The Rearing of *Phlebotomus* Sandflies (Diptera: Psychodidae) II. Development and Behavior of Panamanian Sandflies in Laboratory Culture[1]

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ABSTRACT

Observations on the immature stages and adults, in laboratory cultures, of 33 of the 65 known Panamanian *Phlebotomus* sandflies, of which 24 species have been reared to the adult stage, include the following: (i) General observations on larval food; temperature; feeding habits and other behavior of larvae; feeding habits of adults; copulation; fertility of eggs; longevity; diapause and quiescence. (ii) Detailed observations on the six principal man-biting species, notes on 27 other species, with tables showing numbers of eggs per female and the times of development from oviposition to adult and for individual stages.

We have been concerned with rearing Panamanian species of *Phlebotomus* as a part of investigations on leishmaniasis. We have been particularly interested in establishing the principal man-biting species in laboratory cultures for use in transmission experiments. Other species have been reared as opportunity presented itself, to settle taxonomic uncertainties in the matching of males and females and to provide immature stages for future taxonomic study as well as incidental biological information.

Eggs have been obtained from 33 of the 65 species known for Panama (63 described, 2 undescribed): 24 species have been reared to the adult stage and 4 more probably would have completed their cycle but were preserved as fourth instar larvae for taxonomic study. We failed to rear five species; in four of these the eggs hatched but the larvae died in the first instar, and in one the eggs did not hatch. Laboratory-reared females of 8 species have taken a blood meal in the laboratory, out of 11 species given the opportunity to feed on various hosts; 6 of the 8 were carried through at least a second generation, with 2 species, *sanguinarius* and *gomezi*, now in their 13th and 16th generations respectively (table 1). Most of the species dealt with are now being reared through at least one generation with relative ease.

The majority of the wild-caught females were freshly fed and were taken by our field crews on bait animals, mostly horses and themselves. Many of the wild-caught gravid females were collected by our colleague, Mr. Wilford J. Hanson, in the course of ecological studies (Hanson 1961).

The methods and equipment used in handling, feeding and rearing sandflies[2] are described in the preceding paper, Part I of this series (Hertig and

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[2] Although not in accordance with the recommendations of the Committee on Common Names of Insects of this Society, this name was set solid in this paper at the request of the junior author.

Table 1.—Panamanian sandflies, wild-caught: oviposition and rearing in the laboratory.

Species	No. of pools or individual ♀♀ ovipositing in laboratory	Condition when collected		No. of rearings to adult	Lab.-reared ♀♀ fed in laboratory	Stage attained	
		Gravid	Freshly fed, man, horse			Lab. generations	Not reared to adult
PRINCIPAL MAN-BITING SPECIES							
P. sanguinarius (Mar'57-May'59)	273 pools	+	+	258	+ (man, spiny rat, guinea pig)	13 (current)	
P. gomezi (Mar'57-May'59)	64 pools	+	+	52	+ (" " ")	16 (current)	
P. trapidoi (Mar'57-Oct'59)	135 pools	+	+	98[a]	+ (" " ")	2	
P. ylephiletor (Mar'57-Jul'59)	81 pools	+	+	64[a]	+ (" " ")	3	
P. panamensis (Mar'57-May'59)	187 pools	+	+	127[a]	+ (" " ")	2	
P. pessoana	36 pools	+	+	4[a, b]	0	1	
OTHER SPECIES							
P. aclydiferus	1	+		1		1	
P. apicalis	5	+	+	1		1	
P. arborealis	4	+		4		1	
P. barrettoi	3	+					Instar I
P. camposi	3	+		0[a]			Inst. IV, preserved
P. carpenteri	6	+					Inst. I
P. cayennensis	3	+		3		1	
P. cruciatus	1	+		1		1	
P. dysponetus	1	+		0[a]			Didn't hatch
P. galindoi	3	+	+	1[a]		1	
P. geniculatus	8		+				Inst. IV, prsvd
P. hamatus	1	+		1[a]		1	
P. hartmanni	2	+	+	1		1	
P. isovespertilionis	20[c]	+		20[c]		1	
P. nordestinus	1	+		1[a]		1	
P. ovallesi	5	+	+	4[a]		1	
P. pinealis	1	+					Inst. I
P. rubidulus	3	+		0[a]			Inst. IV, prsvd
P. runoides	1	+					Inst. IV, prsvd
P. serranus	3		+	2[a]	+ (man)	2	
P. shannoni	14	+	+	9		1	
P. spinosus	1	+		1		1	
P. trinidadensis	12	+		6[a]	+ (gecko)	1	
P. triramulus	2	+		2	+ (man)	1	
P. vespertilionis	23[c]	+		23[a, c]		1	
P. vexillarius	3		+	1		1	
Warileya rotundipennis	6	+	+				Inst. I

