A NEW METHOD FOR REARING \textit{Lutzomyia Trapidoi} (DIPTERA: PSYCHODIDAE), WITH OBSERVATIONS ON ITS DEVELOPMENT AND BEHAVIOR IN THE LABORATORY$^1$

By Byron N. Chaniotis$^2$

Abstract: A new technique for rearing the Panamanian phlebotomine sand fly \textit{Lutzomyia trapidoi} (Fairchild & Hertig) is described. Experimental data are provided on sugar- and blood-feeding, mating, oviposition and culture of immature stages. As many as 70\% of females survived oviposition when maintained in styrofoam chambers and fed concentrated sucrose solutions.

\textit{Lutzomyia trapidoi} (Fairchild & Hertig) is widely distributed in Panama (Fairchild & Hertig 1952). It bites a wide variety of vertebrates, including man (Chaniotis et al. 1971, Tesh et al. 1972), and is known to be a vector of cutaneous leishmaniasis (Johnson et al. 1963), as well as a number of arboviruses (Tesh et al. 1974). Attempts to colonize this species have not been successful. Hertig & Johnson (1961) and Johnson & Hertig (1961) in a major effort to rear Panamanian sand flies succeeded

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$^2$Gorgas Memorial Institute of Tropical and Preventive Medicine, Middle America Research Unit, Box 2011, Balboa Heights, Canal Zone.
in rearing 24 of 33 species to the adult stage, but only *L. sanguinaria* (Fairchild & Hetvig) and *L. gomezi* (Nitzulescu) were maintained continuously for many generations. High larval mortality and inability to induce females to take a blood meal were given as reasons for failure to maintain *L. tarpidoi* beyond the 2nd generation.

Rearing and colonization of phlebotomine sand flies is generally a delicate and arduous operation. The principal factor that throttles efforts to establish viable colonies of Panamanian sand flies appears to be their narrow range of tolerance to physical conditions. This report presents a new rearing method and bionomical data from an attempt to establish *L. tarpidoi* in the laboratory. The colonization of this species, however, remains to be tested with this new method.

**EQUIPMENT**

*Rearing vessels:* An essential requirement for rearing immature stages of phlebotomine sand flies is a constantly moist substrate. Since 1922, when sand fly rearing was first initiated, a variety of techniques has been employed in the Old and New World. A comprehensive review of the evolution of methods and equipment used is provided by Hetvig & Johnson (1961).

Undoubtedly the most satisfactory and most commonly utilized rearing vessel has been a small, porous, unglazed clay pot coated internally with plaster of Paris. In my experience, clay pots have the following shortcomings: they are costly and not always readily available, their porosity is not rigidly standardized and deteriorates with each use, they require such labor-consuming practices as frequent sterilization and renewal of the interior plaster coat to maintain them in good condition, and clay is conducive to growth of molds with detrimental results to the immature stages. These considerations prompted me to seek an alternative rearing vessel.

After testing several types of containers, I found 2 which overcame most of the above limitations, and which proved particularly successful for rearing *L. tarpidoi*. First, a combination cage, oviposition chamber, and larval rearing container (Fig. 1) was constructed from an 0.24-liter (8-oz.) styrofoam cup and an inverted plastic tumbler*. A 5-mm diam. perforation was made in the bottom of the styrofoam cup and 50 cc of plaster of Paris mixed with 25 ml of water was poured in. After 30


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**FIG. 1.** Container used as an oviposition chamber as well as rearing and holding vessel for *L. tarpidoi*. The bottom and most of the interior of the styrofoam cup (A) is coated with plaster of Paris. The inverted plastic tumbler (B) is held in place by masking tape (C). The cork (D) seals a hole used for introducing flies into the container. A 5-mm diam. hole (E) at the bottom of the tumbler is covered with fine nylon mesh and is used for ventilation and sugar feeding.

seconds the cup was rotated in such a way as to coat the bottom and 60%-80% of the lower interior wall. The plastic tumbler was inverted over the cup to serve as the top of the cage. To allow ventilation and sugar-feeding, a hole (30-mm diam.) was made on the bottom of the tumbler and covered with fine stretched nylon mesh (carbon tetrachloride glues nylon to plastic permanently). One or 2 cork-stoppered small holes on the side of the tumbler provided a certain degree of flexibility in transferring sand flies in or out. The interior of the styrofoam cup was kept wet by placing the vessel directly in water or on wet sponges. When used to rear immature stages, the bottom of the cup was covered with a thin layer of decomposed leaf litter on which sand fly eggs were sprinkled and larvae developed subsequently.

