[3] Best Foods, CPC International Inc., Englewood Cliffs, NJ 07632.

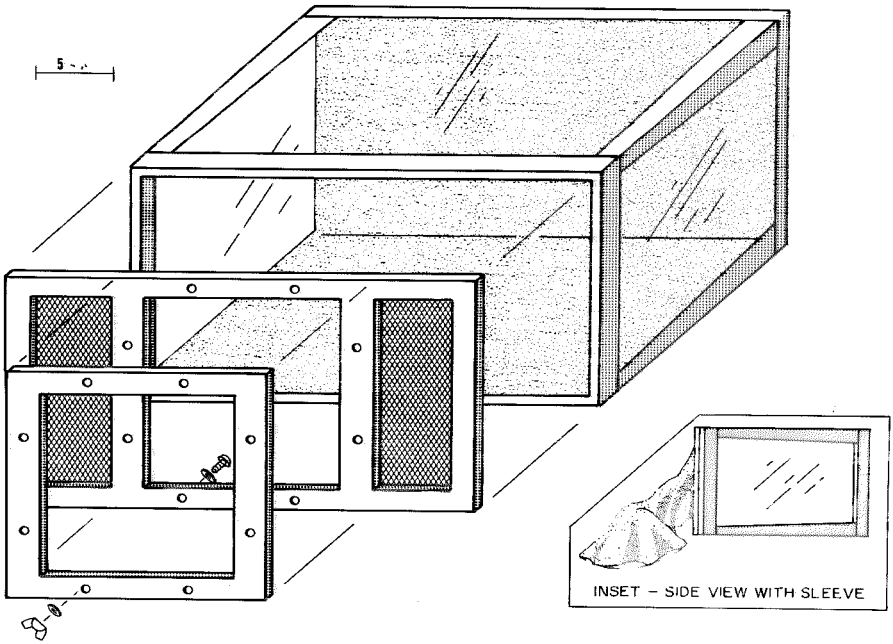


Fig. 4. Modified glass aquarium. A holding cage for adult sand flies. The front two panels are shown disassembled. The inset figure is a lateral view showing cloth sleeve in place.

wire cage (2 mesh/cm), slightly larger in diameter than that of the host. A wad of cotton sealed the opening, and when inserted close to the host, prevented the animal from moving about. The restrained animals were kept in the feeding cage for 2 hours or longer without harmful effects.

We discovered that flies of some species preferred to feed on unrestrained hosts. *Lutzomyia vexator* (Coq.) and L. *cayennensis hispaniolae* (Fairchild and Trapido) females, both lizard-feeders, were among these. A thin piece of tape, covering the mouth but not the nostrils of the lizard, prevented the animal from eating the flies in the feeding cage. For the mammal-feeders that refused to feed on a restrained hamster, we provided an anesthetised hamster, immobilized with ketamine hydrochloride[4] injected intramuscularly with a 27 gauge needle and tuberculin syringe (0.2 ml/adult hamster). The effects of this drug lasted up to 1 hour. We did not remove the hair from the hamster or other mammals because hungry sand flies fed readily on the ears, feet or nose (Fig. 5).

When hosts were not exposed to the sand flies, 3 or 4 circular apple slices, replaced daily, were leaned against the sides of the feeding cage to provide a sugar source. The bottom sixth of each slice was cut off in a straight line for stability.

Mating was commonly observed in the feeding cage, before, during, or more frequently, after a blood meal. For this

[4] Ketaset®, Veterinary Products, Bristol Laboratories, Syracuse, NY 13201.

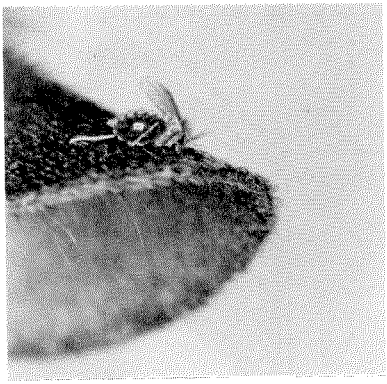


Fig. 5. A female *Lutzomyia anthophora* feeding on the ear of a woodrat, *Neotoma micropus*, in the laboratory.

reason, we allowed blood-fed females to remain in the feeding cage 12 to 24 hr after feeding to insure insemination. Then, each female was placed into a 7 dr vial that was kept in the styrofoam box with the other wild-caught gravid flies.

LABORATORY REARING. We maintained all *Lutzomyia* sand flies at 22–28°, 75–95% RH, and 14:10 LD photoperiod. The Kenyan sand flies were exposed to naturally occurring photoperiod (Beach et al. 1981). No containers or larval food were sterilized except occasionally for mite control (autoclaved for 5 min at 15 psi).

