LABORATORY BIONOMICS, COLONIZATION, AND MORPHOLOGY OF THE IMMATURE STAGES OF THE SANDFLY LUTZOMYIA PANAMENSIS (SHANNON) (DIPTERA:PSYCHODIDAE)

Dissertation for the Degree of Ph. D.
MICHIGAN STATE UNIVERSITY
JOHN HARVEY ZIMMERMAN
1975

This is to certify that the

thesis entitled

Laboratory Bionomics, Colonization, and Morphology of the Immature Stages of the Sandfly Lutzomyia panamensis (Shannon) (Diptera: Psychodidae)

presented by

John H. Zimmerman

has been accepted towards fulfillment of the requirements for

Ph.D. degree in Entomology

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Date June 16, 1975

O-7639

ABSTRACT

LABORATORY BIONOMICS, COLONIZATION, AND MORPHOLOGY OF THE IMMATURE STAGES OF THE SANDFLY LUTZOMYIA PANAMENSIS (SHANNON) (DIPTERA:PSYCHODIDAE)

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The laboratory bionomics, colonization, and morphology of the immature stages of the anthropophilic phlebotomine sandfly Lutzomyia panamensis (Shannon) were studied. Wild-caught blood-engorged females were captured from a horse near Achiote, Canal Zone, Panama, from March 1973 to January 1974.

In an experiment to evaluate the feeding preference of L. panamensis larvae, four experimental food sources (yeast, liver powder, hemoglobin and beef blood serum) were placed on the same plaster lined petri dish. The first three instars preferred yeast to the other food sources. The fourth instar preference for yeast declined with an increased preference for the other food sources, especially hemoglobin. The four food sources were also compared individually on petri dishes to determine the effect of these single diets on the duration of the life cycle, pupation, and adult emergence. The yeast and standard larval food

combination dishes produced the shortest developmental time from the first instar to pupa, with yeast, liver powder, beef blood serum, and hemoglobin, respectively, increasing developmental time. When the developmental times of the larvae reared on single food sources were compared with those of larvae reared on the combination dishes, the combination produced faster and more desirable development. Larvae reared on the combination dishes survived to produce more pupae and adults than those reared on individual diets. The plaster lined petri dish was very satisfactory as a rearing chamber and general observations of larval behavior could be made with ease.

The effects of various saturated sugar solutions on the longevity of laboratory reared females and males of L. panamesis were examined. Fructose and sucrose were the most efficient in prolonging the survival of the females. Fructose, sucrose, honey, Karo syrup and dextrose appeared to provide the same male life expectancy. The styrofoam test vessel was adequate in studying the longevity of these males and females.

Longevity studies also conducted on the wild-caught blood-engorged females indicated that the saturated solutions of fructose, sucrose, and dextrose were the most efficient in promoting longevity. In an analysis of the effects of these saturated sugar solutions on oviposition,

no difference could be detected between the soaked raisin, fructose, sucrose, and dextrose groups.

Four laboratory generations of L. panamensis were reared with 37.7 days being the average length of the life cycle from oviposition to adult. Larval and adult behavior were described including pupation, emergence and mating. The unglazed Boston bean pot was the most satisfactory for rearing L. panamensis in large numbers. The mixture of liver powder, hemoglobin, and beef blood serum added during the third instar to the already present yeast in the pots was shown to be the best larval medium tested. The spiny rat was used successfully as the laboratory host. The double feeding method of using the cloth releasing cage and the plastic feeding cage was the most successful in feeding the largest number of laboratory reared adults with little injury to the females.

The morphology of the immature stages of L. panamensis was redescribed and clarified. The dorsal and ventral aspects of the first and fourth instars and the lateral aspect of the pupa were illustrated. Measurements of the prominent setae were very useful in differentiating changes between instars and other closely related species. Scanning electron photographs of the fourth instar served as an aid in describing the nature of the cuticular surface. The scanning electron microscope was used to describe the

John Harvey Zimmerman

egg surface ultrastructure of L. panamensis and other anthropophilic species L. pessoana, L. gomezi, L. sanguinaria, L. trapidoi, and L. ylephiletor.

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By

John Harvey Zimmerman

A DISSERTATION

Submitted to
Michigan State University
in partial fulfillment of the requirements
for the degree of

DOCTOR OF PHILOSOPHY

Department of Entomology

1975

To Mary

ACKNOWLEDGMENTS

I would like to extend my sincere appreciation to my advisor Dr. Harold D. Newson for his guidance and moral support throughout my Ph.D. program.

I am indebted to Drs. James E. Bath, Roland L. Fischer, and Herbert W. Cox for serving on my committee and for continued assistance during this project.

Special gratitude is extended to Dr. John M. Hunter of the Latin American Studies Center for providing the financial support during my stay in Panama. Also, a special thanks goes to Drs. Gordon E. Guyer and James E. Bath for their continued financial assistance during the remainder of my program.

A special thanks goes to Drs. Howard A. Christensen and Arístedes Herrer of the Leishmaniasis Department, Gorgas Memorial Laboratory, Republic of Panama for their guidance and technical assistance, use of laboratory facilities and equipment, and logistical support during my stay in Panama. I am especially grateful to Dr. Martin D. Young, the director of the Laboratory, for his continued support and encouragement. Special recognition goes to the following technicians of the Leishmaniasis Department: Angélica Ceballos, Anita Velasquez, Millie Vieto, Belinda de Merel

Choy, Marta Chacón, Aurelio Powers, Roberto Rojas, Samuel Scott, and Enrique London for their friendship and assistance while in Panama.

A very special thank you is extended to Enrique Van Horn who accompanied me on my jungle sandfly collecting expeditions. His enthusiastic willingness to work with me and to share his knowledge of sandflies and the jungle, was greatly appreciated. My stay in Panama was enriched for having known him.

Thanks goes to Dr. Byron Chaniotis and Major Louis Rutledge (U.S. Army, Ret.) for their support and assistance early in my sandfly research efforts.

Two other individuals deserve recognition for their assistance during this project: Dr. Gary Hooper for his guidance in scanning electron microscopic techniques and Mrs. Natalie Knobloch for her instruction in drawing techniques for my illustrations.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

INTRODUCTION

At present 68 phlebotomine sandfly species are known to occur in Panama, of which Lutzomyia panamensis (Shannon), L. gomezi Nitzulescu, L. ylephiletor Fairchild and Hertig, L. sanguinaria Fairchild and Hertig, L. trapidoi Fairchild and Hertig, L. pessoana Barretto and L. olmeca-bicolor Fairchild and Hertig are the most abundant anthropophilic species. Experimental studies on these species have been limited primarily to attempts at laboratory rearing. Of these species only L. gomezi and L. sanguinaria were maintained in colonies at the Gorgas Memorial Laboratory (GML), Republic of Panama, when the present study was initiated in March 1973. Studies by Chaniotis (1974) on L. trapidoi dealt primarily with the sugar feeding behavior of this species under experimental conditions and were a significant contribution to the very limited knowledge of the behavior and bionomics of sandflies.

The purpose of this study was to contribute to the existing information of the man-biting species Lutzomyia panamensis by laboratory investigations of its biology and external morphology of its immature stages. This sandfly is an important species in Panama and other areas of Central

and South America. Leishmania braziliensis s. lat., the causative agent of cutaneous leishmaniasis, has been isolated from naturally infected L. panamensis in Panama (Christensen et al., 1969).

L. panamensis has been reared in the laboratory by Mirsa (1952) and Pifano et al. (1960) through one generation. Efforts to colonize this species by Hertig and Johnson (1961) were hampered by the sandflies' reluctance to feed on a laboratory host (man and spiny rat), thus preventing colonization beyond the second generation. The description of the external morphology of L. panamensis immature stages has been limited to the fourth instar and brief statements about the first instar and pupa (Hanson, 1968). The egg stage of L. panamensis and other man-biting species has not been adequately described. The scanning electron microscope (SEM) has been used successfully to differentiate the egg surface ultrastructure of mosquitoes but has not previously been used to compare the surface structure of sandfly eggs.

The specific objectives of this study were to determine: (1) the biological parameters needed for successfully rearing and colonizing L. panamensis; (2) the effects of various diets on the longevity and egg production of wild-caught blood-engorged females and laboratory reared males and females of this species; and (3) the times during the female life cycle when various sugar diets were imbibed;

and (4) the effects of different feeding regimens on larval growth. In addition, the external morphology of the larval and pupal stages were redescribed and clarified and the egg surface ultrastructure of L. panamensis and other anthropophilic sandflies including L. pessoana, L. trapidoi, L. ylephiletor, L. sanguinaria, and L. gomezi were studied by means of the SEM.

LITERATURE REVIEW

Taxonomic Status

Phlebotomus panamensis was first described in 1926 by Shannon from specimens he collected in Cano Saddle, Canal Zone, Panama. At that time there were no other published records of *Phlebotomus* from Panama. He described both the male and female terminalia in detail. Dyar (1929) could not find the subdivision in the middle (upper) claspers of the male terminalia as pictured in Shannon's figures and created a new subgenus, *Shannonomyia*, thinking it was a peculiar species that required a separate subgenus. This was later changed to *Shannonomyina* by Pratt (1947) because the subgenus *Shannonomyia* had already been proposed for a genus of Tipulidae. Root (1934), after examining both Dyar's and Shannon's specimens, disagreed with Dyar and had no doubt that Shannon's figure and description of the middle (upper) clasper were correct. Barretto (1946) examined the type specimens of *P. panamensis* and described additional details of the median (upper) claspers. Ortiz (1950) described the setae on the parameres from specimens collected in Venezuela and he compared his drawings with those drawn by Stone in Barretto's paper.

The most complete and accurate set of figures and description of P. panamensis were made by Fairchild and Hertig (1951) and are as follows (Figures 1-9):

> A medium sized sandfly with markedly infuscated mesonotum and the abdomen clothed with flat white recumbent scales. The male genitalia have not previously been adequately figured, the original sketch by Shannon being quite misleading. Stone's sketch in Barretto (1946) shows the proportions, but is admittedly diagrammatic and does not indicate the broad blade-like character of the setae on the parameres, ascoids simple, not quite reaching the ends of their respective segments, paired on all segments, but absent from the terminal three segments in the female, the terminal six in the male. Terminal three segments in both sexes noticeably shortened, pearshaped. Newstad's scales scattered sparsely over the distal two-thirds of palpal segment III. Eyes rather large in both sexes. Palpi as figured, those of the male about one-third shorter than the female. Stem of genital fork rather slender and pointed in dorsal view. Gonapophyses of the eighth sternite short and slender. Spermathecae as figured, the common duct mostly very thin-walled and difficult to see. Annulations of spermathecae variable in number, 10 to 11 in our material, though others show up to 13, the terminal annulation markedly asymmetrical. Cerci rather slender. Cibarium as figured. Pharynx fairly broad and well sclerotized, its posterior end provided with numerous fine transverse wrinkles, apparently beset with minute spinules. Venation as figured, the veins and margin bearing numerous fine long hairs but no scales, though the hairs at the extreme base of the costa are somewhat lorate.

While describing the Panamanian sandflies, Hertig and Fairchild (1950) found a new character (the second sternite) that was useful in the taxonomy of Phlebotomus (Figure 9). Its size and proportions were very similar in both sexes.

Figure 1. P. panamensis, pump and genital filaments.

Figure 2. Same, male genitalia, inner aspect; the two long hairs on the coxite are apparently the same as the deciduous hairs on the outer surface, but one or two commonly persist in this position in our series of specimens.

Figure 3. Same, spermathecae, ducts and genital fork, dorsal aspect; note asymmetrical terminal annulation, terminal knob bent to one side.

Figure 4, 5. Same, female cibarium entire, showing armature, chitinous arch, pigment patch, salivary pump; detail of armature at higher magnification. The central rows or patch of very heavy erect teeth are characteristic of most of the group; two different females.

Figure 6. Same, head, female.

Figure 7. Same, antennal segments II-IV and palps, male and female; antennal segment IV with ascoids enlarged.

Figure 8. Same, wing, male, costal portion.

Figure 9. Same, sternites I and II, female.

Fairchild (1955) attempted to subdivide the New World Phlebotomus into subgenera as Theodor (1948) had proposed for the Old World species but was hindered in his classification because less than half of the described species were known in both sexes. He placed P. panamensis in the subgenus Psychodopygus Mangabeira (=Shannonomyia Dyar, Shannonomyina Pratt), group Panamensis (=Shannonomyina Fairchild and Hertig, 1951). Barretto (1955) revised the systematics of the subfamily Phlebotominae (Rondani) and placed P. panamensis in the genus Sergentomyia, an Old World genus. His paper had many errors and contained no new characters. Theodor (1965) proposed a new classification of American Phlebotominae because the evolution of these phlebotomines has apparently followed different lines from those in the Old World. Barretto (1962) elevated the subgenus Lutzomyia Franca to generic status which included most of the New World sandflies. In this classification Lutzomyia panamensis was retained in the subgenus Psychodopygus where it remains today. Forattini (1971) elevated Psychodopygus to generic status, but as of this writing his classification has not been generally accepted.

Morphology of the Immature Stages

Morphological studies of the immature stages of New World phlebotomine sandflies have been undertaken by only

a few investigators, largely because they are difficult to find in nature and to rear in the laboratory. Barretto (1940, 1941) described the immature stages of ten neotropical species and established the taxonomic nomenclature of the larvae and pupae. Other scientists have since described the immature stages: Mangabeira (1942a-e), Addis (1945), Sherlock (1957a,b), Mangabeira and Sherlock (1962), Sherlock and Carneiro (1963), Carneiro and Sherlock (1964), and Guitton and Sherlock (1969). Hanson's (1968) dissertation described the immature stages of the subfamily Phlebotominae of Panama, primarily the fourth instars and pupae of 32 species including L. panamensis. Ward (1972) drew and described the four larval and pupal stages of Psychodopygus wellcomei Fraiha, Shaw, and Lainson and was the first to include comparative measurements of the setae. He did not mention Hanson's work and apparently was not aware of its existence.

Geographical Distribution

Lutzomyia panamensis has a wide geographical distribution: Mexico through Central America and South America (Venezuela, Colombia, Peru, and Brazil). Barretto (1947, 1950) in his critical analysis of the known sandfly literature up to 1950 reported L. panamensis from Colombia, Panama, Peru, Venezuela, and the Canal Zone. Pifano and

Ortiz (1952) and Iriarte (1952) listed the Venezuelan states in which L. panamensis had been collected.

Vargas and Nájera (1953) reported L. panamensis from three localities in Mexico. Fairchild and Hertig (1959, 1960) stated that this sandfly occurred in Mexico, Costa Rica, Nicaragua, British Honduras (Belize), and Panama. Lewis and Garnham (1959) collected L. panamensis in several localities in British Honduras. Nájera's (1963) studies extended the range of this sandfly in Mexico to Veracruz. De Biagi (1966) and de Biagi et al. (1966) found this species in Quintana Roo, Mexico. Martins et al. (1963) collected L. panamensis for the first time in Brazil in an area close to Venezuela. The geographic distribution of this phlebotomine in Colombia was discussed in articles by Osorno-Mesa et al. (1967), Barretto (1969), and Morales et al. (1969).

Disease Relationships

Phlebotomine sandflies comprise an important group of hematophagus Diptera because they can transmit certain bacterial, viral, and protozoal disease agents. In the New World, sandflies have been incriminated as vectors of bartonellosis, various viral diseases, and forms of leishmaniasis. Pifano and Ortiz (1952), in their epidemiological investigations of tegumentary leishmaniasis in

Venezuela, suspected that L. panamensis was a vector of Leishmania braziliensis Vianna. Five of the 72 specimens they captured while feeding on the border of leishmania lesions of man revealed leptomonad forms which were very similar to cultured L. braziliensis. In 1959 Pifano et al. continued their investigations in northern Venezuela and demonstrated the transmission of tegumentary leishmaniasis to a human by the bite of L. panamensis.

Using sandflies found in nature and infected experimentally in the laboratory, several groups have attempted to demonstrate flagellate forms of L. braziliensis in the digestive tract of L. panamensis. The time from feeding to the detection of flagellate forms was 8-20 days. Pifano et al. (1960) dissected 75 specimens of L. panamensis collected in the Yaracuy Valley, Venezuela, during an outbreak of cutaneous leishmaniasis and found three sandflies infected with leishmanial and leptomonad forms morphologically indistinguishable from the cultured L. braziliensis. Johnson et al. (1962, 1963) in studies of natural infections in Panama found leptomonad infections in 11 out of 579 specimens of L. panamensis dissected. These were confined to the hind-gut, with the hind triangle usually the only area infected. Biagi et al. (1965) reported Lutzomyia flaviscutellata (Mangabeira) naturally infected with leptomonad flagellates but found no infections in dissected specimens of L. panamensis.

Christensen et al. (1969) found that four out of 306 dissected L. panamensis from several localities in Panama had flagellate infections. One out of four infections was, by its morphology and behavior in vitro, indistinguishable from those parasites responsible for human cutaneous leishmaniasis in Panama. This strain was considered to be Leishmania braziliensis s. lat. and was maintained in laboratory culture and in the golden hamster.

As mentioned previously, phlebotomines are of increasing importance in the transmission of various viral agents. Chaniotis et al. (1974) reported an isolation of virus of the Phlebotomus Fever group from L. panamensis. Sabin et al. (1944) and Velasco (1973) have reviewed the existing literature on sandfly transmitted viruses with special attention given to sandfly fever virus, which does not occur in Panama.

Bionomics--Adult

Vertical Distribution

In British Honduras, Williams (1970a) studied the vertical distribution of phlebotomine sandflies and found that L. panamensis was the only common man-biting species having greatest biting activity at ground level (0-3 m). Only a few specimens of this species were caught at 25 feet and even fewer at 40 feet. The specimens at these levels

were collected only when feeding also occurred at ground level. These findings confirmed those of Disney's (1968) in which L. panamensis appeared to search for blood meals near the ground, though an occasional specimen took its blood meal above the ground. Resting flies were taken by Disney during the day in greatest numbers near the ground. In light trap collections, L. panamensis was the most numerous species at 25 and 40 feet with very few flies captured at ground level. Williams (1970b) postulated that between blood meals females remained close to their preferred resting places high above ground in tree foliage and the physiologically hungry females descended to the forest floor for a blood meal and then remained in close contact with the breeding site for oviposition.

Studies were also conducted in Panama by Chaniotis, Neely et al. (1971) and Chaniotis, Correa et al. (1971). Their light trap collections showed that L. panamensis was the second most numerous sandfly captured at the ground level and in the canopy (28 meters). Daytime collections from the tree trunks rarely yielded L. panamensis. A total of 420 specimens were captured biting man of which 90.7% were taken at ground level. Christensen et al. (1972) reported that 18 females of L. panamensis were collected by a light trap at ground level and 125 females at 35 feet.