A large number of cups can be coated in a short
time and, since the cost is relatively small, it is a useful vessel for large-scale experiments that require rearing the progeny of individual females. The vessel is disposable, thus eliminating the need for sterilization and plastic renewal.

The 2nd type of vessel, suited primarily for rearing the immature stages, was a 500-ml capacity plastic jar containing a bottom layer of fine sand covered with a 100-cc layer of peat moss or decomposed leaf litter (FIG. 2). A hole (30-mm diam.) was made in the plastic lid and stoppered with a screened cork to allow ventilation. This vessel requires minimum care to maintain the proper degree of moisture necessary to the immature stages. In several trials I found no need to add water to the substrate during the entire period of sand fly development lasting from 4 to 6 weeks.

Cages: Cages are indispensable for maintaining, feeding, mating, and releasing flies. Essentially, any medium-sized mosquito cage with fine screening is satisfactory. However, preference should be given to a cage which provides few hiding places and unobstructed visibility of its contents. A simple and convenient cage consists of 3 wooden or plywood panels for the back (35 cm high × 30 cm wide), and top and bottom (30 cm wide × 25 cm long). The front corners of the top and bottom panels are slightly rounded to fit a continuous sheet on nylon marquisette (35–40 meshes per linear cm) without need for vertical supports. A sleeve was attached to a hole (15-cm diam.) in the back panel to allow access into the cage. The interior surface of the panels was painted white to enhance visibility of resting flies. The cage was provided with 1 or 2 flasks (500-ml) of water with a wick of wet paper towel protruding above the rim of the flask to provide drinking water and a wet resting surface for the adult flies. Cages were kept in an insectary with temperature ranging from 23°–27°C and high humidity. Optimal humidity conditions have not been established, but experience dictated that relative humidity below 80% was associated with high adult mortality.

Suction tubes: Aspirators were made from Pasteur pipettes by cutting near the constriction point where the diameter is approximately 2.0–2.5 mm. The proximal end was screened with fine nylon mesh and inserted into a 40-cm long flexible rubber tubing. This aspirator, operated by mouth, was used to transfer flies from one vessel to another by light suction and blowing. This device eliminated the use of CO₂ gas anesthesia, a practice which caused fly mortality despite cautious administration.

Transport containers: Wild-caught sand flies are normally the primary source of flies for experimental rearing, and frequently they are essential for strengthening a declining laboratory colony. In addition, experimental studies of disease vectors often depend upon an abundant supply of wild-caught specimens. It is thus essential to have a safe and efficient method of transporting flies from the field to the laboratory, particularly in the tropics where the daytime heat is detrimental to the frail adults. In initial trials of small Barraud cages (Barraud 1929) placed in moist insulated containers, fly mortality was frequently high during the 4- to 5-hr trip by foot and vehicle from the field station to the laboratory. Mortality was substantially reduced by using coffee cans (13 cm diam. × 16 cm high) with snap-on plastic lids and the interior coated with plaster of Paris. These provided a rigid resting wall and optimum moisture for a prolonged period of time when properly soaked with water. A hole (30-mm diam.) in the lid allowed the use of a suction device for collecting flies directly from their resting sites into the can and provided ventilation during transport when stoppered with a screened cork. Even more satisfactory than tin cans are containers made of compressed paper, as they do not rust and are heat resistant. The cans may be protected from excessive heat during long trips by placing them inside insulated boxes.

SUGAR-FEEDING

Provision of sugar for adults is absolutely essential
to maintain sand flies under experimental conditions. Without sugar, laboratory-reared *L. trapidoi* did not survive more than 4 days, as compared to a maximum of 35 days when provided sucrose or fructose solution (Chaniotis 1974).

Perhaps the most interesting finding was that proper sugar-feeding combined with a suitable vessel promoted survival of ovipositing females. Failure of females to survive the 1st gonotrophic cycle has been a stumbling block in experimental transmission studies of leishmaniasis and arboviruses. I was able to improve post-oviposition survival by utilizing plastic-lined styrofoam cups as holding vessels, by maintaining relatively low temperature (23°-25°C), and by supplying the flies constantly with 30%-50% sucrose solution. In a typical experiment, flies were either provided with sucrose solution (Group I) or were denied it (Group II). Of 63 females in Group I, 44 (70%) laid their eggs and subsequently survived 2 to 7 days; none of the 25 flies in Group II survived more than 1 day post-oviposition.