Following oviposition, the female flies were released once again into the feeding cage for refeeding. The screen lid of the 7 dr vial containing the eggs was replaced with a solid plastic lid that had been punctured several times with a fine needle. Additional tap water was added to the plaster of paris bottom, again insuring that no standing water remained. Most eggs were deposited on the plaster. These usually hatched within 10 days following oviposition, but staggered hatch, ranging from 14 to 40 days, was observed in some batches of *L. cruciata* (Coq.) from Florida, *L. diabolica*, (Hall) and *L. vexator*.

The 120 ml specimen containers were also used as rearing chambers. Fifteen to 20 engorged females and 8–12 conspecific males were kept together in each container capped with a screen lid.

Larval food of aged rabbit feces and laboratory rabbit chow (Young et al. 1981) and stored moist in a closed container, was lightly sprinkled on the surface of the plaster of paris anytime before egg hatch. We reared 1 to 50 larvae per 7 dr vial and up to 200 larvae per 120 ml specimen container, adding more food and water if needed. The larvae were examined once a week, or more often, if dictated by the objectives of the study. The developmental time from first instar through the fourth varied from 20–40 days.

Mature larvae pupated on the spent larval food and/or on the sides and top of the rearing container. When adults appeared, they were released into the feeding cage with apple slices or into another 120 ml specimen container with drops of sugar solution on the screen lid. The stock sugar solution (1:1 mixture of Karo syrup or honey and water) was kept in a glass dropper bottle and refrigerated at 10°C. New solution was made weekly.

Adult females took their first blood meal 1 to 6 days after eclosion depending on species. Adults kept in the feeding cage were offered hosts in the manner already outlined. For convenience, we exposed sand flies to their hosts during the daytime, the feeding cage darkened or not according to species preference. *Lutzomyia cayennensis hispaniolae* females fed only in light.

For those adult sand flies kept in the 120 ml specimen container, instead of the larger feeding cage, we simply pressed the screen lid to a rabbit's ear or hairless skin of another host. *Lutzomyia shannoni* (Dyar) and *L. diabolica* fed readily through the screen and also through a silicone membrane (Davis et al. 1982) that was substituted for the screen.

In one test, 2 out of 50 *L. shannoni* females became infected with cultured *Leishmania chagasi* after they fed or probed through the membrane placed di-

rectly on NNN culture medium heated to 37°C in a petri dish.

MODIFICATION AND MAINTENANCE OF REARING AND FEEDING CAGES. A. *7 dr polystyrene vial*[5] (Fig. 3). Construction grade plaster of paris, mixed with tap water, was poured into the bottom third of each vial and allowed to dry for 48 hr at room temperature. For making screen lids, we cut a circular hole, 2 cm diam in the solid lid with a cork borer. A circular piece of nylon screen (18 mesh/ cm), slightly larger than the hole was glued over the hole with Weldwood® contact cement or other non-water soluble glue. Lids were cleaned after use by soaking them in 5% Chlorox® solution for 20 min., then rinsing twice in tap water. The spent larval food in the vials was discarded and boiling water was poured into each for mite control before further use. Screen lids were used when adult flies were held inside; solid lids when immatures were being reared.

B. *120 ml specimen container*[6] (Fig. 2). These transparent plastic containers, normally used for urine sample containers in medical laboratories throughout the world, were modified for rearing immature sand flies and for holding adults. Thirty to 50 ml of plaster of paris, mixed with water, was poured into the bottom of each container. A circular hole 18 mm diam was made by pressing a hot cork borer on the side of the container above the plaster of paris. Two pieces of thin latex rubber, cut from a surgical glove, were glued with rubber cement over the opening; one on the inside, the other on the outside of the container. Perpendicular slits, "+ shaped," were then cut through both pieces of latex to provide a fly-proof opening for insertion of the aspirator. Some lids were modified by cutting a 35 mm dia hole in the top with a scalpel or scissors, filing the edges smooth, and covering with nylon screen affixed with contact cement. Solid lids were also kept on hand for rearing immature stages.

C. *Feeding cage* (Fig. 4). The bottom of a 31×17×21 cm glass aquarium was covered with a 1 cm layer of plaster of paris. After drying for 24 hr, the layer was saturated with water. The back side was then similarly covered with plaster. Additional plaster, up to 2.5 cm thick, was poured in the upper corners, cracks and crevices, only after the other layers had been again rewet. Thus, freshly mixed plaster was not allowed to contact dry, previously hardened plaster of paris because it prevented proper hardening and manipulation.

The front of the feeding cage, attached to the aquarium with silicone glue, was transparent 6 mm thick acrylic plastic (Plexiglas®). The rectangular openings on each side of the center opening were covered with nylon screen to provide ventilation. We used epoxy glue to attach the screen to the lateral openings as it did not weaken at high humidity. A cloth sleeve, measuring approximately 36 cm long × 15 cm wide, was secured to the front panel, over the middle opening, by compressing it between the panel and a removable Plexiglas frame using brass screws and wingnuts (Fig. 4).

The plaster of paris on the bottom and back of the feeding cage served as a resting site for the sand flies which were easily seen and captured against the white background. The plaster was kept dry in the feeding cage to reduce fungal growth and to absorb excess water. In nature, most sand flies that rest on tree trunks in humid forests are nearly always found on the driest parts.

