

Colonization of *Lutzomyia trinidadensis* and *L. vespertilionis* (Diptera: Psychodidae)¹

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ABSTRACT

The laboratory colonization of 2 species of New World phlebotomine sand flies, *Lutzomyia trinidadensis* (Newstead) and *L. vespertilionis* (Fairchild & Hertig) was accomplished through modifications of standard rearing techniques used at Gorgas Memorial Laboratory, Panama. The development of a new adult sand fly feeding

and maintenance cage contributed to consistent host-feedings by females facilitating subsequent colonization. The colony of *L. trinidadensis* was terminated after 6 generations. The colony of *L. vespertilionis*, presently in its 6th generation, is being maintained.

Approximately 15% of the more than 250 described species of New World phlebotomine sand flies have been reared from immature stages to adults. Coloni-

zation of these flies, however, has been limited to only a few species. *Lutzomyia sanguinaria* (Fairchild & Hertig) and *L. gomezi* (Nitzulescu) colonies, initiated by Hertig and Johnson (1961) in January and February 1959 at Gorgas Memorial Laboratory in Panama, are presently in their 56th and

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64th respective generations. Unpublished records, kept by this research organization, also document the colonization of *L. longipalpis* (Lutz & Neiva) by these workers. This colony was subsequently maintained through 21 successive generations by staff members within the Department of Leishmaniasis. Chaniotis (1967) reared *L. vexator occidentis* (Fairchild & Hertig) through 7 generations in California.

The rarity of phlebotomine colonies attests to the difficulties encountered in their establishment. Factors which preclude colonization of some *Lutzomyia* species are obscure. Partial successes with other species, however, should encourage workers to concentrate their efforts in discerning the limiting factors.

This report deals with the successful colonization of 2 additional species, *L. trinidadensis* (Newstead) and *L. vespertilionis* (Fairchild & Hertig), through the modification of methods used for many years at Gorgas Memorial Laboratory.

MATERIALS AND METHODS

The rearing vessels, oviposition vials, larval food and handling techniques used are essentially those devised by Hertig and Johnson (1961). The non-glazed larval rearing pots are seated on cotton pads within plastic petri dish halves placed in large shallow pans containing tap water to a depth of 1 cm. The surrounding lip of each petri dish is perforated with 4 equidistant holes to allow continuous wetting of the cotton pads, since larvae of both species were found to benefit from a very moist environment. The water in the pans serves as a barrier to extraneous insects, and as a source of moisture for the rearing vessels.

A cage (Fig. 1), modified from a plastic cake cover, was devised for the feeding and maintenance of adults. The cage is 15 cm high and has a base diameter of 30 cm. An entrance 10×14 cm and 2 vents, each 3×5 cm, were cut out of the circular cage wall. An additional vent 7×7 cm was made in the center of the top of the cage. All vents were screened with fine-mesh nylon. The inside wall was etched lightly by swabbing with acetone. Galvanized wire mesh was bolted 1 cm from the inside wall. Rubber tubing ¼ in. diam was laid around the inside cover between the wire and wall near the cage top. The ends of the tubing emerged through 2 holes in the cover top near the cage entrance. Small holes were made an inch apart along the length of tubing inside the cage before applying a 2-cm-thick layer of plaster of paris to the inside wall. A muslin sleeve was glued to the cage entrance. The cover was then seated tightly on its base with modeling clay. Water was poured into the perforated rubber tubing once or twice each week, wetting the inside wall and thus providing optimal humidity conditions for adult sand fly survival. Raisins were placed on the nylon screened top vent to provide a sugar source for adults.

Forest geckos, *Thecadactylus rapicaudus*, and several species of bats were used as host animals for

L. trinidadensis and *L. vespertilionis*, respectively. The geckos were collected by hand or noose from tree buttresses, old buildings, and caves. They were maintained in the laboratory on a diet of meal worms and mosquitoes. The bats were captured in caves using hand nets, and from forested areas with mist nets. The fruit-eating species were maintained in small rodent cages on a banana diet.