Seasonal Distribution

The seasonal distribution of sandfly populations was studied by Chaniotis, Neely et al. (1971) in Panama to discern some of the factors important in the complex interplay between the biotic potential of various sandfly species and the "environmental resistance," consisting of a number of physical and biotic variables. Their data suggested that the amount and distribution of rainfall might affect the sandfly density by transforming the breeding conditions on the ground. For their study they divided the year into three climatological periods. L. panamensis showed sharp density peaks during the early wet season (May to August) with decreasing abundance during the late wet season (September to December). Christensen et al. (1972) indicated that L. panamensis was a wet season species having the highest densities in June and September. Fairchild and Hertig (1951), Hertig et al. (1968), Biagi and de Biagi (1953), and Disney (1968) stated that L. panamensis was a rainy season species.

Diel Periodicity and Biting Behavior

Chaniotis, Correa et al. (1971) determined the daily man-biting activity of anthropophilic sandflies. L. panamensis was found to feed on humans throughout the 24 hour period with primary activity peaks occurring from dusk to 2200 hours and at dawn; thus, its primary biting

activity was crepuscular. In Panama they also found L. panamensis feeding on man during the daylight hours.

Williams (1966a) studied the biting rhythms of some anthropophilic sandflies in British Honduras and found L. panamensis to be the most numerous fly biting man. It was collected during all hours of the day and night, with peak biting activity at dusk from 1800 to 1859 and from 2100 to 2159. Day biting activity was greatest at sunrise and least in the early afternoon when the temperatures were the highest and the relative humidity the lowest. To discern what part of the human body was preferred by sandflies, Williams (1966b) collected L. panamensis feeding on the ear, but subsequent observations by Williams (1970b) revealed that when collectors worked without shirts most L. panamensis landed on the arms and torso. The comparative flying and biting activity of Panamanian sandflies in a mature forest and adjacent open space was studied by Chaniotis and Correa (1974). Fewer L. panamensis and other anthropophilic species were taken in the open space than in the forest by both light trap and human bait collections.

Diurnal Resting Habitats

In Panamanian forests the diurnal resting sites of phlebotomine sandflies were studied (Chaniotis et al., 1972). The habitats studied included animal burrows,

tree hollows, leaf litter, green plants, tree trunks and buttresses (ground level to 0.6 meters and 0.6 to 2.0 m), and tree trunks (5.0 m, 9.0 m, and 15.0 m). Specimens of L. panamensis were found on tree trunks (0.0 to 0.6 m) and green plants and in leaf litter and tree hollows. Females and males were most frequently collected in leaf litter. Hertig et al. (1960) flushed L. panamensis from green leaves within a few feet from the ground. Hanson (1961) and Johnson and Hertig (1961) collected this phlebotomine from the dead leaf microhabitat on the forest floor. Disney (1968) collected L. panamensis from under leaves in British Honduras. Williams (1965, 1970b) did not often find this sandfly on tree buttresses but frequently collected it beneath leaves on the forest floor.

Host Preference

A number of studies have been conducted to determine the natural host of phlebotomine sandflies. Several traps have been used utilizing various animals as bait. Disney (1966) developed an animal-baited cage trap situated over a pan of castor oil so that after a fly had fed, it usually dropped off into the oil. He noted that only a few specimens of L. panamensis were collected in the traps baited with the rat Ototylomys phyllotis Merriam but that he and his assistant were attacked by many more L. panamensis than were caught in the rat-baited traps during the collection

period. Williams (1965) also used the Disney fly traps with different bait animals. L. panamensis was rarely taken and then only in the traps baited with Ototylomys.

Disney (1968) collected L. panamensis in traps baited with the opossums Didelphis marsupialis L. (common opossum) and Philander opossum L. (four-eyed opossum) and the rats Heteromys desmarestianus Gray, Oryzomys cousei (Alston), Tylomys nudicaudus (Peters), O. phyllotis, and Sigmodon hispidus Say and Ord. He questioned whether or not sandflies fed on all animals to which they were attracted. He found only one freshly engorged L. panamensis in a trap baited with Didelphus and none in rat baited traps. To determine if sandflies showed any preference for a particular bait he erected traps in pairs, baiting one trap with Ototylomys and the other trap with the alternative bait. The numbers of L. panamensis trapped were too low to have any significance.

Fairchild and Hertig (1951) and Hertig et al. (1959) stated that L. panamensis fed on horses and pigs. Thatcher and Hertig (1966) compared the baited trap technique with the direct collection of sandflies from animals and preferred the latter method in their host distribution studies. They collected L. panamensis from Philander and Potos flavus (Schreber) (kinkajou) with the kinkajou the most attractive for all sandfly species collected. They found that maximum

feeding took place on warm nights with no wind and that an 8-10 mph wind inhibited sandfly activity. Thatcher (1968) studied the sandfly host preference of L. panamensis in the same general area as my project. He found that L. panamensis was the second most abundant species captured, with 95% being taken in traps at ground level and only a few trapped at the 10 to 13 meter level. This phlebotomine was more attracted to Didelphis than to Potos and was the only sandfly attracted to all the bait animals used, including the chicken. Christensen et al. (1972) indicated that L. panamensis was the second most numerous sandfly trapped by the animal-baited (spiny rat or rice rat) castor oil trap method; however, it represented less than 1% of the total catch.

Tesh et al. (1971) tried to determine the natural hosts of Panamanian sandflies by the precipitin test. Seven freshly blood-engorged specimens of L. panamensis collected from the field were tested with three class-specific antisera. They all reacted with mammal antiserum. Tested with six order-specific mammalian antisera these same flies reacted positively to one rodent and one marsupial antisera. In studies continued and expanded by Tesh et al. (1972) only six L. panamensis were tested against nine order-specific mammalian antisera. One reacted with rodent, two with edentate, two with carnivore, and one with perisodactyl antisera. Most flies used in the above two studies

were collected from tree buttresses early in the morning, a resting place usually not sought by L. panamensis.

Sugar-Feeding Behavior

The feeding habits, including the sugar-feeding habits of biting flies belonging to Nematocera, were well documented by Downes (1958). Both sexes feed on sugars from various sources in nature such as plant juices, nectar, and ripe fruits. In the laboratory they feed on raisins, fresh fruit, honey, solutions of assorted commercial syrups, and solutions of pure sugars, as well as the pure sugars themselves. The literature contains very little information concerning the sugar-feeding behavior of tropical phlebotomine sandflies. Lewis and Domoney (1966), Chaniotis (1974), Barretto (1942), and Downes (1958) have reviewed the existing literature on this subject.

Lewis and Domoney (1966) examined the sugars in the dissected crops (esophageal diverticulum) of sandflies from British Honduras by thin-layer chromatography and found sucrose, fructose, and glucose. If there were other sugars present they were indistinguishable with this method because of their small quantities. They concluded that the liquid normally present in the crop always contains some type of sugar.

Chaniotis (1974) used the sandfly Lutzomyia trapidoi to determine some of the optimal laboratory sugar-feeding

conditions necessary to maintain this sandfly. Out of the 11 sugars screened sucrose, fructose, maltose, raffinose, and glucose were preferred by the wild-caught sandfly females, Chaniotis concluded that the rate of sugar acceptance was unaffected by concentration, pH, NaCl content, color of the solution, or temperature. In an experiment using laboratory reared L. trapidoi, the sucrose solution concentration had no appreciable effect on sandfly longevity. The highest feeding rates were obtained using the styrofoam cup holding vessel developed by Chaniotis. He described the optimal sugar meal as a highly concentrated solution of sucrose or fructose in distilled water, with or without added coloring.

Bionomics--Immature Stages

Very little information is known about the natural breeding habitat of immature phlebotomine sandflies. This is largely due to the difficulty in finding and isolating the immature stages from the soil. Very few randomly taken soil samples contain the immature stages because of their discontinuous breeding habitat. Early investigations of the breeding sites of New World phlebotomines by Ferreira et al. (1938), Coutinho and Barretto (1941), Pifano (1941), Hertig (1942), Forattini (1954), and Deane and Deane (1957) uncovered only about 60 specimens.

In an effort to further the knowledge of the immature stages of New World sandflies, Hanson (1971) conducted an investigation in Panama to discern the breeding habitats of these flies, especially the anthropophilic species. A total of 370 soil samples were processed by the screening flotation method. This method, in combination with direct examination of the soil and debris in the field, yielded 2,258 larvae and pupae, of which 600 were reared to the adult stage and identified. The breeding places investigated were soil between tree buttresses, from burrows, under roots, at base of trees, and dead leaves in forest litter. The immature stages of L. panamensis were found only among the dead leaves of the forest floor habitat. The larvae were on moist areas of either the upper or the lower surfaces of decaying leaves when the leaves were lying loosely. Hanson speculated that the feces of various animals along with fragments of insects and other dead arthropods formed part of the natural food supply. He mentioned that various bacteria, fungi, and algae might also contribute to the diet. Two other studies were conducted in Panama to learn more about the immature habitats, but they did not yield any more information about L. panamensis (Thatcher, 1968b; and Rutledge and Mosser, 1972).

Rutledge and Ellenwood (1975c) used emergence traps to determine the species composition of sandflies on the open forest floor in Panama. They reported that populations of L. panamensis reached their maxima during the early part of the wet season (May to August), when the soil moisture conditions were relatively moderate, and reached their minima during the late wet and dry season (September to April) when the moisture conditions were at the extremes in wetness and dryness. They found that L. panamensis bred regularly in the soil or litter of the open forest floor. Rutledge and Ellenwood (1975a) also determined the hydrologic and physiographic features of this species' breeding habitat. They indicated that the forest litter tended to be removed from steep slopes and valleys and accumulated on gentle sopes and prominences. The forest litter occurred in stable, residual deposits on hilltops, while relatively unstable, alluvial deposits were more common on stream banks and hillsides. L. panamensis tended to be more abundant in the hilltop regions. No effects of non-destructive inundation or of physiographic aspects could be detected.

The forest vegetation and its local effects on the sandfly breeding habitat on the open forest floor was studied by Rutledge and Ellenwood (1975b). L. panamensis was most abundant in association with large trees of the genus Ancardium (Sapindales, Ancardiaceae). They were also

found in association with litter of Oenocarpus (Principes, Palmae) a tall columnar tree; Scheelia (Principes, Palmae) tall columnar tree; Inga (Rosales, Leguminosae) medium-sized branching tree; Croton (Geraniales, Euphorbiaceae) medium-sized branching tree; Ourouparia (Rubiales, Rubiaceae) large liana; and Sabicea (Rubiales, Rubiaceae) small liana. L. panamensis was especially abundant in palm forest of Oenocarpus and Scheelia. They concluded that sandfly-plant interactions largely determine the sandfly composition in the forest floor breeding habitat.

Rearing and Colonization

Phlebotomine sandflies have been reared and colonized by several individuals, with varying degrees of success, in connection with various investigations concerning sandfly-borne diseases. Most of the early rearing techniques were developed by individuals working with Old World sandflies: Waterston, 1922; Whitingham and Rock, 1922; Smith, 1925; Shortt et al., 1926; McCombie Young et al., 1926; Christophers et al., 1926; Ashner, 1927; Roubaud and Colas Belcour, 1927; Adler et al., 1938; Young and Hertig, 1941; Unsworth and Gordon, 1946; Eldridge et al., 1963; Schmidt, 1964; Hafez and Zein el Dine, 1964; Safianova, 1963; and Mesghali and Lofti, 1968. Some of the more important studies which dealt with the laboratory

development of New World sandflies were done by Hertig, 1942; Addis, 1945; Sherlock and Sherlock, 1959; Chaniotis and Anderson, 1964; Chaniotis, 1967; Christensen, 1972; Sherlock and Sherlock, 1972; and Ward, 1972. The essential basic requirements for sandfly rearing determined in their studies were: (1) a moist surface for oviposition for the gravid females, (2) a moist environment for development of eggs and immature stages, (3) suitable food for the larvae, and (4) containers or cages for the emerging adults and their subsequent confinement, feeding, and experimental manipulation (Hertig and Johnson, 1961).

The first attempt at rearing L. panamensis was made by Mirsa (1952) using flat-bottomed test tubes (9 cm by 2.5 cm) with paper wadding in the bottom two centimeters. Two circles of filter paper placed over the wadding provided the rearing substrate. A mixture of dry pulverized rabbit and sheep excrement was placed on the filter paper after the appearance of the first instar. The food source was sterilized to prevent the establishment of the fungus Aspergillus niger Tieg. and mites. Water was added with an eyedropper to maintain the humid conditions of the rearing container. Development of L. panamensis from egg to adult took 29 days at 26-28° C. No attempt was made to colonize this sandfly.

Pifano et al. (1960) utilized the technique developed by Young and Hertig (1926) of earthen pots lined with plaster of Paris for rearing L. panamensis. Dried pulverized sterile rabbit feces mixed with dry pulverized soil from the sandfly habitat was used as the food source. Laboratory developmental temperature was 26-28° C with the relative humidity above 65%. The period of development from egg to adult was 42 days. After emergence, adults were maintained at a temperature of 21-23° C at a very high humidity and lived for two weeks on a diet of the juice in boiled raisins.

The observations made by Johnson and Hertig (1961) on the development and behavior of Panamanian sandflies in laboratory culture provided the background information for the present study. Their rearing techniques were generally the same as those used in this study although certain modifications were necessary to successfully rear L. panamensis. Collected gravid females were kept at 25.5° C in an air conditioned room until oviposition. Larval development was successful at 26.5° C but both hatching and development were more satisfactory at higher temperatures. Daily temperatures ranged from 26-29° C and occasionally rose to 30-31° C in the rearing room. During the dry season (January-April) the relative humidity ranged from 54-92% and in the rainy season (May-December) it was usually above 90%.

Johnson and Hertig (1961) observed that larvae of L. panamensis were attracted to the soft, pigmented material of the inner surface of dipteran eye capsules, which were part of the larval food mixture. The larvae were always found on the surface of the food material perhaps because they had long caudal bristles which caused difficulty in movement if they crawled under and into the substrate material. The eggs were dark, thick-shelled, and had a sticky substance which purportedly cemented them to the substrate. Certain larval reactions to some external stimuli were noted. The sandfly larvae did not react to changes in light intensities. When the caudal bristles were touched, the larvae quickly flattened them down to the substrate on the same level as the body and would then remain motionless for several seconds. They were observed to clean their caudal bristles of debris by bending backward dorsally, grasping the base of the bristle between their mandibles and pulling the bristle through. This process was repeated with each bristle. L. panamensis larvae were regarded as slow-moving and would remain in one place for prolonged periods. They were observed to crawl as far up the pot walls as possible before pupating, but not to the point of leaving the moistened substrate.

For sandflies in general, it is known that a higher proportion of females feed on a laboratory host six to seven

days after the first adults emerged in a given culture. Johnson and Hertig (1961) speculated that a slight change in the environment such as light intensity, temperature, or humidity, might lead to a feeding response in physiologically hungry females. Although the spiny rat (Proechimyes semispinosus (Tomes)), guinea pig (Cavia porcellus (L.)), and man were used as laboratory hosts, L. panamensis females did not readily feed on any of these animals under any conditions tried. The reluctance of L. panamensis to feed in these studies prevented the species from being carried beyond the second generation.

They designed an experiment to show the length of time from engorgement to oviposition, and found that ten laboratory fed L. panamensis females laid eggs four days after feeding. In three of 127 pools of wild-caught blood-engorged females, one or more of the females laid eggs one and two days after feeding, while in the other 124 pools, eggs were laid after three to five days. This indicated that the females which laid eggs after one or two days had had a previous blood meal because normal developmental time was four days.

Johnson and Hertig (1961) observed quiesence in one culture of L. panamensis that had produced adults and then was overlooked and allowed to become very dry. Viable fourth instars and pupae were still present two weeks after

adults first appeared and after the pot was moistened they continued development and normal adults were produced. Uneven rates of hatching were also noted in some cultures of L. panamensis started in the dry season. The first hatching took place within nine to ten days, but three to four weeks later, with most of the larvae in the fourth instar, newly hatched larvae were discovered. Batches of eggs that did not hatch within 21 days were routinely discarded.

They reported that the life cycle of L. panamensis averaged 38 days from oviposition to adult emergence. Wild-caught females laid an average of 28 eggs. The larvae of all stages had very long caudal bristles. The first instars were a dark grey in color and flattened dorsoventrally. The first and second instars had dark brown heads, but the heads of the third and fourth instars became lighter than the body. The heads of the last two instars bore greatly enlarged conical antennal bases which, they suggested, resembled a profile of a cat's head. They noted almost all larvae, while on leaves in the culture, constantly manipulated their mandibles, presumably scraping the leaf surface. The pupae were very slender and dark brown in color. Larval mortality in the cultures was high and pots started with 200-300 eggs might only yield 30-50 adults.

Ultrastructure of Egg Stage

Until now no attempt has been made to use the SEM to demonstrate the fine ultrastructure of anthropophilic phlebotomine sandfly eggs. Barretto (1941) used the light microscope to obtain photographs of the eggs of 12 sandfly species but they were difficult to interpret because of the low magnification and light glare.

The SEM has been used by many authors to study the fine surface structure of mosquito eggs. Hinton (1968a,b) and Hinton and Service (1969) used the SEM to characterize the function and structure of Anopheles, Culex, and Aedes egg surfaces. Matsuo et al. (1972) described the egg surface structure of five species of Aedes and one species of Armigeres from Japan and Malaysia. Matsuo et al. (1974) continued their study of the egg surface structure with 13 Aedes species from Taiwan.

MATERIALS AND METHODS

Collection Site

Field collections of L. panamensis were initiated March 26, 1973, in an area near Achiote, Colon Province, Panama, inside the Canal Zone boundary. This site was selected for two reasons: (1) past collections conducted by Gorgas Memorial Laboratory (GML) personnel showed that L. panamensis was the most abundant species in man-biting counts; and (2) the Atlantic drainage of the Canal Zone was considerably wetter than the Pacific, resulting in minimal fluctuations of the sandfly populations. The collection site was adjacent to the large forested area which forms part of the Fort Sherman jungle training preserve. The town of Achiote and the Fort Sherman training area in the Canal Zone were known to be endemic for human cutaneous leishmaniasis (Thatcher, 1968).

Collection Procedure

Plaster of Paris lined 5-dram glass vials (55 X 27 mm), used to collect sandflies, were modified in this study from the original design of Hertig and Johnson (1961). The inside walls were coated with plaster to a distance of

approximately 10 mm from the shoulder, leaving an opening for observing the sandfly without removing the cap. Two small notches were cut through the glass opposite each other on the bottom edge of the vial to allow moisture from the substrate to penetrate the plaster in the vial.

The method employed to collect wild-caught blood-engorged females of L. panamensis was the standard procedure adopted by the Leishmaniasis Department of GML. On collection mornings, approximately 150 vials were moistened and placed in lunch boxes described by Hertig and Johnson (1961). Late in the afternoon shortly before dusk, a horse was tethered at the collection site. Sandflies soon began to feed on the horse and shortly before a fly had fed to repletion, a moistened plaster lined vial was placed over the fly (Figure 10). The sandfly would then fly up into the vial permitting the rapid placement of the cap. The vials were placed in the kits for return to the laboratory.