[a] Hanson (1961) reared 600 larvae, recovered from natural breeding places, to the adult stage, as follows: *panamensis* 8, *pessoana* 4, *trapidoi* 12, *ylephiletor* 2, *camposi* 1, *dysponetus* 24, *galindoi* 15, *hamatus* 437, *hansoni* 16, *nordestinus* 6, *ovallesi* 24, *rubidulus* 1, *serranus* 36, *trinidadensis* 13, *vespertilionis* 1.

[b] Including one very successful culture in a large aquarium.

[c] *Isovespertilionis* and *vespertilionis* can be identified only as adult males; 20 rearing attempts which were unsuccessful or produced only females are therefore not included.

Johnson 1961). The present paper (Part II) is concerned with the cycle of development and the behavior of the immature stages and adults of the various species which we have reared.

GENERAL OBSERVATIONS

Temperature.—On moving the sandfly cultures from an air-conditioned room maintained usually within a degree of 25.5° C., to another air-conditioned room with a temperature range averaging about 26.5°, it was found that certain species which were not doing well in the first room immediately began to thrive and the "hardier" species did even better at the slightly higher temperature. With this demonstration of the near-critical importance of a degree or two of temperature, we tried keeping cultures in various phases of development at the differing temperatures of rooms with and without air-conditioning. The following combination of temperatures has been found satisfactory for all our principal species.

The wild-caught females, in their individual plaster-lined vials, are kept in an air-conditioned room at about 25.5° until eggs are laid. While larvae do well at 26.5°, both hatching and larval development are more satisfactory at a still higher room temperature.

The rearing pots with eggs and larvae are kept in a room with open windows and through ventilation, with a daily range usually between 26° and 29°, with mid-day and afternoon temperatures occasionally reaching 30° or 31°. During the dry season, January through April, the relative humidity in this room ranges from 54% to 92%, but during the rainy season, May through December, the humidity is usually above 90%. When the adults begin to emerge the pots are transferred to an air-conditioned room maintained at about 26.6° during the day, but between 27° and 28.5° at night. The relative humidity during the dry season averages 77% at night and 71% during the day, with wet-season averages of 85% and 76% respectively. At the lower daytime temperature, a reversal of the conditions in nature, the adults feed more readily in the laboratory during the day.

In the latter air-conditioned room evaporation from the moat, the moist cotton and the pots themselves, lowers the temperature of all three so that the water averages 2.6° cooler than the air, and the food material in the bottom of the pots, with a range of 24.9° to 26.9°, averages about 1.0° cooler than the air. In the warmer open room with higher humidity these differentials are usually smaller, although there is considerable fluctuation. The interior of the pots with the eggs and larvae, ranging from 25.5° to 28.7°, average about 1.0° warmer than in the air-conditioned room.

Feeding Habits of the Larvae.—Most of the species we have reared in the laboratory have been observed ingesting molds found on the culture media as well as feeding on organic matter of the food proper. Frequently a larva will approach a colony of mold and remain without advancing for 24 hours or more while it swings from side to side eating a semicircular swath. This is particularly true of larvae of the slower-moving, surface-feeding species such as *ylephiletor* and *trapidoi*. A species of *Helminthosporium*, a mold first found in a pot of *gomezi* larvae, seems particularly attractive to *gomezi* and *ylephiletor* larvae and is eaten in preference to other molds in the pot.

Parts of insects are also utilized by the larvae. The five species we have dealt with most (*sanguinarius*, *gomezi*, *ylephiletor*, *trapidoi* and *panamensis*) are all particularly attracted to the soft, pigmented material on the inner surface of the eye capsule of Diptera.

Most of our species fall roughly into two classes according to their behavior in culture. (i) One group is always found on the surface of the food material, never burrowing under it. From this behavior we were able to predict that *panamensis*, *pessoana*, and probably also *trapidoi* and *ylephiletor*, would be found in nature on the surface of objects such as rotting leaves. This was confirmed for all four of these species by Hanson (1961) who found the larvae on dead leaves and debris scattered over the forest floor. The larvae of this group have long caudal bristles and the eggs are dark, thick-shelled, and have a sticky substance which presumably cements the eggs to the leaves or other forest litter, and thus prevents their being washed away by the rain. The larvae of *ylephiletor*, when very numerous in an aquarium culture, have been observed to consume all but the skeleton of decomposing leaves. Other litter- and leaf-dwelling species may also ingest material from the leaf proper although many times the larvae appear to be merely scraping off the leaf surface, presumably eating bacteria and fungi.