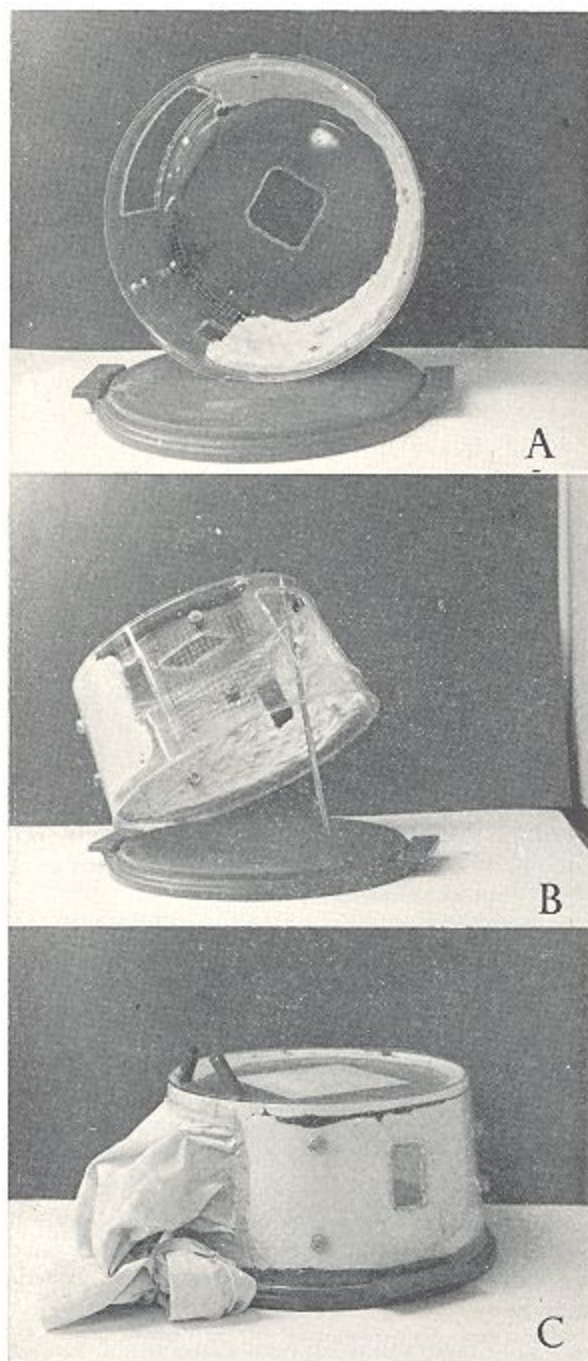


FIG. 1.—Adult sand fly feeding and maintenance cage. A, interior and B, exterior views during construction; C, appearance after completion.

RESULTS

Colony Initiation.—Large numbers of blood-engorged or gravid *L. trinidadensis* were collected from tree buttresses at the Chagres Boy Scout Camp in Madden Forest, Canal Zone, during a 2-month period in 1969. Individual females were maintained until oviposition occurred in shell vials lined with plaster of paris and fitted with a nylon top. Fresh raisins were placed on the nylon top and changed every other day. Eggs from vials containing *L. trinidadensis* were removed by rinsing with distilled water. Lots of 100 to 200 eggs were transferred to individual rearing pots and inspected every other day. Larval food was added as needed and excessive fungal mycelia were removed with a fine forceps.

The colony of *L. vespertilionis* originated from the progeny of the blood-engorged females collected in a large cave on the continental divide about 3 km north of Buena Vista in central Panama. Collections made in November, 1970, provided sufficient material to initiate and sustain the colony. The procedures for handling the wild-caught females for subsequent colonization were identical to those outlined for *L. trinidadensis*.

Immature Stages.—Ambient temperature in the insectary ranged from 21 to 28°C. Developmental rates of immature stages of both species are shown in Table 1.

Adult Feeding and Maintenance.—*L. trinidadensis* refused to feed on man, new-born mice, guinea pigs, sloths, or bats. The forest gecko, *T. rapicaudus*, proved to be an acceptable laboratory host. Adult flies which emerged in the rearing pots were released inside the cake-cover cage. A wild-caught *T. rapicaudus* was transferred to the colony cage and allowed complete freedom of movement. The cages were then stored in a dark cabinet overnight. The gecko was removed the following morning and all blood-engorged flies were collected in individual oviposition vials. The resulting eggs were pooled into lots of 100 to 200 and transferred to rearing vessels.