Laboratory Handling

The next morning each vial was examined. Dead specimens were discarded and live specimens were placed in individual vials. The vials were then placed in clay dishes, approximately 36 per dish (Figure 11). Moisture was maintained in the vials by placing about one-half inch of water in the dish every other day for three minutes or until the

Figure 10. Sandfly collection from a horse.

Figure 11. Collection vials; also used for longevity studies with wild-caught blood-engorged L. panamensis females.

plaster was visibly moist. The excess water was then dumped out. The vials were examined every day at the same time and the following information recorded: (1) whether or not oviposition had occurred, and (2) the number of females surviving.

Early in the study, when a female died, she was removed from the vial and placed on a well-slide containing 92% phenol for clearing, identified to species, and the number of eggs retained in the abdomen recorded. The number of eggs deposited in the vial was also recorded to give the total number of eggs developed by each female. The vials were then numbered and placed according to the day oviposition occurred.

Rearing Procedure

The standard breeding vessel used at GML, the unglazed clay pot (Boston bean pot, outside dimensions 80 mm high by 85 mm in diameter at widest part, 70 mm at lip, 64 mm at constriction below lip, flat bottom 65 mm in diameter), described by Hertig and Johnson (1961), was employed in this study as the principle rearing chamber. Three other rearing chambers were tested for colonization attempts but only one (plaster of Paris lined petri dish) proved to be satisfactory and is discussed in the larval experimental techniques section. The other two chambers

are described as follows. Chaniotis (1974) used an 8 ounce styrofoam cup as a sugar feeding vessel for L. trapidoi and also as a rearing chamber (Chaniotis, personal communication). Both techniques were adopted in the colonization procedures of L. panamensis in this study. Approximately 80% of the inside cup surface was lined with plaster and a small hole placed in the bottom of the cup permitting the plaster to contact the moist sponge on which the cup was placed. A plastic petri dish half served as a cover for the rearing chamber. Pressed peat moss disposable potting cups with the same dimensions as the styrofoam cups (8 cm high and 7.5 cm in diameter at the top) were also used. Plastic petri dish halves were used as covers. These cups were placed on sponges to maintain the moisture content.

Eggs from the same oviposition day were transferred to the rearing chambers in the following manner. They were carefully brushed from the sides and bottom of the plaster in each vial and washed into a beaker. Care was taken not to injure the eggs during this process. The eggs were washed three times with sterile water and transferred to the rearing chamber with a pipet. Approximately 500 eggs were placed in the center of the bean pot and 200 into the styrofoam or peat moss cups. In initial rearing attempts, a water-yeast solution was given to the first instars and increasing amounts of a mixture of autoclaved ground dried

leaves and animal feces (standard larval food) were provided beginning with the third instar.

Adult Feeding

The cloth releasing cage described by Hertig and Johnson (1961) was utilized in this study to transfer adults from one holding vessel to another and for some feeding trials. A new feeding cage was developed along the same design of the Wohlbach louse feeding cage (Wohlbach et al., 1922) to feed up to 50 L. panamensis females in close proximity to the host. The round plastic feeding cage was 7 cm in diameter by 3 cm in depth with openings of 4.5 cm in diameter in the cover and the bottom. The opening in the bottom was covered by a piece of nylon cloth (9 meshes per linear centimeter) and fastened tightly into place by acetone placed on the plastic. Another piece of nylon cloth not fastened to any plastic surface (22 meshes per linear centimeter) having an outside dimension 4 cm larger than the cover, was used as a covering under the plastic top (Figure 12). To facilitate the easy entry of adults into the feeding cage, a small plastic entry tube, approximately 25 mm in length was placed half way through the center of the cloth and glued into position. The inner plastic surface of the bottom portion of the cage was coated with plaster, which was moistened before use to provide the

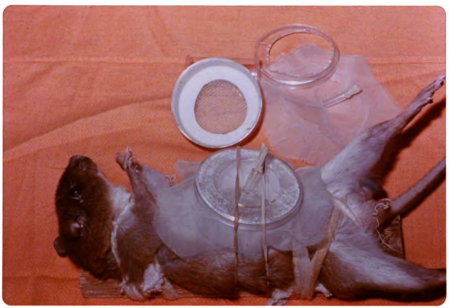


Figure 12. Plastic feeding cage and laboratory host spiny rat.

Figure 13. Holding and test vessel for laboratory reared adults.

humid conditions necessary during feeding. The cage was assembled by placing the top cloth over the plastic bottom and placing the cover over the cloth, securing the cloth tightly in place. After feeding was completed, the feeding chamber was placed in the releasing cage and the top removed allowing the fed adults to be transferred to the holding chamber.

The aspirator used to transfer the adult flies from one container to another was made from a disposable pipet with the small end filed off, leaving a hole just large enough for the entry of the adults (Figure 13). A 20 inch piece of rubber tubing (6 mm in diameter) was attached to the pipet, with a piece of cloth placed between the glass and tubing.

Larval Experimental Techniques

The food sources used were Bacto-Beef Blood Serum, Bacto-Liver Powder, Bacto-Hemoglobin (Difco Laboratories, Detroit, Michigan), Fleishmann's package yeast (Standard Brands Inc., N.Y., N.Y.) and the standard larval food (ground leaves and rabbit feces). These substances were autoclaved prior to use.

A rearing chamber, similar to the one used by Gemetchu (1971), was utilized to study the feeding preferences of L. panamensis larvae. A small hole

(6 mm in diameter) was made in the bottom half of a 9 cm plastic petri dish. Plaster of Paris was poured into the bottom half to a depth of about 7 mm three hours prior to the placement of the eggs. The plaster seeped through the hole and became even with the underside of the plastic bottom. Test dishes were made in the following manner. Eggs selected were from wild-caught females who had oviposited approximately 25 or 50 eggs on either the fifth or sixth day after blood engorgement. The amount of food initially placed on the dish accommodated about 25 larvae so those females who deposited 50 eggs had their complement divided in half. Eggs were placed in the center of the plaster 24 hours before the anticipated emergence of the first instars. The foods, mixed with sterile water, were placed in the dishes with a microcapillary tube about six hours before the anticipated first instar emergence. Small mounds of food (24), between 4-5 mm in diameter, were placed in rows (6), alternating each food source, equidistant from each other. When only one food source was used per dish, care was taken to place the same number of rows and mounds on each dish. The dish cover was then positioned with a rubber band fastened around it and the dish was placed on a watch glass in a pan of water to protect it from ants and mites. Daily, after recording the pertinent data, the dishes were placed on a moist sponge for about three minutes

to maintain the proper humidity. The Chi-square test was used to evaluate the feeding preferences of the larvae.

Adult Experimental Techniques

The carbohydrate sources used in these experiments were: fructose and sucrose (General Biochemicals, Chagrin Falls, Ohio); dextrose (Allied Chemicals, Morristown, N.J.); maltose (Fisher Sci. Co., Fair Lawn, N.J.); honey, Karo syrup and soaked raisins. Saturated sugar solutions were made fresh each week from reagent grade sugars mixed with distilled water, placed into screw-cap vials and refrigerated. All sugar sources were colored with green food coloring to facilitate easy detection in the ventral diverticulum of the sandfly.

A holding and test chamber was used to determine the effects of various carbohydrate sources on the longevity of laboratory reared adult L. panamensis (Figure 13). The styrofoam plaster lined bottom used was described previously. The top for the holding chamber was constructed from a nine ounce plastic cup (9 cm in diameter at the top, 5 cm in diameter at the bottom and 7.5 cm in depth). A hole 3.5 cm was made in the bottom of the cup and a small hole (9 mm) was placed about midway between the top and bottom. The bottom hole was covered with a nylon cloth (22 meshes per linear centimeter) and glued to the plastic by acetone.

A No. 0 cork was used to plug the hole in the side. The plastic cup was inverted and placed on top of the styrofoam cup and held together by two opposing strips of masking tape to form the holding or test chamber.

Five drops of each sugar were spotted on the nylon mesh of each test chamber. Five females and five males, less than 24 hours old, were aspirated into each container via the side hole. The chamber was then situated on a moist sponge to maintain the humidity in the vessel. Standard procedure was to keep up to 50 or 60 adults in these holding containers when they were not being used for test purposes. Life tables were used to determine observed life expectancies of the males and females feeding on the various sugar sources (Barretto, 1942).

The plaster lined vials were used as test chambers for the wild-caught blood-engorged females to determine longevity and egg production. Specimens from each field collection were divided into groups and placed in the clay dishes (Figure 11) with one drop of a sugar or a soaked raisin spotted on the nylon mesh. The maintenance procedures and data collected are outlined in the Laboratory Handling section. The comparison of various sugar diets on mean percentage of eggs oviposited by L. panamensis was determined by an analysis of variance with replications of unequal size and an arcsin transformation (Snedecor and Cochran, 1973).

The ambient rearing temperature throughout the colonization and experimental studies varied from 22° to 28° C with the relative humidity averaging 75%.

Ultrastructure of the Egg Stage

Eggs were obtained from wild-caught blood-engorged females captured from a horse near Achiote, Canal Zone, Panama. The collected females were placed in moistened plaster of Paris lined glass vials, described previously, and allowed to oviposit. Two days after oviposition the eggs were fixed in 5% glutraldehyde and then passed through an alcohol dehydration series and allowed to dry. The specimens were then sent to the Center for Electron Optics, Michigan State University, East Lansing, Michigan, for further processing. The eggs were mounted on specimen stubs, sputter coated with approximately 300 Å of gold, and examined in an AMR-900 SEM at varying beam currents, voltages, and tilt angles.

The eggs are very fragile and if allowed to dry before eclosion, will collapse. Although it was difficult to keep the eggs from collapsing with the techniques employed in this study, the surface structure remained unchanged.

Morphology of the Larvae and Pupa

The immature stages described in this study were obtained from the laboratory colony, mounted in Hoyer's medium and ringed with nail polish. Measurements of the body structures and setae of ten specimens of each instar and pupa were made with the Wild ocular micrometer and camera lucida tracings. Drawings of the dorsal and ventral aspects of the first and fourth instars and the lateral aspect of the pupa were made with the aid of the Wild drawing tube.

RESULTS AND DISCUSSION

Species Composition

Twenty-seven collections were made beginning March 26, 1973 and ending January 15, 1974. The species distribution of anthropophilic phlebotomine sandflies on a horse in the primary forest near Achiote, Canal Zone, is shown in Table 1. Lutzomyia panamensis was the most numerous species with an average of 135.6 per collection, followed by L. trapidoi, L. pessoana, L. gomezi, L. ylephiletor, and L. sanguinaria in decreasing order of abundance.

The numbers of sandflies in the collections increased with the onset of the rainy season (early May) and decreased late in the wet season (late September). This decrease in the late wet season has been reported by others (Chaniotis and Neely et al., 1971).

Feeding Preferences--Larvae

Four experimental foods (yeast, liver powder, beef flood serum, and hemoglobin) were placed together, but separately, on plaster of Paris lined petri dish bottoms to determine which were preferred by L. panamensis larvae.

Table 1. Seasonal occurrence of phlebotomines--Achiote, Canal Zone, 1973-74

	Lutzomyia												
	panamensis		gomezi		pessoana		sanguinaria		trapidoi		ylephiletor		
Date	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	Total
26-III-73	58	98.3	1	1.7	0	0	0	0	0	0	0	0	59
28-III-73	92	78.6	13	11.1	1	0.9	4	3.4	7	6.0	0	0	117
2-IV-73	97	94.2	2	1.9	0	0	1	1.0	1	1.0	2	1.9	103
4-IV-73	99	75.6	9	6.9	1	0.8	2	1.5	16	12.2	4	3.1	131
10-IV-73	37	90.2	2	4.9	0	0	0	0	0	0	2	4.9	41
18-IV-73	59	92.2	5	7.8	0	0	0	0	0	0	0	0	64
3-V-73	187	99.5	1	0.5	0	0	0	0	0	0	0	0	188
15-V-73	230	97.9	0	0	4	1.7	0	0	1	0.4	0	0	235
29-V-73	257	96.6	0	0	7	2.6	0	0	2	0.8	0	0	266
7-VI-73	205	99.0	0	0	2	1.0	0	0	0	0	0	0	207
14-VI-73	173	100.0	0	0	0	0	0	0	0	0	0	0	173
5-VII-73	225	100.0	0	0	0	0	0	0	0	0	0	0	225
12-VII-73	151	100.0	0	0	0	0	0	0	0	0	0	0	151
26-VII-73	185	100.0	0	0	0	0	0	0	0	0	0	0	185
9-VIII-73	192	97.0	0	0	4	2.0	0	0	2	1.0	0	0	198
23-VIII-73	190	90.5	0	0	5	2.4	0	0	15	7.1	0	0	210
6-IX-73	189	96.9	1	0.5	1	0.5	0	0	4	2.1	0	0	195
24-IX-73	98	93.3	0	0	1	1.0	0	0	1	1.0	5	4.8	105
22-X-73	82	91.1	0	0	3	3.3	1	1.1	0	0	4	4.4	90
5-XI-73	133	97.1	1	0.7	3	2.2	0	0	0	0	0	0	137
20-XI-73	47	94.0	0	0	0	0	2	4.0	1	2.0	0	0	50
27-XI-73	166	99.4	0	0	0	0	1	0.6	0	0	0	0	167
4-XII-73	153	98.1	0	0	1	0.6	1	0.6	1	0.6	0	0	156
11-XII-73	30	100.0	0	0	0	0	0	0	0	0	0	0	30
18-XII-73	133	87.5	0	0	5	3.3	4	2.6	10	6.6	0	0	152
8-I-74	69	71.1	0	0	0	0	4	4.1	24	24.8	0	0	97
15-I-74	123	76.4	0	0	0	0	2	1.2	32	19.9	4	1.5	161
Total	3,660	94.0	35	0.9	38	1.0	22	0.6	117	3.0	21	0.5	3,893

A total of ten plastes and 236 eggs were used in the experiment.

The proportion of first, second, third, and fourth instar larvae feeding on each food was observed on each day following eclosion. The assumption was made that the number of larvae feeding on each of the four food sources was equal and that they had no preference for a particular food source during the feeding period of each instar. This hypothesis was tested by the following method. Since the feeding of a larva on one day may not be independent of its feeding on other days, the mean number ($\bar{N}$) of larvae and the mean proportion ($\hat{P}_1$, $\hat{P}_2$, $\hat{P}_3$, $\hat{P}_4$) of larvae feeding on each food was computed for the days that each proportion was approximately constant. The number of larvae feeding on each food per day was then computed by multiplying $\bar{N}$ by each $\hat{P}_i$ ($i = 1,2,3,4$). Using the values of $\hat{P}_i$ specified by the null hypothesis (i.e., $\hat{P}_1 = \hat{P}_2 = \hat{P}_3 = \hat{P}_4 = 0.25$), the expected number of larvae feeding on each food was computed. The Chi-square test was used to evaluate the hypothesis for the first, second, and third instars. The tests showed that yeast was significant at the $P < .01$ level for the first, second, and third instars. This clear preference for yeast is demonstrated further in Figure 14 which shows the percentage of larvae feeding on each food source during the days when the proportion feeding was approximately constant.

Figure 14. The percentage of larvae on each food source during the days when the proportion feeding was approximately constant.

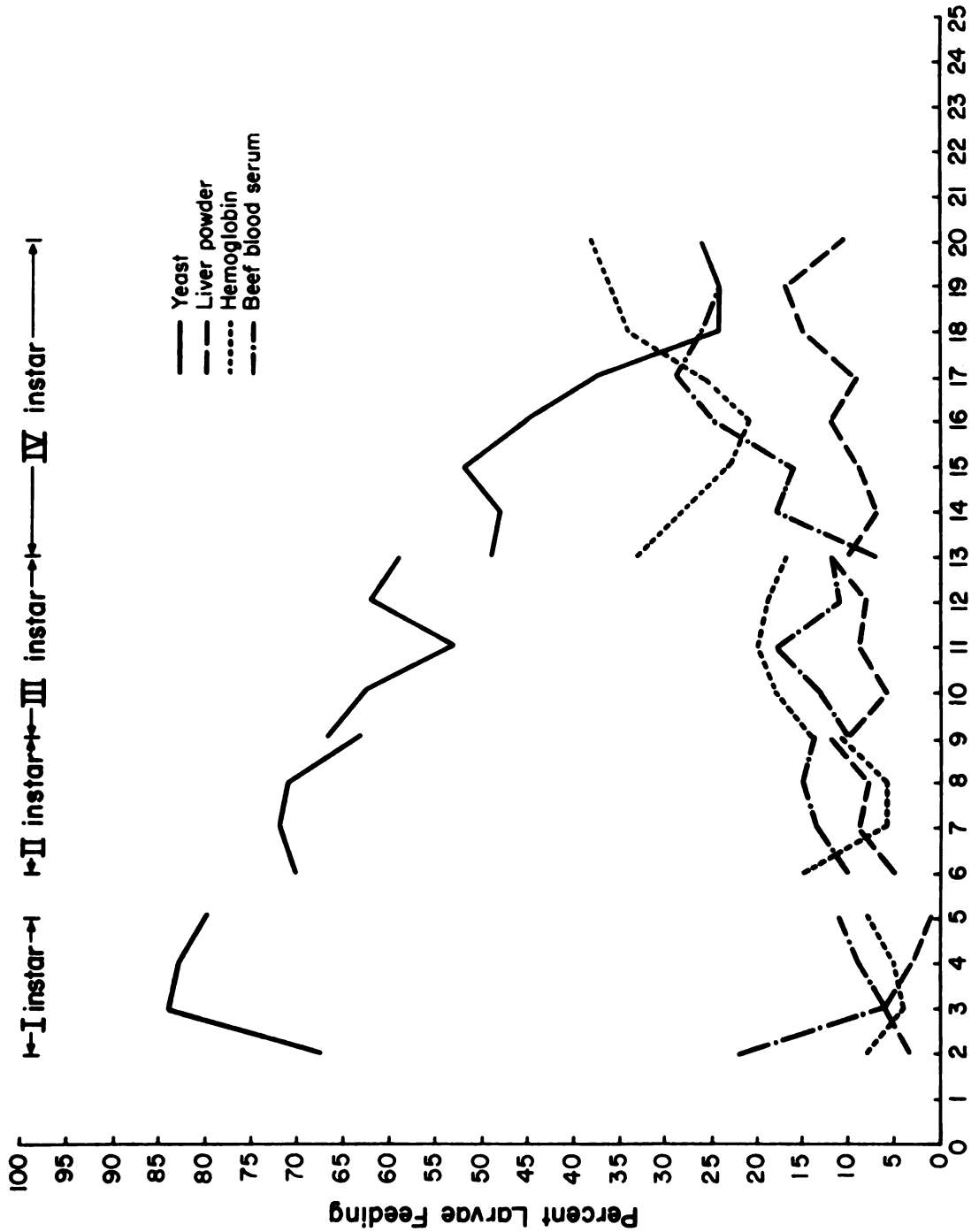

I instar
II instar
III instar
IV instar
Yeast
Liver powder
Hemoglobin
Beef blood serum
Percent Larvae Feeding
DAYS
0
5
10
15
20
25
30
35
40
45
50
55
60
65
70
75
80
85
90
95
100
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25

Figure 14

There was no period when the proportion of fourth instar larvae feeding on each food source was nearly constant. Those feeding from day 15 through 18 on yeast decreased linearly at a rate of about 9.2 larvae per day; the number feeding on liver powder and beef blood serum generally increased in a non-linear trend. The number feeding on hemoglobin decreased from day 13 through 16 but then steadily increased through day 20. These results differed from those of a similar study conducted by Gemetchu (1971) in which the Phlebotomus longipes first through third stage larvae preferred yeast and liver powder equally and were less attracted to beef blood and rabbit feces. However, no clear preference could be detected for a particular food source by the fourth instar in his study which substantiates the results obtained for the fourth instar in this study. Based on the findings of these two studies, the larvae of sandfly species appear to have different food preferences early in their development but have no clear preference during the fourth instar. Therefore, before colonization is attempted, studies should be conducted to find the best possible larval medium for each developmental stage.