Sugar-feeding was a prerequisite to initiation of blood-feeding in laboratory-reared flies. These flies refused to take their first blood meal from an animal host until after they had fed on sugar. For instance, newly-emerged *L. trapidoi* females kept at 23°-25°C in plastic-lined styrofoam cups were offered water in dental wicks and fresh sucking hamsters as long as they survived. Male flies were present with females in some tests. Of 41 flies tested in 7 separate tests, 40 failed to feed and died during a 4-day period.

**BLOOD-FEEDING**

*L. trapidoi* has not been reported to be autogenous. The 2 field populations I dealt with in the laboratory also did not exhibit autogeny. Even when larva were provided with a high protein diet (liver powder), and adult females fed on a mixture of sucrose and fetal calf serum, there was no ovarian development or oviposition. Blood meals from animal hosts were thus indispensable for maintaining this species in the laboratory.

In nature, *L. trapidoi* is known to feed on a broad spectrum of animals that belong to at least 7 mammalian orders (Tesh et al. 1972). In the laboratory it was found expedient to utilize sucking hamsters (3-5 days old). These small animals were easily restrained on tongue depressors with narrow strips of masking tape and remained acceptable to flies for at least 12 hr in this condition. Sucking rats were likewise acceptable, but sucking mice were less satisfactory.

Flies were fed in cages by introducing 1 or more restrained suckling animals or fed in small styrofoam cups with a single restrained host. As a general rule, most flies fed readily within 1 hr after host introduction. Some took longer to feed, but a few did not feed even when offered fresh hosts on several succeeding days. Laboratory-reared females began to probe proffered animal hosts and take a blood meal approximately 1 day post-emergence. The feeding rate increased from 8% (6/77) for 1-day-old females to 37% (27/73) and 73% (85/116) for 2-day-old and 3-day-old females, respectively. Thereafter, biting was erratic, indicating that prolonging the starvation period did not induce the flies to feed more readily. The maximum bloodfeeding rate attained was 82%.

As pointed out earlier, a sugar meal was necessary to elicit blood-feeding. However, the degree of satiation with sugar solution affected to some extent not only the initiation of biting, but the volume of blood ingested, as well. Females with highly distended abdomens due to excessive intake of sugar either did not feed for several days, or took only a small volume of blood. For this reason, females to be offered animal hosts were supplied sugar solutions by means of cotton balls rather than by means of drops on stretched nylon mesh, as the latter method permits the flies to ingest excessive sugar meals.

Temperature between 23°C and 29°C had no effect in inducing depleted wild-caught *L. trapidoi* to take a blood meal in the laboratory. Utilizing sucking hamsters or suckling rats as hosts, and styrofoam cups as holding vessels, the overall feeding rate in 20 separate tests (during a 3-hr host exposure period) was 46% both at 23°C-25°C and 29°C for 192 and 172 females, respectively. However, when the presence or absence of light was tested as a condition of blood-feeding, significantly more females fed in total darkness than in artificial light. Specifically, the feeding rate was 41% (40/98) in light vs 50% (49/98) in darkness at 23°-25°C, and 42% (37/88) in light vs 54% (40/74) in darkness at 29°C.

**MATING**

On many occasions I observed *L. trapidoi* mating in the laboratory in cages of various sizes, at temperatures from 23°C to 30°C, in bright natural and artificial light, and irrespective of the time of day. Johnson & Hertig (1961) did not indicate that mating was a cause for failing to rear this species beyond the 2nd generation. I found mating a vexing problem at times when small numbers of laboratory-reared adults were confined in small holding vessels,
such as styrofoam cups or clay rearing pots.

An attempt to define the optimal mating conditions for *L. trapidoid* in the laboratory was started but was not concluded. The problem was complex as well as time-consuming. Preliminary experiments provided no clear-cut indication as to the conditions which favor mating activity and egg fertilization. Mating did occur at 23°-25°C as well as at 27°-30°C, under constant light, constant darkness and dark-light photoperiod, with blood-fed and blood-starved females. Mating behavior was generally very erratic based on the criterion of egg fertility, although egg infertility does not always indicate lack of mating or even insemination. Replicate experiments with laboratory-reared flies and 1 to 1 sex ratio provided dissimilar results.