(ii) The second group of species burrow in the food mixture. Some are found with equal frequency above and below the surface of the food, as for example, *sanguinarius*, *trinidadensis* and *hartmanni*, while others, like *galindoi*, usually conceal themselves within or under aggregations of food particles, and do not occur on the surface. These species may have pale brown or black eggs, often with thin shells, which lack the adhesive substance occurring on eggs of *panamensis*, etc., and usually have the caudal bristles much shorter than those of the leaf- and litter-dwellers. Hanson (1961) found larvae of several of the species which have burrowing habits in culture in the top 2 inches of soil taken from between tree buttresses.

A few of the species we have reared do not fit either of the above categories. *P. gomezi* is a species with dark, thick-shelled eggs, but the larvae have short bristles and always occur on the surface of the food. *P. arborealis*, on the other hand, has very long caudal bristles but will crawl under the food.

Larval Reactions to Stimuli.—Most species show slight, if any, reaction to sudden increases in light intensity when the pot is opened under the microscope light. Neither the burrowing species, which presumably live in darkness in nature, including among others *sanguinarius*, *trinidadensis* and *hamatus*, nor the species which live exposed to constantly changing light intensities on upper surfaces of the leaf litter on the forest floor, such as *panamensis* and *pessoana*, appear to react to changes in light intensity. Larvae of *ylephiletor* and *trapidoi*, particularly the younger instars, may show increased activity and flick the caudal bristles for a few seconds after exposure to the microscope light. These species, which live as larvae in leaf litter next to the ground, are probably exposed to differences in light intensity mainly when the litter is disturbed, as by predators.

The usual response elicited by touching the caudal bristles of any species is a quick flattening backward of the bristles to the plane of the body. Usually the larva does not make avoidance reactions and we have observed that *geniculatus* and *panamensis* often will remain motionless for several seconds after such disturbance. *P. geniculatus* will also become completely motionless if water is dropped on it.

At times *ylephiletor* and *trapidoi* larvae near an area of the pot where mold is being removed with a dissecting needle will rear back with their heads near the tail end and with mandibles working. There may or may not be disturbance of the food particles on which such larvae are resting. If this is a defensive reaction, it should be remarked that touching or pushing the larvae directly elicits no such response.

P. trapidoi, ylephiletor, sanguinarius, gomezi and *panamensis* have been observed to clean the caudal bristles and body hairs. The larva bends back dorsally, grasps the base of a bristle between its mandibles, and straightening itself out, draws the bristle through the mandibles. The process is repeated with the remaining bristles. Cleaning body hairs is accomplished in a random fashion, also with the mandibles. Since at times mold spores may completely cover a larva and its caudal bristles with no apparent effect on the larva and without stimulating any attempt at cleaning, the factors leading to bristle grooming are not understood.

Movement of Larvae; Places of Pupation.—*P. sanguinarius, triramulus, trapidoi, ylephiletor, galindoi, panamensis* and *geniculatus* are all slow-moving species and may remain in one place for extended periods. *P. gomezi, cayennensis,* and to a lesser extent *spinosus,* are constantly moving in the culture. *P. gomezi* and *cayennensis* are voracious feeders and, interestingly, are among those with the shortest life cycles of all the species we have reared.

Although all the species have a tendency to pupate on the side of the pot rather than on the food material or debris on the bottom, *panamensis, pessoana* and *triramulus* are particularly prone to crawl as far up the sides of the pot as possible before pupating. In the case of *sanguinarius, gomezi, ylephiletor* and *trapidoi,* climbing the walls by prepupal larvae is most common at the time pupation is just beginning while the later pupae often occur on the bottom as well as the walls of the pot. Probably in nature larvae have a tendency to crawl upward before pupating. This would be expected especially of burrowing species which would pupate in soil cracks or very near the surface of the soil in such a way that the adult could emerge from the pupal skin and get to the surface without injury. Within the artificial environment of the rearing pot the crowding of larvae may add an irritating factor which strengthens the tendency to travel upwards, particularly at the beginning of pupation when many larvae are still feeding and active.