Rearings of L. panamensis using only one food source were conducted to determine the effects of specific foods on the duration of the life cycle, the pupae and adults resulting from first instar larvae, and the percentage of adults

emerging from pupae. In addition to these individual comparisons, studies were made to determine at what stage in their life cycle larvae actually started to feed on the standard larval food rather than on yeast. Approximately 25 eggs were used in each of eight petri dishes for each individual food source experiment except for beef blood serum in which only six dishes were used.

The development from the first instar L. panamensis to pupa varied with the diets provided: 19.6 days (yeast and standard larval food); 21.6 days (yeast); 22.4 days (liver powder); 40.7 days (beef blood serum); and 50.5 days (hemoglobin), compared with 18.7 days for the combination of the four foods.

For rearing sandflies in the laboratory, it is important that a larval diet be developed that will assure maximum pupation and adult emergence. Table 2 summarizes the larval diets tested in this study and their effects on pupation and adult emergence of L. panamensis. The combination of the four foods was most efficient in producing the maximum number of pupae (91.5%) and adults from pupae (94.6%), whereas liver powder, yeast, hemoglobin, and beef blood serum showed decreasing efficiency, respectively. The larvae reared on the yeast and standard larval food plus yeast alone seemed to develop normally through the first three instars, but during the late developmental period of

Table 2. Comparison of larval diets and their effects on pupation and adult emergence of *L. panamensis*

Diet (no. plates-no. eggs)	Percentage of pupae from surviving first instar larvae	Percentage of adults from surviving first instar larvae	Percentage of adults emerging from pupae
Combination: yeast, liver powder, beef blood serum, hemoglobin (10-236)	91.5 (205)[a]	86.6 (194)[b]	94.6 (224)[c]
Liver powder (8-198)	75.9 (123)	67.9 (110)	89.4 (162)
Yeast and standard larval food (8-206)	64.2 (120)	59.6 (111)	92.5 (187)
Yeast (8-212)	36.8 (67)	27.5 (50)	74.6 (182)
Hemoglobin (8-194)	20.6 (33)	10.0 (16)	48.5 (160)
Beef blood serum (6-156)	10.4 (12)	7.8 (9)	75.0 (115)

[a]Total number of pupae.

[b]Total number of adults.

[c]Total number of first instar larvae.

the fourth instar some developed a darkening of the eighth and ninth abdominal segments and died. One can only speculate at this time as to the cause. It may have been due to the lack of some nutrient in the yeast or standard larval food or a bacterial or viral infection. This phenomenon was also noticed when these same two foods were used in my early colonization attempts but was not seen in the larvae feeding on the other test food sources.

The larvae reared on the yeast and standard larval food plates showed a clear preference for yeast through the third instar. No larvae began feeding on the standard larval food until the second or third day of the fourth instar, and then only to a limited extent. Although it seemed that the larvae derived some nutritive value from the standard larval food, they did not do so until the fourth instar when the mouth parts might have been better developed to handle the larger pieces of leaf and feces.

The effectiveness of the combination food source as a larval medium is demonstrated further in Table 3, which shows the developmental time range of each stage of L. panamensis utilizing the different experimental diets. This table shows that the combination and yeast and standard larval food have approximately the same developmental ranges, with yeast, liver powder, beef blood serum, and hemoglobin increasing in developmental time, respectively.

Table 3. The effects of experimental diets on the development of L. panamensis

Diet	Instar I	Instar II	Instar III	Instar IV	Pupa	Adult
Combination	1-10[a] (10)[b]	5-13 (8)	8-19 (11)	11-28 (17)	18-28 (10)	25-34 (9)[c]
Yeast and standard larval food	1-11 (11)	5-14 (9)	8-18 (10)	12-31 (19)	18-30 (12)	25-36 (11)
Yeast	1-12 (12)	5-17 (12)	8-22 (14)	12-37 (25)	19-30 (11)	26-38 (12)
Liver powder	1-14 (14)	6-18 (12)	9-32 (23)	14-46 (32)	20-39 (19)	27-50 (23)
Beef blood serum	1-21 (21)	9-28 (19)	15-44 (29)	21-62 (41)	30-53 (23)	37-60 (23)
Hemoglobin	1-27 (27)	11-35 (24)	17-61 (44)	25-83 (58)	35-74 (39)	43-77 (34)

[a] Developmental time range in days for each stage.

[b] Total number of days in each developmental period.

[c] Time required to complete emergence of all adults.

Some general observations were made on the behavior of these larvae during their development. The first instars, upon eclosion, seemed to move randomly over the surface of the plaster for about six hours before they began to feed. Usually when the first instar has located a preferred food source it either climbed on the food source or fed around the edges, frequently remaining there during its entire first instar feeding period. Shortly before ecdysis, it stopped feeding and usually moved away from the food and did not start to feed again until the moulting process had been completed. The second, third, and fourth instars tended to wander from food mound to food mound during their developmental periods. The only time that the larvae left the plaster substrate and crawled on the plastic was shortly before pupation. This occurred either on the plaster, on the plastic side of the dish bottom, or on the plastic surface of the top. The pupae apparently did not require a semi-moist substrate since the moist environment of the petri dish appeared to be adequate.

Covered petri dishes lined with plaster of Paris were very satisfactory as rearing vessels for observing the feeding behavior and feeding preference of L. panamensis. The advantages of using this technique for rearing and colonization are: (1) the larvae can be observed at any time without removing the cover; (2) the desired amount of

food can be placed on the plates prior to eclosion; and (3) the plates are free of mites and fungi that would compete for food and ensnare the larvae, as long as the dishes remain on glass plates in a pan of water. This technique would be ideal for rearing larvae hatched from eggs produced from blood-engorged wild-caught females, where only the female was known and the larvae and the male needed to be reared and described.

Sugar Feeding Studies--Laboratory Reared Females and Males

The styrofoam holding and testing vessel developed by Chaniotis (1974) proved to be very satisfactory in studying the effects of various saturated sugar solutions (fructose, sucrose, dextrose, maltose) and commercial honey and Karo syrup on the longevity of laboratory reared L. panamensis. The complete life expectancies of the females and males for each sugar source are shown in the life tables in Appendices A-G. The average life expectancies for adults feeding on each sugar are summarized in Table 4. Of the sugars tested, females feeding on fructose and sucrose resulted in the longest average female life expectancies (13.9 and 13.5 days, respectively). There were no apparent differences between females which fed on honey (10.6 days), Karo syrup (10.7 days), and dextrose (9.8 days). Maltose (4.6 days) was the least effective diet for the females and

also the males (3.0 days). No difference was observed between fructose (10.0 days), sucrose (9.1 days), honey (8.8 days), Karo syrup (8.4 days), and dextrose (7.9 days) in the average observed life expectancies of the males. The females and males that received no sugar had average life expectancies of 1.8 and 1.3 days, respectively.

The percentage of females surviving on each day is shown in Figure 15, with the number of days to 100% mortality listed in Table 4. The survival time ranged from 22 days (maltose) to 29 days (dextrose). The percentage of males surviving on each day is shown in Figure 16, with the maximum number of days to 100% mortality shown in Table 4. Survival time ranged from 19 days (maltose and Karo syrup) to 23 days (dextrose). Males and females without sugar lived a maximum of three days. Barretto (1942) stated that females reared in his laboratory lived up to seven days without a sugar source.

Female L. panamensis lived longer on each sugar diet than did the males. The females imbibed more of the sugar solutions than the males. Both females and males began to feed on the sugar solutions immediately after the drops were placed on the nylon mesh, averaging two minutes per feeding. The green color of the sugar solutions was observed through the body wall of the sandflies and it persisted until shortly before death. The flies extracted

Table 4. Longevity of laboratory reared L. panamensis females and males feeding on various saturated sugar diets

Sugar diets	Maximum no. of days to 100% mortality		Average length of life at emergence (days)	
	F[a]	M	F	M
Fructose	26 (70)[b]	22 (70)	13.9	10.0
Sucrose	28 (70)	21 (69)	13.5	9.1
Honey	24 (61)	21 (72)	10.6	8.8
Karo syrup	27 (67)	19 (70)	10.7	8.4
Dextrose	29 (70)	23 (70)	9.8	7.9
Maltose	22 (64)	19 (69)	4.6	3.0
No. sugar	3 (65)	3 (66)	1.8	1.3

[a]F = female; M = male.

[b]Total number of sandflies.

additional moisture from the plaster of Paris in the test vessels. The reagent grade saturated sugar solutions did not visibly change in consistency during the experiment. However, the honey and the Karo syrup did appear to become less viscous as the experiments progressed and became partially fouled with mold after the eighth day. This did not seem to interfere with feeding unless the feeding surface was completely covered with the mold.

Figure 15. Longevity of L. panamensis females feeding on various saturated sugar diets.

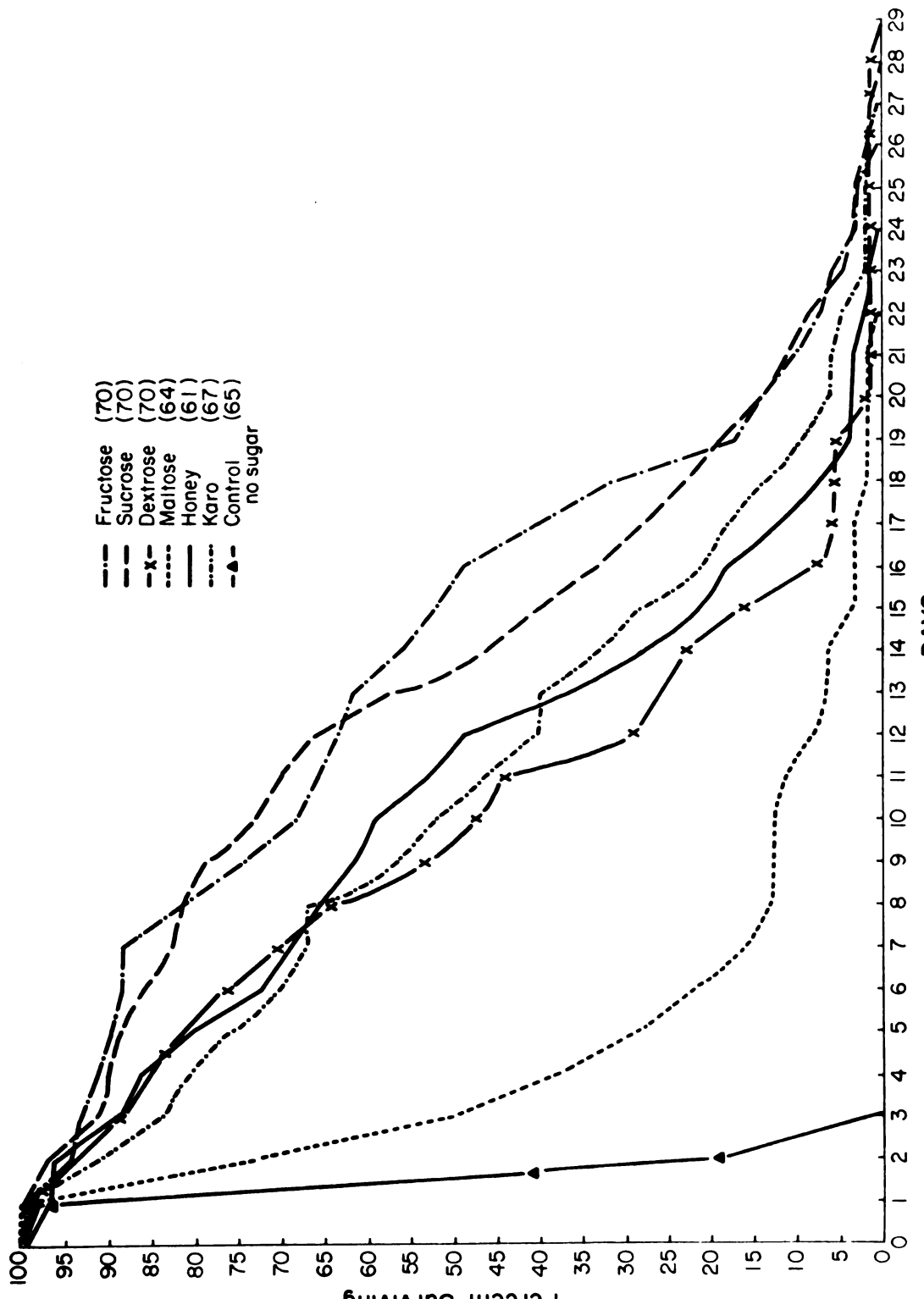

Fructose (70)
Sucrose (70)
Dextrose (70)
Maltose (64)
Honey (61)
Karo (67)
Control (65)
no sugar
Percent Surviving
100
95
90
85
80
75
70
65
60
55
50
45
40
35
30
25
20
15
10
5
0
0 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25 26 27 28 29
DAYS

Figure 15

Figure 16. Longevity of L. panamensis males feeding on various saturated suagr diets.

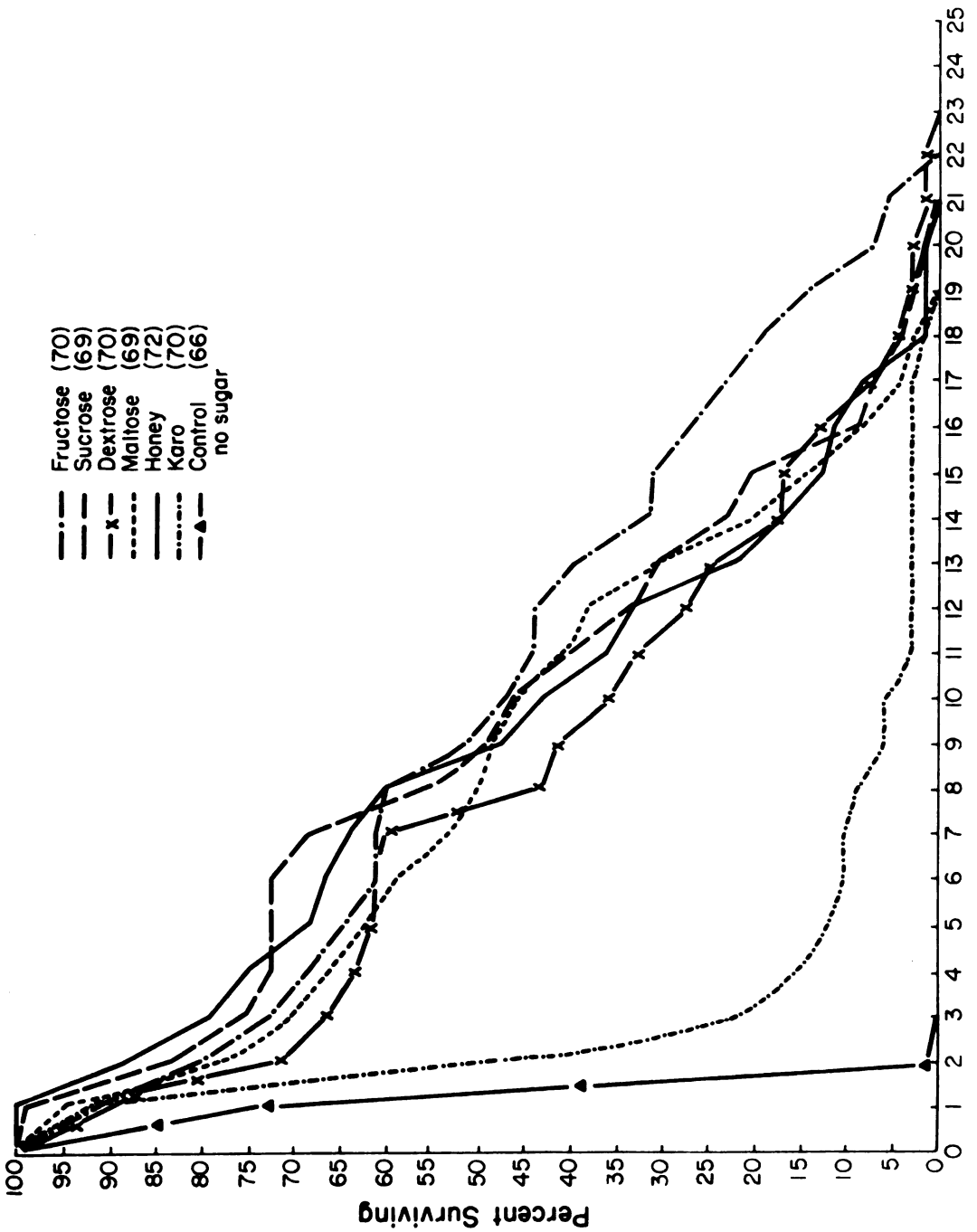

Fructose (70)
Sucrose (69)
Dextrose (70)
Maltose (69)
Honey (72)
Karo (70)
Control (66)
no sugar
Percent Surviving
100
95
90
85
80
75
70
65
60
55
50
45
40
35
30
25
20
15
10
5
0
0 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25
DAYS

Figure 16

Sugar-Feeding Studies--Wild-Caught Females

The effects of various saturated sugar solutions and the water-soaked raisin fluid on longevity and egg production of wild-caught blood-engorged females of L. panamensis are presented in this section. The females were maintained in five dram plaster of Paris lined glass vials for the entire test period except when they were transferred to another vial to permit the eggs to be counted and transferred to rearing chambers. Two main sugar-feeding periods were observed with the aid of green food coloring in the sugar diets. The first was the day after the sugar was added to the nylon mesh of the vial cap and the other was the day after oviposition. Many flies started to feed immediately after the sugars were made available, even though they had fed to repletion on blood less than 24 hours previously. Once a female had fed on a sugar the green coloring could be observed in the abdomen usually until she died.