**OVIPOSITION**

The plaster-coated styrofoam cup with the inverted tumbler on top (Fig. 1) was the most satisfactory oviposition vessel. Blood-fed females were aspirated from the feeding cage and were blown lightly into the cup through a hole in the tumbler. As many as 50 females could be introduced into 1 cup. Eggs were invariably laid on the wet plaster coating. From the oviposition vessel eggs were washed into a small beaker (100-ml) with water and a fine camel-hair brush. A small amount of a commercial dishwashing detergent (1 drop/50 ml water) added to the beaker helped in sinking floating eggs. A large syringe was found useful in removing the detergent water without disturbing the eggs on the bottom of the beaker. Eggs were washed 2 to 3 times and then transferred with a Pasteur pipette onto the organic substrate in the rearing vessel.

Fecundity data obtained from wild-caught and laboratory-reared females fed on suckling hamsters showed parallel results. The mean numbers of eggs laid by 329 wild-caught and 62 laboratory-reared flies were 24.2 and 25.3, respectively. The maximum number of eggs laid by 1 female was 69.

Provision of sugar to blood-fed females resulted in prolonging the gestation period and improving fecundity. For instance, the maximum gestation period at 23°-25°C was 4 days without sugar and 9 days with sugar. The mean numbers of eggs laid by 59 sugar-deprived and 53 sugar-provided females were 20.8 (1-44) and 27.4 (1-53), respectively. The mean difference is statistically significant (*t* = 2.41 with 110 d.f.; expected *t* values are 1.98 and 2.61 for 5% and 1% level of probability, respectively).

**IMMATURE STAGES**

Development of these stages was accomplished best in Nalgene jars containing sand and organic detritus (Fig. 2). Eggs were transferred to these vessels from the oviposition cups and sprinkled directly on the organic substrate. Survival of the highly susceptible 1st-instar larvae was thus aided by their proximity to proper diet upon eclosion. Fungus growth, common in plaster-lined vessels, was contained by natural bacterial action, and mortality of eggs and young larvae was minimal. With the appearances of the 2nd-instar larvae, liver powder (Gemetchu 1971) was sprinkled in small amounts every 2nd or 3rd day till pupation occurred. Liver powder proved to be superior to boiled lettuce, as it accelerated and synchronized development of larvae.

Each jar could easily sustain development of 200-300 larvae. The number of eggs introduced depended upon a general knowledge of their fertility rate. In most instances, fertility was over 60% for eggs obtained from wild-caught females. Fertility of eggs from laboratory-reared females was quite variable depending on sex ratio in each instance and conditions of mating.

Development of immature stages occurred at temperatures from 23°C to 30°C. However, the higher temperatures in this range are preferable, as they shorten the length of development and result in a relatively synchronous adult emergence. The most obvious conditions which affected the length of life cycle were temperature and larval diet. With peat moss or decomposed leaf litter as organic substrate, and liver powder as larval diet, the minimum time between egg deposition and earliest adult emergence was 30 days at 29°C, 32 days at 27°C, and 45 days at 23°-25°C. With boiled lettuce as larval diet, the earliest adult emergence occurred 37 days after egg deposition at 29°C, and 50 days at 23°-25°C.

**DISCUSSION**

The principal aim of this report is to introduce a new approach in rearing *L. trapidoid*, an important anthropophilic species which has not done well in laboratory culture using conventional methods. More work is essential not only to improve rearing but also to test the feasibility of establishing viable, long-lasting colonies. Emphasis should be placed on improving egg fertility and defining the optimum range of physical conditions (e.g., temperature, humidity, light) that promote maximum survival of the adult stage. It is suggested that temperature requirements for adults and for immature stages may be different. Relatively high temperature (27°-30°C) enhanced the speed and synchronized
development of immature stages, whereas relatively low temperature (21°–25°C) promoted survival of adults without adverse effects on blood-feeding, mating or oviposition. However, it is not known whether rapid development of immature stages gives rise to adults with smaller than normal size and with behavioral characteristics unfavorable to colonization.

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LITERATURE CITED