The only exception to these procedures for the handling of *L. vespertilionis* concerned the host. This

sand fly species refused to feed on new-born mice, guinea pigs, or sloths. Only 2 of several hundred females fed on man after repeated exposures in the laboratory. A variety of bat species, including *Saccopteryx bilineata*, *Artibeus lituratus*, *A. jamaicensis*, *Carollia perspicillata*, *Phyllostomus discolor*, and *Glossophaga soricina*, were found to be acceptable hosts for *L. vespertilionis*. Bats released inside the maintenance cage would cling to the plaster of paris wall in an inverted position and were fed upon readily by the flies.

DISCUSSION AND CONCLUSION

Johnson and Hertig (1961) reported that the number of eggs laid by wild-caught individual females, of *L. trinidadensis* ranged from 39 to 100, \bar{x} 73, (12 ♀ observed); the shortest developmental time from eggs to adults required 38 days (2 observations). These workers also noted that this species feeds on geckos in nature. McConnell and Correa (1964) observed fresh nonnucleated red blood cells in the blood meal of one wild-caught *L. trinidadensis*, indicating that this specimen had fed upon a mammal.

During the early stages of colonization the gecko hosts frequently fed on the sand flies resting on the cage wall. Blood-engorged females, which were somewhat reluctant to fly when approached by geckos, were especially vulnerable. A small nylon hood, fastened by a draw string over the gecko's head, prevented further predation of the flies. Concerning wild-caught *L. vespertilionis*, Johnson and Hertig (1961) stated, "Number of eggs laid by individual females: 13 to 64; average for 21 females: 38. Total days oviposition to adult: 45 (one observation)." Hanson (1968)² discussed the wild-caught immature stages of many Panamanian species, including *L. trinidadensis* and *L. vespertilionis*.

The majority of colony adults of both species, dissected after feeding and oviposition during concurrent trypanosome transmission studies, retained a portion of their eggs. Since egg retention occurs infrequently among wild-caught flies, oviposition is more efficient in nature and egg production undoubtedly is greater than that reported in Table 1.

McConnell and Correa (1964) tried unsuccessfully to feed *L. vespertilionis* on 3 species of bats, a guinea pig, an opossum, and a spiny rat. Johnson and Hertig (1961) noted the vagarious nature of adult female sand flies given the opportunity to feed on hosts in cloth cages, and discussed some possible explanations for this phenomenon.

Both *L. trinidadensis* and *L. vespertilionis* produce active and robust larvae which develop well under present laboratory rearing conditions. The principal factor underlying the failure to colonize both species previously involved the refusal of the stenophagous adult females to take blood meals.

The cake-cover cage was designed to provide high humidity conditions essential to adult sand fly sur-

Table 1.—Oviposition and development of immature stages of *L. trinidadensis* and *L. vespertilionis*.

	<i>L. trinidadensis</i>	<i>L. vespertilionis</i>
No. cultures ^a	31	38
No. ♀♀	114	202
No. eggs	3,083	4,241
No. eggs/♀♀	2-80; \bar{x} 27.0	1-70; \bar{x} 21.0
Days development time		
Eggs	8-16; \bar{x} 12.2	9-16; \bar{x} 12.7
1st instars	4-13; \bar{x} 6.9	3-10; \bar{x} 5.6
2nd instars	3-11; \bar{x} 6.0	3-9; \bar{x} 5.8
3rd instars	4-8; \bar{x} 5.7	4-9; \bar{x} 6.1
4th instars	7-19; \bar{x} 10.2	5-11; \bar{x} 8.3
Pupae	7-29; \bar{x} 13.8	6-18; \bar{x} 11.3
Oviposition to adults	42-75; \bar{x} 64.5	41-60; \bar{x} 47.7

^a Individual rearing vessels inoculated with 100-200 eggs.

² W. J. Hanson, 1968. The immature stages of the subfamily Phlebotominae in Panama (Diptera: Psychodidae). Ph.D. thesis, Univ. of Kansas, 104 p.

vival. Its construction allows flies protracted access to unrestrained hosts in the same stable environment. Most adult females, 1 day old or more, feed within 12 to 24 hr. Similar exposure times in cloth cages would result in desiccation of flies.

The colony of *L. trinidadensis* was terminated after 6 generations because of difficulties encountered in the procurement and maintenance of its only known host, *T. rapicaudus*. *L. vesperilionis*, also in its 6th generation, has been in colony for almost 1 year, and will be maintained in the insectary.

The cake-cover cage will be used in future attempts to colonize other species of New World Phlebotominae. Particular emphasis will be given to anthropophilic species incriminated as vectors of leishmaniasis in the Neotropical Region.

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