This method of maintaining wild-caught blood-engorged females for experimental purposes proved to be very satisfactory. The adults could be observed in the vials at all times and the number of eggs deposited were counted without difficulty. The sugar sources, except for the raisin, could be placed on the nylon mesh of the cap

and did not have to be replaced for the duration of the test. As mentioned previously, the reagent grade sugar sources fructose, sucrose, dextrose, and maltose did not lose their consistency or develop any visible mold or fungal deposits on the drop of sugar during the test.

Oviposition began on the third day after blood-engorgement, but the majority of females oviposited on days four, five, and six and an occasional female did not oviposit until day ten. Some split their complement of eggs and oviposited on two different days, waiting as long as nine days after the first oviposition period of 18 days from blood-engorgement.

Figure 17 shows the longevity of wild-caught blood-engorgement females of L. panamensis feeding in the laboratory on various saturated sugar sources and soaked raisins. The females survived for 39 days (dextrose), 37 days (fructose), 29 days (sucrose), 26 days (maltose), 12 days (raisin), and 6 days (control). No sugar source was given to the control group. Female mortality increased rapidly with the onset of oviposition, especially in the control group and those feeding on raisins and maltose.

Table 5 summarizes the effects of the sugar solutions on the oviposition of the wild-caught females. The percentages of females surviving long enough to oviposit were 92.1% (fructose), 92.9% (sucrose), 88.5% (dextrose),

Figure 17. Longevity of wild-caught blood-engorged females of L. panamensis feeding on various saturated sugar diets.

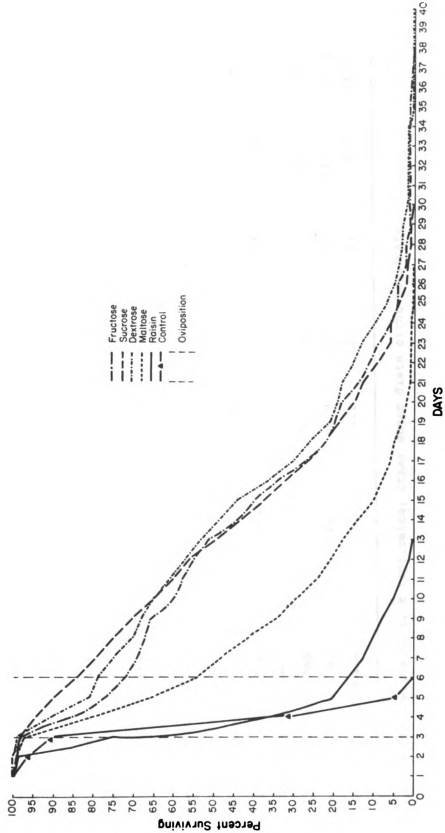

Percent Surviving
DAYS
Fructose
Sucrose
Dextrose
Maltose
Raw Milk
Control
Oviposition

Figure 17

Table 5. A summary of the effects of various saturated sugar solutions and soaked raisins on oviposition by wild-caught blood-engorged L. panamensis females

	Sugar diets					
	Fructose	Sucrose	Dextrose	Maltose	Raisin[a]	Control[b]
Total no. of females	290	281	270	287	134	314
Average no. females	36.2	35.1	33.8	35.9	33.5	39.3
Ave. no. females ovipositing	33.4	32.6	29.9	31.6	25.0	29.1
% of females ovipositing	92.1	92.9	88.5	88.1	74.6	74.0
Number eggs oviposited	1079.6	1203.8	1080.9	927.6	902.3	593.1
Number eggs retained	165.0	175.6	146.9	279.3	79.8	438.1
Total number eggs	1244.6	1379.4	1237.8	1206.9	981.1	1031.2
No. eggs oviposited/female	32.4	36.9	36.3	29.3	36.9	20.2
No. eggs retained/female	4.9	5.5	5.0	8.9	3.4	15.0
Total number eggs/female	37.3	42.4	41.3	38.2	40.3	35.2
% eggs oviposited/female	86.4	86.9	87.9	76.9	91.7	57.4
% eggs retained/female	13.6	13.1	12.1	23.1	8.3	42.6

[a]Based on the average of four replicates; other sugar diets eight replicates.

[b]Control group received no sugar source.

88.1% (maltose), 74.6% (raisin), and 74.0% (control). More than 25% of the females in the control and raising groups died either shortly before oviposition or at the beginning of the oviposition period. None of the females in the control group lived beyond the sixth day while some females receiving sugar lived beyond their oviposition period.

An analysis of variance with replications of unequal size and an arcsin transformation of the mean percentages of eggs oviposited per female was conducted testing results obtained with the various sugar sources. The mean percentages of eggs oviposited per female presented in Table 6 were separated with the LSD test at the 5% level of error. The analysis of variance test revealed a significant difference in the effects of the various sugar solutions on the percentage of eggs deposited by each female. The soaked raisin, fructose, sucrose, and dextrose solutions were superior to maltose, which in turn outperformed the control group in promoting a high percentage of oviposition. Although the soaked raisin appeared to promote the highest percentage of eggs to be deposited, it did not increase female longevity past the oviposition period to any appreciable degree. It is speculated that the females imbibed only small quantities of the sugar from the raisin prior to oviposition and did not have the energy upon completion of egg deposition to refeed. The raisin, unless

Table 6. Comparison of various sugar diets on the mean percentage of eggs oviposited by L. panamensis

Variants:	Raisin	Fructose	Sucrose	Dextrose	Maltose	Control[a]
Mean:[b]	91.7	86.4	86.9	87.9	76.9	57.4
Relationship:	a	a	a	a		
					b	
						c

[a]Control group received no sugar diet.

[b]Means with same letter are not significantly different at P = 0.05.

changed daily, promoted mold growth on the surface of the nylon mesh which might have inhibited the feeding of the adults and decreased longevity.

The average number of eggs oviposited was 32.4 for females fed on fructose, 36.9 on sucrose, 36.3 on dextrose, 29.3 on maltose, and 20.2 control. The range for all groups was 1 to 110. Johnson and Hertig (1961) stated that L. panamensis females laid an average of 28 eggs with a range of 1 to 90. Each group of females that was provided a sugar diet in this study produced, on the average, a higher number of eggs than that observed by Johnson and Hertig.

Colonization

The colonization of L. panamensis was an extremely difficult project. The unglazed Boston bean pot proved to be the only vessel in which L. panamensis could be reared in large numbers. The plaster of Paris lined styrofoam cup and the compressed peat moss cup were unsatisfactory. Moisture could not be regulated in the styrofoam cups so the plaster substrate became too dry or too moist. Also, these cups could not be autoclaved prior to use, consequently fungus enveloped the first instars so they became entangled and died. The peat moss cups also became overgrown with fungus and bacteria as soon as they were moistened and did not provide a smooth substrate for the first instars.

In this study early attempts at rearing L. panamensis in the bean pots met with little success. Though the bean pots were standard in size and shape, each one apparently had certain unknown features that affected the rearing of a particular culture. As was noted earlier in the Larval Experimental section, the first instars would wander randomly for about six hours before they began to feed. Thus, if the yeast, which was usually added the day before the eggs hatched, was not placed at convenient locations on the pot bottom and sides the larvae wandered away from the food source and died. Nevertheless, some larvae

died because of this wandering habit no matter how much care was taken in the placement of the yeast.

Moisture and a smooth substrate were also problems. The first pots used were too moist and the first instars drowned or became stuck in the very moist yeast. To correct this, the pots were placed on moistened cotton in glass petri dish bottoms. Certain modifications had to be made for each pot because the porosity of the clay varied from pot to pot. The first instars required a relatively smooth plaster substrate. After several cultures had been reared in one pot, the substrate became rough and pitted, which proved to be detrimental to the first instars, so it had to be removed and replaced.

The life history of laboratory reared generations of L. panamensis are presented in Table 7. The four laboratory generations were completed during this study with very little variation in the total number of days for each generation (blood meal to adult). The average length of time for the four generations to develop from egg deposition to adult (37.7 days) is almost identical to the results obtained by Johnson and Hertig (1961) of 38 days (average) for two generations. However, the average developmental time, oviposition to adult, differed from the 29 days reported by Mirsa (1952) and the 42 days of Pifano et al. (1960). They reared only single generations of this species, using different laboratory conditions and techniques.

Table 7. Developmental time of L. panamensis in the laboratory

Laboratory generations	Blood meal to egg	Egg	Larval instars I	Larval instars II	Larval instars III	Larval instars IV	Pupa
1st (42.9)[a]	159[b] 4.4[c] (3-10)[d]	159 9.5 (8-13)	156 5.3 (4-7)	156 3.5 (1-7)	155 5.5 (2-10)	144 7.2 (4-17)	144 7.5 (3-12)
2nd (42.9)	74 4.0 (3-5)	74 9.2 (9-10)	67 6.6 (5-9)	64 3.4 (2-7)	57 4.4 (2-8)	45 7.3 (5-12)	41 8.0 (5-11)
3rd (41.0)	15 4.1 (4-5)	15 9.2 (9-10)	12 6.4 (5-9)	12 3.1 (3-4)	12 4.8 (3-6)	10 6.0 (5-7)	8 7.4 (7-9)
4th (40.2)	6 3.8 (3-4)	6 9.3 (9-10)	5 6.8 (6-8)	5 2.8 (2-3)	3 4.0 (4)	2 6.5 (6-7)	2 7.0 (7)

[a]Total number of days in each generation.

[b]Number of cultures.

[c]Average number of days for each stage.

[d]Range of days for each stage.

The developmental times of the egg and the fourth instar were the most variable in the life cycle; the developmental times of the egg stage have already been discussed. Quiescence in the fourth stage of L. panamensis, observed by Johnson and Hertig (1961), was not seen in this study. However, the extreme variability, especially in the developmental time of the first generation fourth instar reared in the laboratory, may have been due to adverse conditions in some pots. This variability was demonstrated very clearly in the larval feeding experiments in which different food sources were used. They showed extremes in developmental times in all immature stages.

The behavior of the larvae in the pots was essentially the same as that observed in the petri dishes. All stages were very sensitive to the touch of other larvae. If contact was made between two larvae, very violent flexing actions of the body occurred in an effort to escape. However, larvae that fed in very close proximity to each other without touching did not show any type of avoidance reaction. Second, third, and fourth instars were observed to clean their caudal setae from time to time with their mandibles. The larva reared backwards, dorsally grasped one of the caudal setae with its mandibles and gradually drew the entire seta through the mandibles. This process was sometimes repeated with the remaining setae but more often only the one seta irritating the larva was cleaned.

Pupation took place anywhere on the inner surface of the pot, usually in the drier regions along the sides around the rim and was occasionally observed on the dry clay surface. These pupae remained viable because of the humid environment of the pot. When the fourth instar was ready to pupate a substance was apparently secreted from the anal region of the larva anchoring it to the substrate. A split in the dorsum of the thoracic segments allowed the pupa to break through the larval integument. The pupa, which was white in color during this process, forced the cast integument of the fourth instar to the base of the pupa by peristaltic movements. Two large prominences on the mesonotum (mesonotal tubercles) were also used to push the cast integument downward by rearing backwards and rubbing the tubercles against the receding integument. The pupal position after this process was perpendicular to the substrate. The pupal stage reacted violently to the touch of a foreign object by snapping its body forward to the substrate, then returning to its original position.

The adult emergence from the pupal skin began by the protrusion of the mesothorax through the dorsal split in the thoracic region of the pupa. The thoracic region of the adult was gradually freed from the pupal skin by a peristaltic-like action. The head was the next body part freed, along with the mouth parts and the antennae and

followed by the wings and legs. The body of the adult extended backwards pulling the long femur and tibia out of the exuvia. The adult remained in this position for about two minutes. Then the tarsal segments were pulled out freeing the legs completely. A sudden jerk of the body freed the remaining abdominal segments and the adult landed on the substrate next to the pupal exuvia. The adult was able to crawl immediately but could not fly for a few minutes. The whole process from the break in the pupal skin to the complete freedom of the adult took about 15 minutes.

The adults reared from the cultures were placed in the styrofoam holding vessels and drops of the saturated sugar solutions were placed on the nylon mesh to provide a carbohydrate source for the newly emerged adults. Before the experimental sugar feeding studies were completed, fructose and sucrose were used for this purpose because of the success Chaniotis (1974) had with them. After the completion of the experimental tests, fructose and sucrose were selected as the primary carbohydrate sources.

Early attempts at feeding the adults on a laboratory host (suckling mice and hamsters), suspended in the holding vessels were unsuccessful. The few adults that were obtained from early larval rearing attempts severely limited initial efforts to find a successful host, feeding technique, and feeding chamber.

In the initial first generation laboratory cultures, adults were obtained when yeast and the standard larval food were used as the larval medium. The average number of eggs per pot in all cultures was 550. In 41 cultures using this food combination adult emergence averaged 10.3% (>.1%-34.1%). Because of the low production of adults, the larval feeding experiments, cited previously, were set up to develop a better larval medium for L. panamensis. In the meantime, liver powder added to the yeast and standard larval food in the first generation cultures during the third instar stage increased the average adult yield in 53 cultures to 15.4% (.1%-36.4%). Upon completion of the larval medium tests, a mixture of liver powder, hemoglobin, and beef blood serum was added to the yeast and standard larval food already present during the third instar stage. This enriched larval mixture increased the average adult yield in 49 first generation cultures to 23.2% (1.4%-55.3%). Not only did the mixture increase the number of adults produced in each culture but the time period of maximum adult emergence was shortened to about six days. Some cultures produced as many as 30 females and 30 males a day which was more than adequate to maintain the colony and also provide sufficient adults for experimental purposes.

At this time adult feeding attempts again were initiated. Adults approximately four days old were released into a damp, cloth-covered releasing cage. Mating was observed almost immediately upon the release of adults into the cage. The adults were left in the cage for about one-half hour until mating was completed. No mating was ever observed in the holding vessels even if more than 100 males and females were placed in the same vessel. The transfer from a small container to a larger cage might have been the stimulus needed for mating.

The mating process was similar to that described by Mukerji (1931) for Phlebotomus argentipes Ann. and Brun. The male usually approached the female from the front, moving its abdomen from side to side flexing its claspers. Once the female was reached he swung his abdomen toward her abdomen, clasping it firmly, remaining in a horizontal position facing each other. The female was now in charge and any movement occurring during the mating process was done by the female. Copulation usually lasted two minutes or longer. The same male often copulated with one or more females.

The first successful feeding attempts occurred in the small metal framed feeding cage of the Wohlbach design used by Hertig and Johnson (1961). The females were aspirated from the releasing cage and placed into the

feeding chamber. A moist cloth then was placed over the feeding cage and the cage placed on the laboratory host which was either man or a spiny rat. If the spiny rat was used, the belly was shaved and the animal was restrained, belly side up (Figure 12). After one-half hour the cage was taken off the host and the flies were freed in the releasing cage. Only a small number of flies could be fed at one time with this method. It was determined that one-half hour was sufficient time for the flies to feed since most of the females fed within the first ten minutes. The females were then aspirated back into another holding vessel and allowed to oviposit in that vessel. No males were placed with the females after blood engorgement because preliminary tests showed that no mating occurred after blood engorgement.

Placing the adults in close proximity to the host seemed to be the key in successfully feeding the females. However, the metal cage was too small to allow large numbers (30-40) of females to be fed at the same time. The plastic cage (Figure 12) was very successful in feeding large numbers of females at one time, thus decreasing the labor involved in manipulating these flies. Using the spiny rat as the host an average of 59% (0%-93%) of the females fed in 66 feeding trials which included first, second, and third generation adults. The lower percentages always occurred when ten or less flies per attempt were used.

To minimize the handling of the flies by aspiration, another method of feeding the females was initiated. After the flies had mated, a restrained belly shaven spiny rat was inserted into the releasing cage. The flies were allowed one-half hour to feed on the rat, after which it was removed from the cage. The engorged females were then transferred into the new holding vessel while those that had not fed were aspirated into the plastic cage and placed directly on the rat's belly. The females in the cloth releasing cage were handled once, while the females aspirated into the plastic cage were handled twice. This method reduced injury and trauma to the fragile blood-engorged females. This double feeding attempt method was successful and an average of 62% (17%-100%) of the females fed in 28 feeding trials. These feeding trials also included batches of females from the first, second, and third laboratory generations. Very slight differences occurred in the percentage of feeding successes between the generations. In only two out of the 28 trials did all the females in the plastic cage fail to feed on the host.

The human host, my arm, was used only when a spiny rat was not available for feeding. The results were as successful as with the spiny rat, and, I must add, very painful.

Egg Morphology

Sandfly eggs are generally elongate and ellipsoidal in shape. The sculptured outer layer (exochorion) is sticky which allows a species like L. panamensis to lay its eggs singly on the oviposition substrate. The eggs remain in place and are difficult to dislodge even with considerable jarring. The term chorion used in the following descriptions refers to the outer layer (exochorion). The described results are presented according to the format of Matsuo et al. (1974). Egg measurements are in microns. The number (N) measured is followed by the range of variation and the mean in parenthesis. L indicates the length and W the width at the widest point. The descriptions follow the nomenclature set forth by Barretto (1941).

Lutzomyia panamensis (Shannon), 1926.

Figures 18-20.

Size: N = 43; L 364-429 (398); W 102-136 (122).

Chorion: Reticulation composed of flattened irregular raised areas shaped like mesas, approximately equidistant from each other; surface of mesas uneven and pitted; the area between mesas very rugous at 2000 X magnification.

Remarks: The chorion is unlike any of the descriptions by Barretto (1941). The egg is similar to but longer and wider than L. pessoana or P. wellcomei. The adults and larvae of these three species are also very similar morphologically.

Figure 18. Egg L. panamensis 200 X.

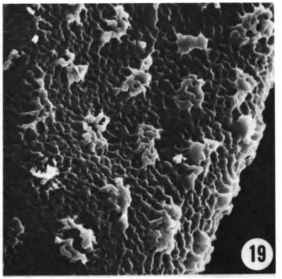
Figure 19. Egg L. panamensis 1000 X.

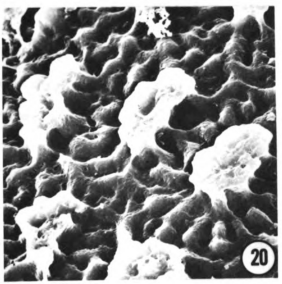
Figure 20. Egg L. panamensis 2000 X.

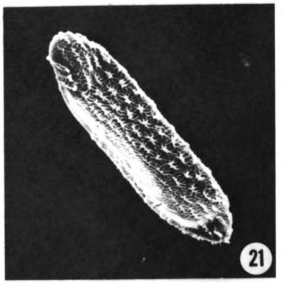
Figure 21. Egg L. pessoana 200 X.