A generalized scheme for rearing phlebotomines is presented in Fig. 6.

RESULTS AND DISCUSSION

Our techniques of rearing sand flies were based on the contributions of other investigators whose papers were cited by Sherlock and Sherlock (1972) and Killick-Kendrick (1978). Following the rationale of Eldridge et al. (1963), we at-

[5] Fisher Scientific Co., Pittsburgh, PA 15219.
[6] Pharmaseal Laboratories, Glendale, CA 91201.

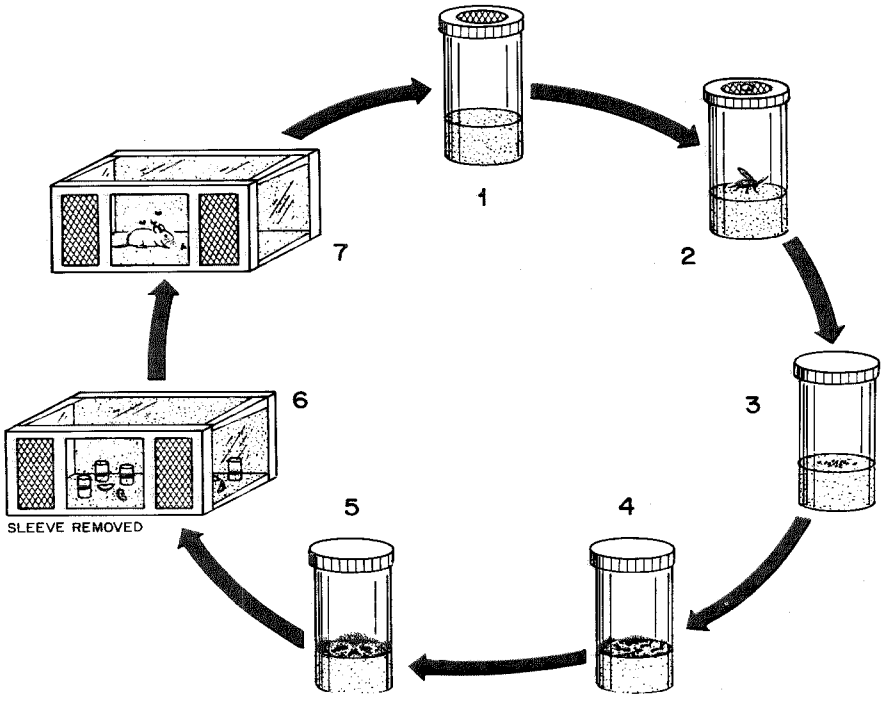


Fig. 6. Generalized steps for rearing sand flies. 1. Plaster of paris at bottom of rearing vial is moistened with tap water. 2. Wild-caught or lab-bred gravid females are placed individually in vials and a drop of sugar solution is placed on each screen lid. 3. A solid lid replaces the screen lid after eggs are deposited. 4. Larval food is sprinkled on the plaster of paris anytime before the eggs hatch. 5. Additional larval food is added as the larvae grow. 6. Lidless vials containing pupae are placed in the feeding cage; apple slices provide a sugar source for emerging adults. 7. For a bloodmeal source, an anesthetized vertebrate is placed in the feeding cage.

tempted to simplify procedures by using readily available, non-sterilized equipment and by eliminating unnecessary handling.

By using transparent rearing and feeding cages, we easily observed all stages of the phlebotomines' life cycles. We did not find it necessary to spread plaster of paris on the sides of the 7 dr vials or specimen containers because the majority of eggs were deposited on the bottom plaster surface. The adults rested there, on the plastic sides or on the nylon screen at the top.

Approximately 5% of the adult flies became stuck and subsequently died in the drops of sugar solution deposited on the screen lids. We have not yet solved this problem. Death of adult flies due to apparent fungal infections was rarely observed. The daily replacement of the Karo syrup—water solution seemed to prevent additional mortality due to pathogenic fungi. Unidentified mites

were commonly seen feeding on larval food and on dead sand flies of all stages but they did not attack living specimens. When reaching high numbers, however, the mites were controlled by treatment with boiling water or by autoclaving as mentioned earlier. Vinyl plastics containing N-butyl phthalate, an elasticizer, were not used because they are toxic to sand flies and other insects (Dr. David Carlson, personal communication).

We presently maintain up to 3000 sand flies per generation, a number adequate to meet current demands of time and research needs. The mount of time required to maintain each colony averages about 18 hours per week.

Approximately 30 larvae were reared on 2 gm of the larval food consisting of laboratory rabbit chow and rabbit feces (Young et al. 1981). The cost of an 11.7 kg (25 pound) bag of the chow is currently less than U.S. $4.00 and when mixed with an equal amount of rabbit feces, the number of sand flies that can be produced approaches 350,000. With further developments of equipment and systems, mass rearing of important vector species for use in biological and/or autocidal control may become economically feasible.

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