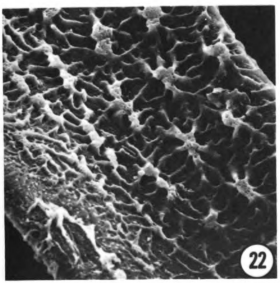
Figure 22. Egg L. pessoana 1000 X.

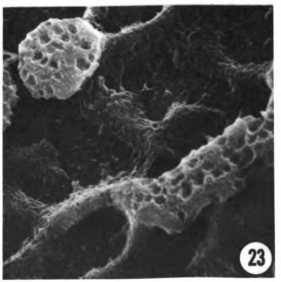
Figure 23. Egg L. pessoana 5000 X.











Figures 18-23

Lutzomyia pessoana Barretto, 1955.

Figures: 21-23.

Size: N = 9; L 357-391 (377); W 95-129 (103).

Chorion: Similar to L. panamensis except mesa-like prominences elongated; not as rugous as L. panamensis with irregular shaped cells formed between lower irregular ridges at 5000 X magnification.

Remarks: The egg is similar but usually shorter than L. panamensis or P. wellcomei.

Lutzomyia sanguinaria Fairchild and Hertig, 1957.

Figures: 24-26.

Size: N = 8; L 344-388 (372); W 95-122 (108).

Chorion: Conspicuous narrow longitudinal ridges more or less parallel; connecting crossridges form pentagonal, hexagonal or polygonal cells; ridges generally continuous except where damaged.

Remarks: The eggs are similar to L. trapidoi. The surface structure is closely related also those eggs described by Barretto (1941).

Lutzomyia trapidoi Fairchild and Hertig, 1952.

Figures: 27-28

Size: N = 14; L 327-378 (350); W 92-122 (104).

Chorion: Conspicuous narrow longitudinal ridges more or less parallel; crossridges of the same consistency forming generally elongated rectangular cells; ridges seem more discontinuous than L. sanguinaria.

Figure 24. Egg L. sanguinaria 200 X.

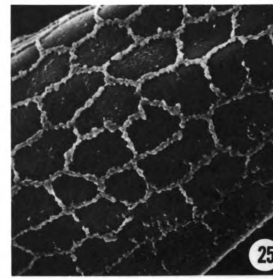
Figure 25. Egg L. sanguinaria 1000 X.

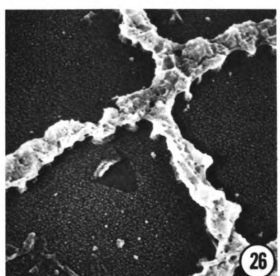
Figure 26. Egg L. sanguinaria 5000 X.

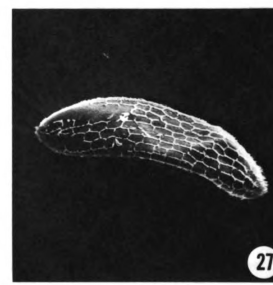
Figure 27. Egg L. trapidoi 200 X.

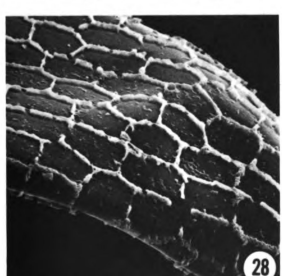
Figure 28. Egg L. trapidoi 1000 X.

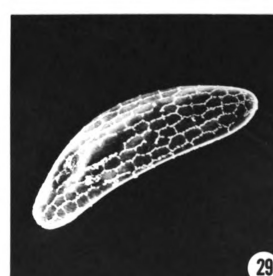
Figure 29. Egg L. ylephiletor 200 X.











Figures 24-29

Remarks: The egg is similar to L. sanguinania but generally smaller in size.

Lutzomyia ylephiletor Fairchild and Hertig, 1952.

Figures: 29-31.

Size: N = 6; L 333-374 (250); W 92-106 (101).

Chorion: Conspicuous flattened longitudinal ridges more or less parallel formed from flattened globular mounds discontinuous in pattern at 2000 X; crossridges formed the same way; ridges may be discontinuous, cells somewhat elongate rectangular to polygonal, no distinct pattern to cells.

Lutzomyia gomezi Nitzulescu, 1931.

Figures: 32-33.

Size: N = 18; L 300-347 (332); W 95-122 (104).

Chorion: Very conspicuous wide flattened longitudinally directed ridges; crossridges narrow and oblique to longitudinal ridges; cells are elongate polygons at 1000 X magnification.

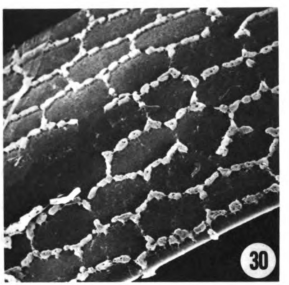
Remarks: The egg is similar to Lutzomyia monticola as described by Barretto (1941).

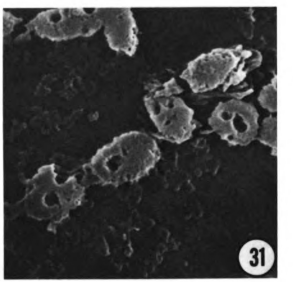
Figure 30. Egg L. ylephiletor 1000 X.

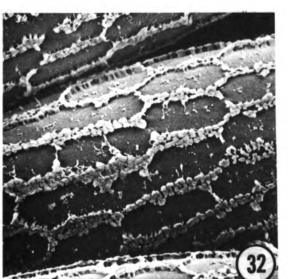
Figure 31. Egg L. ylephiletor 5000 X.

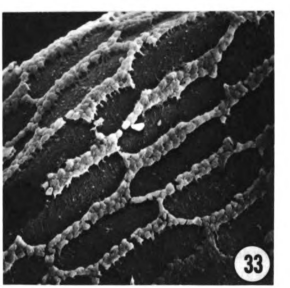
Figure 32. Egg L. gomezi 1000 X.

Figure 33. Egg L. gomezi 1000 X.









Figures 30-33

Morphology of the Larvae and Pupa

The egg stage of L. panamensis has been described previously. The setal nomenclature used in this study follows that of Barretto (1941). Hanson's (1968) description of the fourth instar of L. panamensis supplements the data presented in this work. The average measurements and ranges of the body structures and setae of ten specimens of each instar are presented in Appendix H, with the averages included in the text. The morphology of the fourth instar is described in full and the first, second, and third instars are described where they differ from each other. Only the dorsal and ventral aspects of the first and fourth instars and the lateral aspect of the pupa are figured because the second and third instars are essentially the same as the fourth, except for size.

Fourth Instar

Body length from front of head to base of caudal setae 2.975 mm; flattened dorsoventrally, grey in color, head darker grey; body covered with barnacle-like projections (Figure 37); prominent sclerotized protuberances bear conspicuous setae (Figures 34 and 35).

Head.--Head capsule longer (262μ) than wide (246μ), frontal area prominent, frontoverticular angle approaching 90°. Gular region strongly inflected. Antennal tubercles

Figure 34. Head, thorax, and abdominal segments 1, 8, and 9 of the fourth larval instar; dorsal aspect.

Setal Nomenclature--*Lutzomyia panamensis*--Larvae

Head

ant t	antennal tubercle
ant 1-2	antennal segments
ap	antennal apical process
af	anterior frontals
pf	posterior frontals
lv	lateral vertical
dv	dorsal vertical
c	clypeal
dg	dorsal genal

Prothorax

dsa	dorsal shoulder accessory
aid	anterior internal dorsal
aed	anterior external dorsal
pid	posterior internal dorsal
ped	posterior external dorsal
avl	anterior ventrolateral
pvl	posterior ventrolateral
dl	dorsolateral
as	anterior spiracle

Meso and Metathorax and Abdominal Segments 1-8

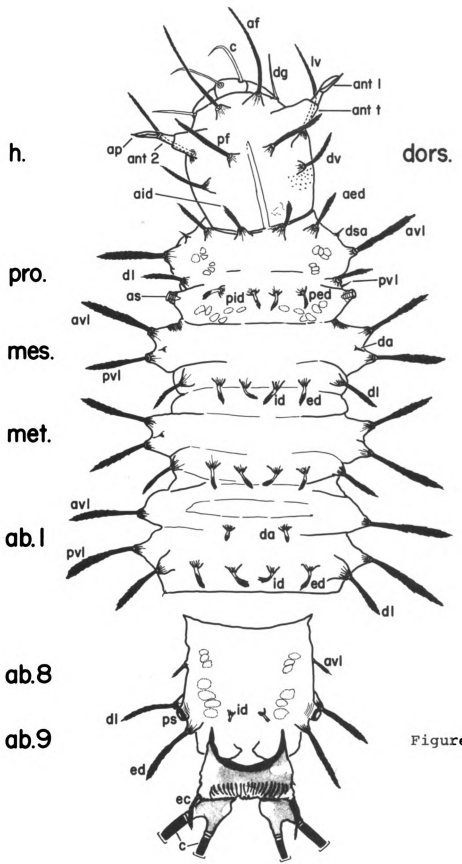
id	internal dorsal
ed	external dorsal
da	dorsal accessory

Remainder of nomenclature as above

Abdominal Segment 9

ps	posterior spiracle
ec	external caudal
c	caudal

Remainder of nomenclature as above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dl
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id
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ed
ec
c

Figure 34

Figure 35. Head, thorax, and abdominal segments 1, 8, and 9 of the fourth larval instar; ventral aspect.

Setal Nomenclature--*Lutzomyia panamensis*--Larvae

Head

lg lateral genal
vg ventral genal

Prothorax

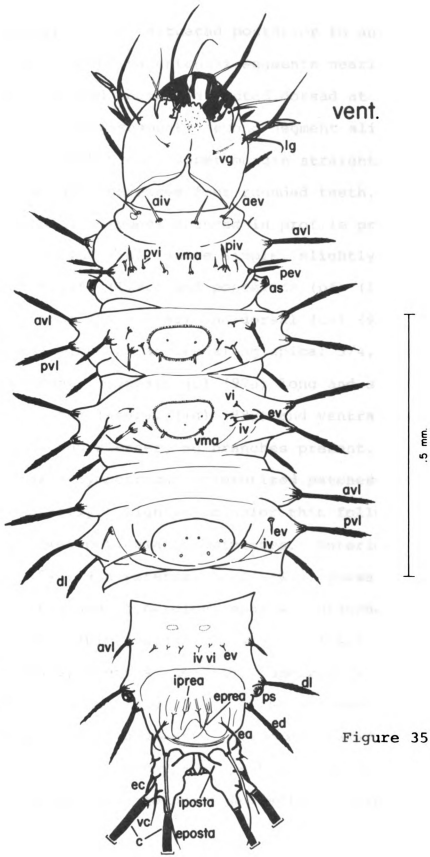
aiv anterior internal ventral
aev anterior external ventral
piv posterior internal ventral
pev posterior external ventral
pvi posterior ventral intermediary
vma ventral median accessory

Meso and Metathorax and Abdominal Segments 1-9

iv internal ventral
ev external ventral
vi ventral intermediary
Remainder of nomenclature as above

Abdominal Segment 9

i pre a internal pre anal
e pre a external pre anal
i post a internal posterior anal
e post a external posterior anal
ea external anal
vc ventral caudal
Remainder of nomenclature as above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.5 mm.

Figure 35

as long as antennae (71μ), situated posterior to anterior frontals. First and second antennal segments nearly equal in length, second segment directed dorsad at a slight angle from first segment; second segment slightly wider. Mandibles dark brown; upper margin straight curving at apex; lower margin possesses four rounded teeth. Mentum dark brown, toothed. Clypeus rounded in profile protruding over labrum-epipharynx. All setae simple, slightly curved anterad; anterior (af) (172μ) and posterior (pf) (121μ) frontals and lateral (lv) (164μ) and dorsal (dv) (90μ) verticals possess minute branches along apical 3/4, darker than head integument; clypeals (c) (97μ) long and slender; dorsal (dg) (80μ) and lateral (lg) (88μ) and ventral (vg) (38μ) genals long and slender, no branches present.

Prothorax.--Symmetrical sclerotized patches present dorsally and ventrally. Lighter in color than following body segments, fewer cuticular prominences. Anterior internal (aid) (54μ) and external (aed) (65μ) dorsals proclinate, on prominent tubercles, sparsely branched, pigmented; dorsal shoulder accessories (dsa) (18μ) laterad to anterior dorsals, short spine-like; posterior external (ped) (32μ) and internal (pid) (29μ) dorsals almost equal in length, brush-tipped, pigmented, directed caudad; dorsolaterals (dl) (65μ) laterad to posterior dorsals and anterad to conspicuous anterior spiracles, curved,

brush-tipped, situated on prominent tubercles; anterior ventrolaterals (avl) (117μ) large, stout, directed slightly anterad and ventrad, anterad to spiracle, pigmented, brush-tipped; posterior ventrolaterals (pvl) (15μ) small, spine-like, directly below dorsolaterals, lightly pigmented. Ventral setae lightly pigmented; anterior internal (aiv) (56μ) and external (aev) (65μ) ventrals simple, projecting anterad; posterior external ventrals (pev) (68μ) arise from large tubercles, stout, brush-tipped; posterior internal ventrals (piv) (43μ) shorter than posterior external ventrals, comb-tipped; posterior ventral intermediaries (pvi) (22μ) short, spine-like, lie anterad between posterior internal and external ventrals; ventral median accessories (vma) (20μ) small, comb-tipped.

Meso and Metathorax.--Dorsal and lateral setae pigmented and brush-tipped except for dorsal accessories. Internal (id) (34μ) and external (ed) (37μ) dorsals larger than those of prothorax, directed caudad and mesad; dorsolaterals (72μ) slightly larger than those of prothorax, arise from prominent tubercles, projected caudad; anterior ventrolaterals (125μ) longer than those of prothorax, curved ventrad at apex and directed slightly cephalad; posterior ventrolaterals (114μ) slightly smaller than anterior ventrolaterals, curved ventrad and directed caudad; dorsal accessories (da) (19μ) slightly dorsad and mesad of

ventrolaterals, spine-like, inconspicuous. Ventral setae lightly pigmented; internal ventrals (iv) (41μ) almost equal to those of prothorax, comb-tipped, directed caudad and ventrad; external ventrals (ev) (72μ) almost equal to those of prothorax, brush-tipped, arise from prominent tubercles, directed caudad and ventrad; ventral intermediaries (vi) (24μ) same as prothorax; ventral median accessories (12μ) smaller than those of prothorax otherwise similar.

Abdominal segments 1-7.--Dorsal and lateral setae pigmented, brush-tipped; internal (28μ) and external (29μ) dorsals equal in length, directed caudad, become smaller progressing from first to seventh segment, shorter than those of thorax; dorsoventrals (109μ) long, stout, arise from large sclerotized tubercles, directed caudad, curved apically, longer than those of thorax; dorsal accessories (16μ) (Figure 37) small, directed caudad, different shaped than those of meso and metathorax; anterior ventrolaterals (118μ) slightly smaller than posterior ventrolaterals (124μ), both setae long, stout, anteriors directed laterad curved ventrad, posteriors directed caudad curved ventrad. Ventrals long and slender, lightly pigmented; internal ventrals (52μ) on edges of large abdominal prolegs, directed caudad, curved apically, longer than those of thorax; external ventrals (42μ) much shorter than those of thorax, 1½ times

longer than those of third instar, curved apically, directed caudad; ventral intermediaries absent.

Abdominal segment 8.--Heavily sclerotized dorsally and laterally, bearing numerous small spines directed caudad. Dorsal and lateral setae pigmented, brush-tipped; internal dorsals (16μ) small directed caudad; external dorsals (121μ) long, stout, directed caudad, curved apically ventrad, much longer than those of other abdominal and thoracic segments; dorsolaterals (94μ) ventrad to large posterior spiracles, shorter than those of other abdominal segments, directed caudad, curved apically ventrad; dorsal accessories and posterior ventrolaterals absent; anterior ventrolaterals (36μ) medium in length, stout, directed caudad, shorter than those of other abdominal and thoracic segments. Ventral setae short, lightly pigmented, directed caudad, curved apically; ventral intermediaries (5μ) present.

Abdominal segment 9.--External caudals (ec) (63μ) long, stout, brush-tipped, directed caudad, curved. Caudal segment bears two bifurcated caudal setae (c) (3.509 mm), each setae bears a point of separation (node) at the base (Figure 36). Ventral setae simple, unbranched, varying in length and pigmented; ventral caudals (vc) (62μ) same length as external caudals, situated ventral to caudal setal fork, directed dorsad; external anals (ea) (72μ) long and slender, directed ventrad, curved apically; internal (i pre a) (35μ)

Figure 36. Caudal setae, fourth instar, L. panamensis, 1000 X.

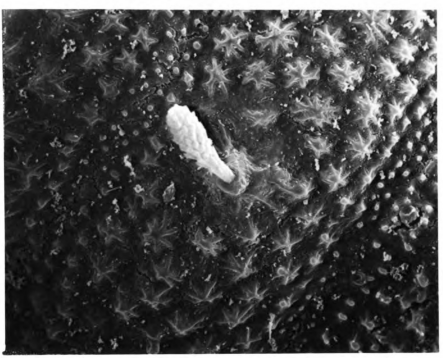

Figure 37. Dorsal accessory seta, fourth instar, L. panamensis and cuticular surface, 1000X.

and external (e pre a) (50μ) pre-anals fine and inconspicuous, directed ventrad, curved apically; external posterior anals (e post a) (151μ) very long and slender, directed ventrad, curved apically; internal posterior anals (i post a) (42μ) slender and inconspicuous, directed ventrad.

Third Instar

Body length 2.118 mm, body grey, head slightly darker; body flattened somewhat dorsoventrally.

Head.--Somewhat longer (180μ) than wide (163μ); first antennal segment darkly pigmented, antennal length 53μ.

Prothorax.--Symmetrical sclerotized patches on cuticle of dorsum.

Abdominal segments 1-7.--Posterior ventrolaterals (68μ) equal to anterior ventrolaterals (70μ) in length.

Abdominal segment 8.--External dorsals (76μ) longer than dorsolaterals (67μ).

Abdominal segment 9.--Four caudal setae (2.48 mm).

Second Instar

Body length 1.423 mm, round in shape, light grey.

Head.--Somewhat square in shape, length 140μ and width 134μ, brown in color, sclerotized. Antennae (40μ) brownish, arising from prominent sparsely spined tubercles. Setal structure same as first instar.

Prothorax.--Anterior internal dorsals (24μ) present, slightly shorter than anterior external dorsals (32μ), both sets proclinate; posterior external (9μ) and internal (10μ) dorsals small, brush-tipped, directed caudad; posterior ventrolaterals (5μ) small. Ventral setae same as other instars.

Meso and Metathorax.--Anterior ventrolaterals (41μ) large, stout with small bristles on apical 3/4, directed anterad; posterior ventrolaterals (21μ) present, large, same as anterior ventrolaterals, directed laterad.

Abdominal segments 1-7.--Posterior ventrolaterals (38μ) present, larger than same meso and metathoracic setae, slightly smaller than anterior ventrolaterals (46μ); external ventrals (10μ) smaller than same in first instar, directed ventrad.

Abdominal segment 8.--External dorsals (49μ), longest setae, much larger than same in first instar, directed caudad.

Abdominal setment 9.--External caudals (30μ) shorter than those of first instar; pair of caudal setae (1.764 mm) bifurcated making four.

First Instar

Body lengths 0.927 mm, round in shape (Figures 38 and 39).

Figure 38. Head, thorax and abdominal segments 1, 8, and 9 of the first larval instar; dorsal aspect.

Figure 39. Head, thorax and abdominal segments 1, 8, and 9 of the first larval instar; ventral aspect.

Setal nomenclature same as fourth larval instar Lutzomyia panamensis.

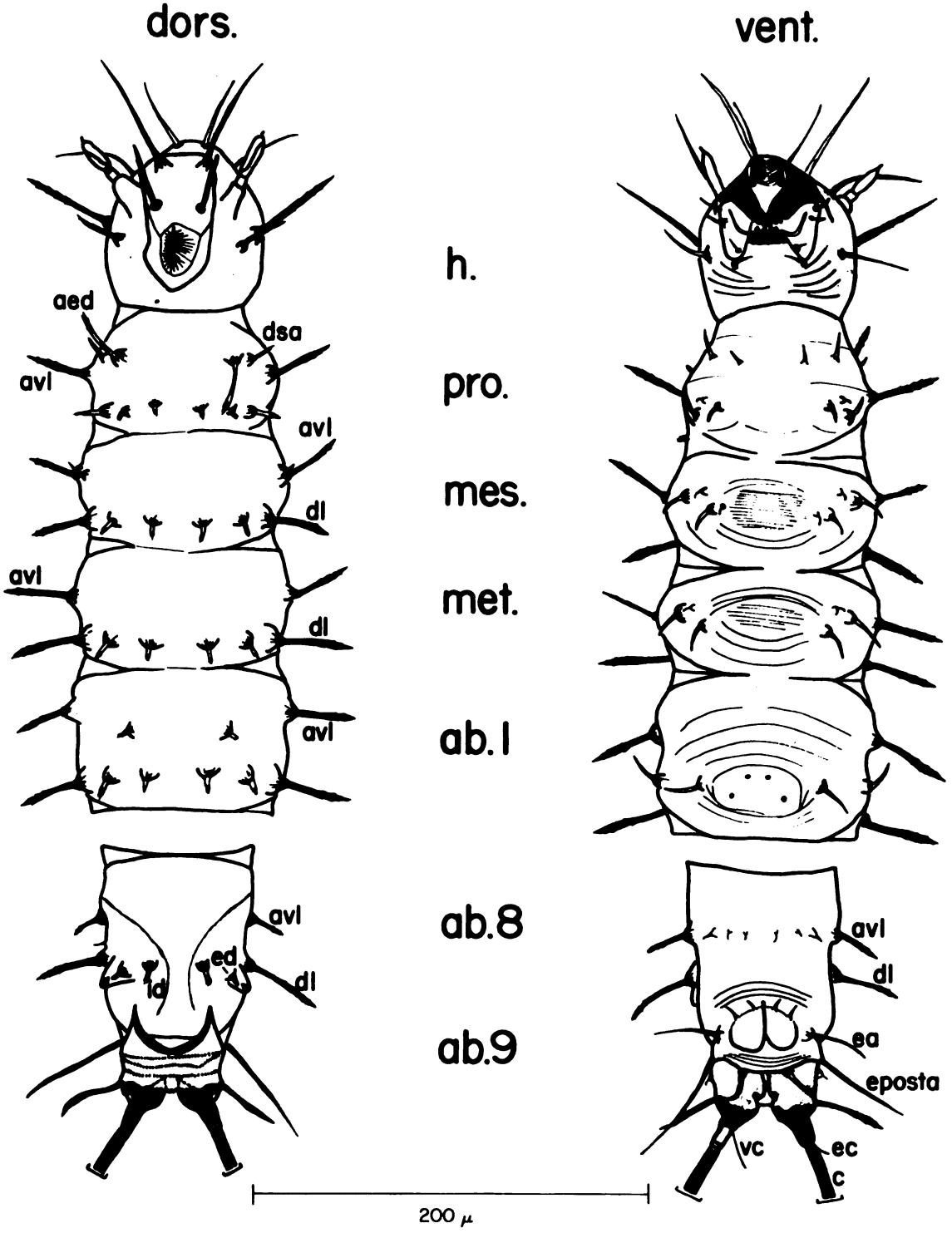

Figure 38

Figure 39

Head.--Head capsule lightly sclerotized, almost square in shape, length 94μ and width 87μ. Antennae (33μ) erect and prominent on erect tubercles. Dorsal setae simple but anterior (64μ) and posterior (29μ) frontals and lateral (45μ) and dorsal (22μ) verticals possess minute spare branches (brush-tipped) almost to base of setae. Ventral setae simple and delicate. Prominent, heavily sclerotized egg burster.

Prothorax.--Anterior spiracles small, indistinct. Anterior interior dorsals absent along with dorsal accessories mentioned by Ward (1972); anterior external dorsals (29μ) directed cephalad to head capsule; dorsal setae minutely branched apically; anterior ventrolaterals (29μ) as long as anterior external dorsals. Ventral setae simple, no branches; posterior external ventrals (16μ) longest.

Meso and Metathorax. Dorsolaterals (29μ) stout, minute branches on apical 3/4; posterior ventrolaterals absent along with anterior dorsals; external (7μ) and internal (6μ) dorsals larger than prothoracic; anterior ventrolaterals (22μ) smaller than prothoracic, directed anterad. Ventral setae similar to those of prothorax.

Abdominal segments 1-7.--Dorsal accessories (4μ) small, directed caudad; external (7μ) and internal (6μ) dorsals directed caudad, progressively smaller from first to seventh segment; ventral intermediaries and ventral

median accessories absent; external ventral (19μ) larger than those of thorax, curved; directed laterad; posterior ventrolaterals absent.

Abdominal segment 8.--Dorsal accessories absent; anterior ventrolaterals (16μ) smaller than those of other abdominal segments; dorsolaterals (31μ) directed caudad; internal dorsals (8μ) larger than external dorsals (5μ), situated on small chitinized area; posterior spiracles larger than anterior spiracles. Ventral setae small, inconspicuous, directed caudad; ventral intermediaries present.

Abdominal segment 9.--External anals (26μ) simple, curved, directed caudad; external (11μ) and internal (8μ) pre-anals simple small, curved ventrad; external (64μ) and internal (15μ) post-anals directed caudad; external caudals (33μ) larger than same in second instar extending past anal protuberance. Caudal setae (two) (1.288 mm) longer than body, conspicuous.

Pupa

Body length of pupa from pronotum to base of abdominal segment nine 2.810 mm (2.609-3.259 mm); color light to dark brown; lateral aspect Figure 40. Respiratory horn and prealar lobe prominent; pre-alar setae 90μ (82-109μ), darkly pigmented, tip blade-like, divided (trifid). Mesonotal tubercles long. Wing sheath slender.

Figure 40. Head, thorax, and abdominal segments 1-6 of the pupa; lateral aspect.

Setal Nomenclature--*Lutzomyia panamensis*--Pupa

h	head
mp	mouthparts
ant	antennae
pr	prothoracic setae
pro	prothorax
pa	pre alar setae
mt	mesontal tubercle
mes	mesothorax
ms	mesothoracic setae
met	metathorax
ab 1-6	abdomen
w	wing
l	leg
abs	dorsal abdominal setae

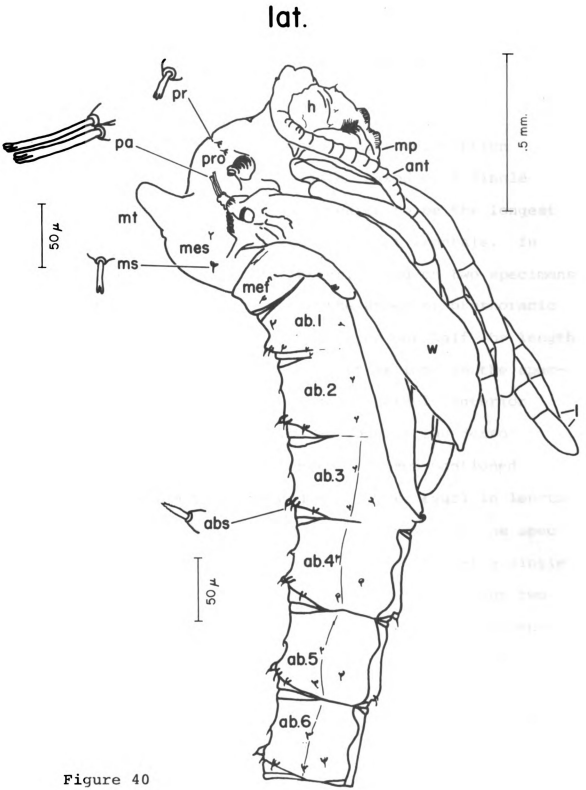
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Figure 40

Abdomen without tubercles. Dorsal setae similar in length but different in shape; pro and mesothoracic setae blade-like; dorsal abdominal setae spine-like.

Discussion

Contrary to Hanson's (1968) short description of the first instar of L. panamensis, based on a single specimen, the anterior frontals appeared to be the longest setae on the head instead of the posterior frontals. In his description of the fourth instar, based on two specimens, the prominent tubercles, on which the anterior prothoracic dorsals were situated, were stated to be one-half the length of the setae; they were only one-sixth as long in the specimens examined in this study. The prothoracic anterior ventrolaterals were almost twice as long as the dorsolaterals instead of equal in length. Hanson mentioned that the meso and metathoracic dorsals were equal in length to those of the prothorax but they were longer in the specimens measured in this study. He also stated that a single pair of anterior prothoracic dorsals were present but two pair were found. Hanson used this character to differentiate L. panamensis from Lutzomyia apicalis Floch and Abonnenc. He stated that L. panamensis fourth instar can hardly be distinguished from L. pessoana. Specimens of L. pessoana were not available for comparison in this study, but setal measuremens of L. pessoana might prove

to be the distinguishing characteristics needed to differentiate these two closely related species. It appears that Hanson was in error in some of his setal comparisons; however, if more than two specimens had been available to him these oversights might not have been made.

Ward (1972), who was not aware of Hanson's dissertation, compared his six specimens of P. wellcomei to Lutzomyia arthuri Fonseca as described by Barretto (1941). If Hanson's work had been available, Ward undoubtedly would have compared his fourth instar larval descriptions with those of L. panamensis and L. pessoana since the four species belong to the same subgenus Psychodopygus and are very similar in many respects.

Ward also used measurements of setae in his descriptions of P. wellcomei which were compared with those of L. panamensis. The average body length of L. panamensis fourth instar was smaller than P. wellcomei but the range was similar. The antennae of P. wellcomei were larger (97μ) than those of L. panamensis (71μ) but the shape was the same. Head setae were similar in size and shape. Prothoracic dorsal accessories of P. wellcomei were absent in L. panamensis. The prothoracic dorsolaterals of L. panamensis were more than twice as long as those of L. wellcomei. The dorsal setae of the meso and metathorax of L. panamensis were more than twice as long as those of

P. wellcomei. The dorsolaterals were also longer in L. panamensis. The ventral setae of L. panamensis were in general longer than those in P. wellcomei. The caudal setae of L. panamensis were longer than those of P. wellcomei. The point of separation (node) at the base of L. panamensis has not been mentioned by other sandfly investigators but is probably present in all larvae and in all instars that possess these long caudal setae. Often larvae were observed with one or two setae missing. The easy removal of these setae might serve as an escape mechanism when the caudal setae become caught or to escape from predators.

The pupal stage of L. panamensis was very similar to P. wellcomei. The pre-alar setae were similar in size and were both trifid. However, the thoracic setae of L. panamensis were blade-like which differ from the spine-like setae of P. wellcomei. The dorsal abdominal setae of both species were spine-like. Hanson indicated that the color of L. panamensis pupae was grey but those in this study were brown to dark brown.

SUMMARY AND CONCLUSIONS

The laboratory bionomics, colonization, and morphology of the immature stages of the anthropophilic phlebotomine sandfly Lutzomyia panamensis (Shannon) were studied. In addition, the SEM was used to examine the egg surface ultrastructure of L. panamensis as well as those of the other common Panamanian anthropophilic sandflies L. pessoana, L. trapidoi, L. gomezi, L. ylephiletor, and L. sanguinaria.

In an experiment to evaluate the feeding preference of L. panamensis larvae, four experimental food sources yeast, liver powder, beef blood serum and hemoglobin were placed on the same plaster lined petri dish. The first three instars preferred yeast to the other food sources. The fourth instar preference for yeast declined with an increased preference for the other food sources, especially hemoglobin. The four food sources were also compared individually on petri dishes in order to determine the effect of these single diets on the duration of the life cycle, pupation, and adult emergence. The yeast and standard larval food combination dishes produced the shortest developmental time from the first instar to pupa,

with yeast, liver powder, beef blood serum and hemoglobin, respectively, increasing developmental time. When the developmental times of the larvae reared on single food sources were compared with those of larvae reared on the combination dishes, the combination produced faster and more desirable development. Larvae reared on the combination dishes survived to produce more pupae and adults than those reared on individual diets. The plaster lined petri dish was very satisfactory as a rearing chamber and general observations of larval behavior could be made with ease.

The effects of various saturated sugar solutions on the longevity of laboratory reared females and males of L. panamensis were examined. Life tables were constructed for each of the six sugars tested and the observed life expectancies at emergence were computed. Fructose and sucrose were the most efficient in prolonging the survival of the females. Fructose, sucrose, honey, Karo syrup and dextrose appeared to provide the same male life expectancy. The styrofoam test vessel was very satisfactory in studying the longevity of these females and males.

Longevity studies also conducted on the wild-caught blood-engorged females indicated that the saturated solutions of fructose, sucrose and dextrose were the most efficient in promoting longevity. In an analysis of the

effects of these saturated sugar solutions on oviposition, no difference could be detected between groups feeding on the soaked raisin, fructose, sucrose and dextrose groups. Both longevity and maximum oviposition are important factors in rearing and colonizing sandflies. With extended longevity it is possible to refeed the wild-caught females on a laboratory host following the first oviposition period and have them produce a second batch of eggs. This would make maximum use out of the sometimes hard to collect wild-caught blood-engorged females. If no sugar source is provided the females do not live to feed a second time and lay only about one-half of their complement of eggs. In all the experimental tests conducted with both laboratory reared adults and wild-caught females, fructose and sucrose were consistently preferred by the adults.

Four laboratory generations of L. panamensis were reared. The average length of the life cycle from oviposition to adult was 37.7 days. The unglazed Boston bean pot was the most satisfactory for rearing L. panamensis in large numbers. The optimum condition of the plaster substrate was damp and smooth in texture. The mixture of liver powder, hemoglobin and beef blood serum added during the third instar to the yeast already present in the pots was shown to be the best larval medium tested. The spiny rat served very well as the laboratory host. The double

feeding method of using the cloth releasing cage and the plastic feeding cage was the most successful in feeding the largest number of laboratory reared adults with little injury to the females.

The morphology of the immature stages of L. panamensis was redescribed and clarified. Hoyer's medium proved to be satisfactory as a mounting and clearing medium. Ink drawings of the dorsal and ventral aspects of the first and fourth instars and the lateral aspect of the pupa were illustrated. Measurements of the prominent setae were very useful in differentiating changes between instars and other closely related species. Scanning electron photographs of the fourth instar served as an aid in describing the nature of the cuticular surface. The use of the scanning electron microscope in describing the egg surface structure of the anthropophilic sandfly species proved to be very useful. Two distinct patterns were shown. L. panamensis and L. pessoana eggs had a surface structure composed of raised mesa-like prominences with the remainder of the surface very rugose in appearance. The egg surface structures of L. trapidoi, L. ylephiletor, L. gomezi and L. sanguinaria were made up of cells of varying shapes bordered by ridges of varying thicknesses.

APPENDICES

APPENDIX A. LONGEVITY OF LABORATORY REARED L. PANAMENSIS FEMALES AND MALES FEEDING ON FRUCTOSE

Age interval days (x)	Number alive at x: ℓ_x		Number dying at x: d_x		Number of days lived $L_x = \ell_x - \frac{1}{2}d_x$		Total number of days lived T_x		Observed life expectancy $e_x = \frac{T_x}{\ell_x}$	
	Females	Males	Females	Males	Females	Males	Females	Males	Females	Males
1	70	70	0	6	70	67	976.0	697.0	13.9	10.0
2	70	64	4	8	68	60	906.0	630.0	12.9	9.8
3	66	56	1	5	65.5	53.5	838.0	570.0	12.6	10.2
4	65	51	1	3	64.5	49.5	772.5	516.5	11.9	10.1
5	64	48	1	3	63.5	46.5	708.0	467.0	11.1	9.7
6	63	45	1	2	62.5	44	644.5	420.5	10.2	9.3
7	62	43	0	0	62	43	582.0	376.5	9.4	8.8
8	62	43	5	1	59.5	42.5	520.0	333.5	8.4	7.6
9	57	42	6	6	54	39	460.5	291.0	8.1	6.9
10	51	36	3	3	49.5	34.5	406.5	252.0	8.0	7.0
11	48	33	2	2	47	32	357.0	217.5	7.4	6.5
12	46	31	2	0	45	31	310.0	185.5	6.7	6.0
13	44	31	1	3	43.5	29.5	265.0	154.5	6.0	5.0
14	43	28	4	6	41	25	222.5	125.0	5.2	4.7
15	39	22	3	0	37.5	22	181.5	100.0	4.7	4.5
16	36	22	2	3	35	20.5	144.0	78.0	4.0	3.5
17	34	19	6	3	31	17.5	109.0	57.5	3.2	3.0
18	28	16	6	3	25	14.5	78.0	40.0	2.8	2.5
19	22	13	10	3	17	11.5	53.0	25.5	2.4	2.0
20	12	10	2	5	11	7.5	36.0	14.0	3.0	1.4
21	10	5	3	1	8.5	4.5	25.0	6.5	2.5	1.3
22	7	4	2	4	6	2	16.5	2	2.4	0.5
23	5		1		4.5		10.5		2.1	
24	4		2		3		6.0		1.5	
25	2		0		2		3.0		1.5	
26	2		2		1		1.0		0.5	

APPENDIX B. LONGEVITY OF LABORATORY REARED L. PANAMENSIS FEMALES AND MALES FEEDING ON SUCROSE

Age interval days (x)	Number alive at x: ℓ_x		Number dying at x: d_x		Number of days lived $L_x = \ell_x - \frac{1}{2}d_x$		Total number of days lived T_x		Observed life expectancy $e_x = \frac{T_x}{\ell_x}$	
	Females	Males	Females	Males	Females	Males	Females	Males	Females	Males
1	70	69	0	1	70	68.5	943.5	630.5	13.5	9.1
2	70	68	2	11	69	62.5	873.5	562.0	12.5	8.3
3	68	57	4	5	66	54.5	804.5	499.5	11.8	8.8
4	64	52	1	2	63.5	51	738.5	445.0	11.5	8.6
5	63	50	1	0	62.5	50	675.0	394.0	10.7	7.9
6	62	50	2	0	61	50	612.5	344.0	9.9	6.9
7	60	50	2	3	59	48.5	551.5	294.0	9.2	5.9
8	58	47	1	9	57.5	42.5	492.5	245.5	8.5	5.2
9	58	38	3	4	56.5	36	435.0	203.0	7.5	5.4
10	55	34	4	2	53	33	378.5	167.0	6.9	4.9
11	51	32	2	5	50	29.5	325.5	134.0	6.4	4.2
12	49	27	3	4	47.5	25	275.5	104.5	5.6	3.9
13	46	23	6	2	43	22	228.0	79.5	4.9	3.6
14	40	21	8	5	36	18.5	185.0	57.5	4.6	2.7
15	32	16	4	2	30	15	149.0	39.0	4.6	2.4
16	28	14	5	8	25.5	10	119.0	24.0	4.2	1.7
17	23	6	4	1	21	5.5	93.5	14.0	4.0	2.3
18	19	5	3	2	17.5	4	72.5	8.5	3.8	1.7
19	16	3	3	1	15.5	2.5	55.0	4.5	3.4	1.5
20	13	2	3	3	11.5	1.5	39.5	2.0	3.0	1.0
21	10	1	2	1	9	0.5	28	0.5	2.7	0.5
22	8		2		7		19.0		2.4	
23	6		3		4.5		12.0		2.0	
24	3		1		2.5		7.5		2.5	
25	2		0		2		5.0		2.5	
26	2		1		1.5		3.0		1.5	
27	1		0		1		1.5		1.5	
28	1		1		0.5		0.5		0.5	

APPENDIX C. LONGEVITY OF LABORATORY REARED L. PANAMENSIS FEMALES AND MALES FEEDING ON HONEY

Age interval days (x)	Number alive at x: ℓ_x		Number dying at x: d_x		Number of days lived $L_x = \ell_x - \frac{1}{2}d_x$		Total number of days lived T_x		Observed life expectancy $e_x = \frac{T_x}{\ell_x}$	
	Females	Males	Females	Males	Females	Males	Females	Males	Females	Males
1	61	72	2	0	60	72	646.5	637.0	10.6	8.8
2	59	72	0	9	59	67.5	586.5	565.0	9.9	7.8
3	59	63	5	6	56.5	60	527.5	497.5	8.9	7.9
4	54	57	1	3	53.5	55.5	471.0	437.5	8.7	7.8
5	53	54	4	5	51	52.5	417.5	382.0	7.9	7.1
6	49	49	5	1	46.5	48.5	366.5	329.5	7.5	6.7
7	44	48	2	2	43	47	320.0	281.0	7.3	5.9
8	42	46	2	3	41	44.5	277.0	234.0	6.6	5.1
9	40	43	2	9	39	38.5	236.0	189.5	5.9	4.4
10	38	34	2	3	37	32.5	197.0	151.0	5.2	4.4
11	36	31	4	5	34	28.5	160.0	118.5	4.4	3.8
12	32	26	4	4	30	24	126.0	90.0	3.9	3.5
13	28	24	6	8	25	20	96.0	66.0	3.4	2.8
14	22	16	5	4	19.5	14	71.0	46.0	3.2	2.9
15	17	12	4	3	15	10.5	51.5	32.0	3.0	2.7
16	13	9	2	1	12	8.5	36.5	21.5	2.8	2.4
17	11	8	4	2	9	7	24.5	13.0	2.2	1.6
18	7	6	3	5	5.5	3.5	15.5	6.0	2.2	1.0
19	4	1	2	0	3	1	10.0	2.5	2.5	2.5
20	2	1	0	0	2	1	7.0	1.5	3.5	1.5
21	2	1	0	1	2	0.5	5.0	0.5	2.5	0.5
22	2		1		1.5		3.0		1.5	
23	1		0		1		1.5		1.5	
24	1		1		0.5		0.5		0.5	

APPENDIX D. LONGEVITY OF LABORATORY REARED L. PANAMENSIS FEMALES AND MALES FEEDING ON KARO SYRUP

Age interval days (x)	Number alive at x: ℓ_x		Number dying at x: d_x		Number of days lived $L_x = \ell_x - \frac{1}{2}d_x$		Total number of days lived T_x		Observed life expectancy $e_x = \frac{T_x}{\ell_x}$	
	Females	Males	Females	Males	Females	Males	Females	Males	Females	Males
1	67	70	0	4	67	68	714.5	585.5	10.7	8.4
2	67	66	6	8	64	62	647.5	517.5	9.7	7.8
3	61	54	5	5	58.5	51.5	583.5	455.5	9.6	8.4
4	56	49	2	3	55	47.5	525.0	404.0	9.4	8.2
5	54	46	3	3	52.5	44.5	470.0	356.5	8.7	7.8
6	51	43	4	2	49	42	417.5	312.0	8.2	7.3
7	47	41	2	4	46	39	368.5	270.0	7.8	6.6
8	45	37	0	2	45	36	322.5	231.0	7.2	6.2
9	45	35	7	1	41.5	34.5	277.5	195.0	6.2	5.6
10	38	34	3	2	36.5	33	236.0	160.5	6.2	4.7
11	35	32	4	4	33	30	199.5	127.5	5.7	4.0
12	31	28	4	1	29	27.5	166.5	97.5	5.4	3.5
13	27	27	0	6	27	24	137.5	69.5	5.1	2.6
14	27	21	5	7	24.5	17.5	110.5	45.5	4.1	2.2
15	22	14	3	4	20.5	12	86.0	28.0	3.9	2.0
16	19	10	5	4	16.5	8	65.5	16.0	3.4	1.6
17	14	6	2	3	13	4.5	49.0	8.0	3.5	1.3
18	12	3	3	1	10.5	2.5	36.0	3.5	3.0	1.2
19	9	2	3	2	7.5	1	25.5	1.0	2.8	0.5
20	6		2		5		18.0		3.0	
21	4		0		4		13.0		3.3	
22	4		1		3.5		9.0		2.3	
23	3		2		2		5.5		1.8	
24-26	1		0		1		3.5-1.5		3.5-1.5	
27	1		1		0.5		0.5		0.5	

APPENDIX E. LONGEVITY OF LABORATORY REARED L. PANAMENSIS FEMALES AND MALES FEEDING ON DEXTROSE

Age interval days (x)	Number alive at x: ℓ_x		Number dying at x: d_x		Number of days lived $L_x = \ell_x - \frac{1}{2}d_x$		Total number of days lived T_x		Observed life expectancy $e_x = \frac{T_x}{\ell_x}$	
	Females	Males	Females	Males	Females	Males	Females	Males	Females	Males
1	70	70	1	7	69.5	66.5	688.0	556.0	9.8	7.9
2	69	63	3	13	67.5	56.5	618.5	489.5	9.0	7.8
3	66	50	4	4	64	48	551.0	433.0	8.3	8.7
4	62	46	2	2	61	45	487.0	385.0	7.9	8.4
5	60	44	3	1	58.5	43.5	426.0	340.0	7.1	7.7
6	57	43	4	0	55	43	367.5	296.5	6.4	7.0
7	53	43	4	1	51	42.5	312.5	253.5	6.0	6.0
8	49	42	4	12	47	36	261.5	211.0	5.3	5.0
9	45	30	8	1	41	29.5	214.5	175.0	4.8	5.8
10	37	29	4	4	35	27	173.5	145.5	4.7	5.0
11	33	25	2	2	32	24	138.5	118.5	4.2	4.7
12	31	23	11	4	25.5	21	106.5	94.5	3.4	4.1
13	20	19	2	2	19	18	81.0	73.5	4.1	3.9
14	18	17	2	5	17	14.5	62.0	55.5	3.4	3.3
15	16	12	5	0	13.5	12	45.0	41.0	2.8	3.4
16	11	12	6	3	8	10.5	31.5	29.0	2.9	2.4
17	5	9	1	4	4.5	7	23.5	18.5	4.7	2.1
18	4	5	0	2	4	4	19.0	11.5	4.8	2.3
19	4	3	0	1	4	2.5	15.0	7.5	3.8	2.5
20	4	2	3	0	2.5	2	11.0	5.0	2.8	2.5
21	1	2	0	1	1	1.5	8.5	3.0	8.5	1.5
22	1	1	0	0	1	1	7.5	1.5	7.5	1.5
23	1	1	0	1	1	0.5	6.5	0.5	6.5	0.5
24-28	1		0		1		5.5-1.5		5.5-1.5	
29	1		1		0.5		0.5		0.5	

APPENDIX F. LONGEVITY OF LABORATORY REARED L. PANAMENSIS FEMALES AND MALES FEEDING ON MALTOSE

Age interval days (x)	Number alive at x: ℓ_x		Number dying at x: d_x		Number of days lived $L_x = \ell_x - \frac{1}{2}d_x$		Total number of days lived T_x		Observed life expectancy $e_x = \frac{T_x}{\ell_x}$	
	Females	Males	Females	Males	Females	Males	Females	Males	Females	Males
1	64	69	0	6	64	66	295.0	206.5	4.6	3.0
2	64	63	17	30	55.5	48	231.0	140.5	3.6	2.2
3	47	33	15	18	39.5	24	175.5	92.5	3.7	2.8
4	32	15	8	5	28	12.5	136.0	68.5	4.3	4.7
5	24	10	6	2	21	9	108.0	56.0	4.5	5.6
6	18	8	4	1	16	7.5	87.0	47.0	4.8	5.9
7	14	7	4	0	12	7	71.0	39.5	5.1	5.6
8	10	7	2	1	9	6.5	59.0	32.5	5.9	4.6
9	8	6	0	2	8	5	50.0	26.0	6.3	4.3
10	8	4	0	0	8	4	42.0	21.0	5.3	5.3
11	8	4	1	2	7.5	3	34.0	17.0	4.3	4.3
12	7	2	2	0	6	2	26.5	14.0	3.8	7.0
13	5	2	1	0	4.5	2	20.5	12.0	4.1	6.0
14	4	2	0	0	4	2	16.0	10.0	4.0	5.0
15	4	2	2	0	3	2	12.0	8.0	3.0	4.0
16	2	2	0	0	2	2	9.0	6.0	4.5	3.0
17	2	2	0	0	2	2	7.0	4.0	3.5	2.0
18	2	2	1	1	1.5	1.5	5.0	2.0	2.5	1.0
19	1	1	0	1	1	0.5	3.5	0.5	3.5	0.5
20-21	1		0		1		2.5-1.5		2.5-1.5	
22	1		1		0.5		0.5		0.5	

APPENDIX G. LONGEVITY OF LABORATORY REARED L. PANAMENSIS FEMALES AND MALES WITHOUT SUGAR SOURCE

Age interval days (x)	Number alive at x: ℓ_x		Number dying at x: d_x		Number of days lived $L_x = \ell_x - \frac{1}{2}d_x$		Total number of days lived T_x		Observed life expectancy $e_x = \frac{T_x}{\ell_x}$	
	Females	Males	Females	Males	Females	Males	Females	Males	Females	Males
1	65	66	1	17	64.5	58.5	118.5	84.0	1.8	1.3
2	64	49	32	48	48	25	54	25.5	0.8	0.5
3	12	1	12	1	6	0.5	6	0.5	0.5	0.5

APPENDIX H. BODY AND CHAETOTAXAL MEASUREMENTS OF THE IMMATURE STAGES OF L. PANAMENSIS

	Average length (range) Instar (10 specimens measured for each instar)			
	Fourth	Third	Second	First
Body length (mm)[a]	2.975 (2.395-3.633)	2.118 (1.650-2.418)	1.423 (1.293-1.534)	0.927 (0.827-1.061)
Head:				
Head length (μ)[b]	262 (245-295)	180 (163-211)	140 (116-157)	94 (86-101)
Head width	246 (218-263)	163 (151-182)	134 (118-155)	87 (81-97)
Antennal length	71 (58-79)	53 (52-57)	40 (36-45)	33 (28-35)
Ant. frontal setae	172 (160-182)	119 (115-124)	87 (77-97)	64 (60-68)
Lat. vertical setae	164 (131-179)	105 (93-111)	68 (63-71)	45 (40-50)
Post. frontal setae	121 (109-144)	71 (66-76)	43 (33-47)	29 (26-34)
Dos. vertical setae	90 (71-108)	50 (45-55)	32 (29-36)	22 (19-29)
Clypeal setae	97 (75-113)	69 (55-81)	46 (40-50)	32 (28-35)
Dors. genal setae	80 (68-96)	59 (55-67)	46 (43-50)	40 (29-45)
Lat. genal setae	88 (73-113)	56 (48-66)	43 (39-48)	29 (27-32)
Vent. genal setae	38 (25-47)	25 (21-32)	19 (15-23)	11 (10-13)
Prothorax:				
Ant. int. dorsal setae	54 (43-66)	35 (29-40)	24 (18-27)	--
Ant. ext. dorsal setae	65 (57-78)	39 (32-44)	32 (26-37)	29 (24-32)
Dors. shoulder acces. setae	18 (10-26)	13 (10-18)	10 (8-13)	8 (6-11)
Post. int. dorsal setae	29 (24-36)	16 (15-19)	10 (8-12)	5 (3-6)
Post ext. dorsal setae	32 (26-40)	16 (14-19)	9 (8-11)	5 (2-8)
Ant. ventrolateral setae	117 (98-147)	71 (62-79)	44 (40-49)	29 (26-32)
Post. ventrolateral setae	15 (11-19)	9 (7-14)	5 (5-7)	--[c]

[a]Body length and caudal setae measured in millimeters.

[b]All other body setae measured in microns.

[c]Setae present but too small to measure.

APPENDIX H--Continued

	Average lengths (range) Instar			
	Fourth	Third	Second	First
Prothorax cont.				
Dorsolateral setae	65 (58-82)	33 (30-36)	19 (16-21)	6 (7-15)
Ant. int. vent. setae	56 (46-63)	32 (23-40)	21 (15-27)	8 (6-11)
Ant. ext. vent. setae	65 (53-77)	40 (34-52)	26 (21-34)	13 (9-16)
Post. int. vent. setae	43 (31-61)	28 (21-37)	22 (15-29)	12 (7-15)
Post. ext. vent. setae	68 (45-90)	33 (27-40)	27 (23-32)	16 (13-19)
Post. vent. intermed. setae	22 (15-27)	13 (10-15)	9 (8-12)	5 (4-7)
Vent. median acces. setae	20 (15-24)	12 (8-21)	6 (4-8)	--[a]
Meso and Metathorax:				
Dors. acces. setae	19 (15-26)	12 (10-17)	8 (7-10)	6 (5-8)
Int. dorsal setae	34 (25-39)	19 (16-25)	12 (10-15)	6 (5-7)
Ext. dorsal setae	37 (31-47)	19 (16-23)	12 (10-15)	7 (5-9)
Dorsolateral setae	72 (56-87)	38 (35-44)	26 (23-30)	29 (23-34)
Ant. ventrolateral setae	125 (104-142)	68 (58-79)	41 (36-45)	22 (17-26)
Post. ventrolateral setae	114 (81-137)	58 (52-65)	21 (17-28)	--
Int. vent. setae	41 (25-50)	26 (18-36)	22 (18-26)	11 (6-14)
Ext. vent. setae	72 (63-81)	33 (23-44)	29 (26-33)	16 (13-19)
Vent. intermed. setae	24 (13-31)	14 (11-17)	10 (8-13)	5 (3-7)
Vent. median acces. setae	12 (8-15)	9 (7-14)	5 (3-7)	--[a]
Abdomin. Segments 1-7:				
Int. dorsal setae	28 (19-37)	16 (11-19)	11 (8-15)	6 (4-7)
Ext. dorsal setae	29 (21-44)	19 (14-24)	11 (8-14)	7 (6-8)
Dorsolateral setae	109 (85-127)	60 (55-71)	37 (32-42)	34 (31-39)
Dors. acces. setae	16 (15-19)	10 (8-11)	6 (5-7)	4 (3-5)

[a]Setae present but too small to measure.

APPENDIX H--Continued

	Average length (range) Instar			
	Fourth	Third	Second	First
Abdom. Segments 1-7--Cont.				
Ant. ventrolateral setae	118 (94-142)	70 (55-81)	46 (44-50)	22 (18-26)
Post. ventrolateral setae	124 (100-146)	68 (57-81)	38 (32-42)	--
Int. vent. setae	52 (30-73)	40 (21-53)	28 (22-34)	11 (8-15)
Ext. vent. setae	42 (31-48)	18 (12-23)	10 (7-17)	19 (16-23)
Abdomin. Segments 8-9:				
Int. dorsal setae	16 (15-18)	14 (11-19)	10 (8-13)	8 (6-11)
Ext. dorsal setae	121 (107-144)	76 (71-81)	49 (43-54)	5 (3-7)
Dorsolateral setae	94 (84-103)	67 (54-77)	47 (39-54)	31 (27-36)
Ant. ventrolateral setae	36 (29-44)	23 (15-31)	18 (15-23)	15 (10-18)
Int. vent. setae	15 (12-18)	9 (7-15)	6 (4-8)	3 (2-4)
Ext. vent. setae	20 (11-25)	12 (9-16)	9 (6-11)	4 (3-6)
Vent. intermed. setae	5 (3-7)	4 (2-7)	2 (2-3)	--[a]
Ext. anal setae	72 (52-89)	43 (37-50)	31 (26-37)	26 (21-36)
Int. post anal setae	42 (31-58)	28 (21-36)	21 (18-24)	15 (11-19)
Ext. post anal setae	151 (123-177)	113 (102-119)	83 (76-92)	64 (53-73)
Int. pre anal setae	35 (29-47)	24 (16-31)	14 (12-17)	8 (5-11)
Ext. pre anal setae	50 (39-71)	34 (28-40)	20 (18-24)	11 (9-13)
Vent. caudal setae	62 (48-73)	37 (31-45)	33 (27-36)	22 (18-28)
Ext. caudal setae	63 (55-68)	43 (39-48)	30 (23-32)	33 (31-36)
Caudal setae	3.509 (3.208-4.174)	2.484 (2.259-2.728)	1.764 (1.537-1.895)	1.288 (1.170-1.357)

[a]Setae present but too small to measure.

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