P. sanguinarius larvae usually do not pupate in close proximity to each other when many are pupating at the same time. In a crowded pot, when first ready to pupate, larvae of this species may climb the sides in large numbers and end up desiccated and dead on the muslin top. This is the only species in which we have observed this suicidal mass migration. On the other hand, *gomezi* larvae in a crowded pot will oftentimes pupate at approximately the same time in groups of five or six, with the individuals all touching one another. *Panamensis,* though it pupates high on the sides, never goes so high that the larvae or pupae die from desiçcation.

Feeding Habits of the Adults.—Females released in a cloth cage containing an animal or a human hand do not react in a constant manner, even females of the same species. At times they begin to feed immediately in the full light of the laboratory; other groups will feed sporadically over a period of an hour while the cage is covered, and thus darkened, with a wet towel; others show the most hunger when the operator removes the wet towel and begins to aspirate the flies into a pot. Sometimes the females refuse to feed at all. In general, a higher proportion of the females feed if they are held until 6 or 7 days after the first adults emerge in a given culture.

We assume that the change from the temperature of the rearing pot to that of the room, the relative humidity in the laboratory, the age of the females and the disturbance incidental to releasing the adults, all play some role in affecting the feeding response. Probably a drop in temperature and corresponding rise in humidity help to provide the stimulus to feed in nature, since these conditions usually prevail during the time sandflies are active and searching for a blood meal. However, the relative humidity of the laboratory, which ranges from 65% to 95% depending on the season, is usually too low to allow survival of adults in cloth cages for more than a few hours unless protected with wet towels. Even so, adults released from a pot with a relative humidity approaching 100% may begin to feed immediately at the lower humidity of the laboratory. The mere fact of a change in the environment, i.e., in light intensity, temperature or humidity, may lead to the feeding response in physiologically hungry females.

Hungry females are usually restless, flying from place to place in the cage and thus exciting the other sandflies, possibly inducing more to feed. A female may insert the piercing stylets three or four or more times in different places on the skin before settling down to feed. The time needed for engorgement differs with the individual female. Some hungry *sanguinarius* and *gomezi* females may feed to repletion in less than a minute, others take as long as 5 minutes.

P. sanguinarius and *gomezi* are our hardiest species and the females are the easiest to feed in the laboratory. They feed readily on man, guinea pigs, or the spiny rat, *Proechimys,* whether in feeding cages or free in a releasing cage. They attempt to bite hamsters but these animals are so difficult to immobilize and react so promptly to the sandfly bite that feeding is rarely successful. When immobilized with drugs hamsters seem to lose their attractiveness for sandflies, possibly because of a drop in skin temperature. By keeping the hamster warm in the operator's hand some feedings have been successful. Various attempts to feed *sanguinarius* and *gomezi* on suckling white mice all failed. *P. ylephiletor, trapidoi* and *panamensis* do not feed readily in the laboratory on any of the animals or under any conditions so far tried.

Copulation; Fertility of Eggs.—We have found that to assure enough fertilized eggs to maintain and increase our laboratory colonies, males must be placed in pots with fed laboratory-reared females, which indicates that copulation in the laboratory often does not occur before the blood meal.

In the laboratory *sanguinarius* females have been observed in copula before feeding, when freshly fed, and when the blood meal was 2 days old. At times for reasons not known, many of the *sanguinarius* adults released from a pot in a cloth feeding cage will immediately pair. Only three times has *gomezi* been observed in copula, once in a pot containing unfed flies, and twice in a releasing cage (one fed female, one unfed). In the laboratory many flies apparently do not copulate at all since the fertility of eggs laid by laboratory-reared females is lower than that of wild-caught females. Probably in nature *sanguinarius* and *gomezi* usually copulate before feeding since the fertility of the eggs of wild-caught females taken in the act of feeding and maintained without males, is high. Of 128 wild-caught *gomezi*, all but six laid fertile eggs. Eggs from wild-caught *sanguinarius* also have a high fertility. Twenty pools, each containing 1 to 10 females, gave the following proportions of eggs hatching and developing to the pupal stage: 9 pools, 90%-100%; 2, 80%-90%; 3, 70%-80%; 1, 60%-70%; 4, 50%-60%; 1 pool (eggs from a single female), 20%-30%.

In another experiment, four groups of *sanguinarius* females, representing wild-caught and tenth laboratory generation populations, laid fertile eggs as follows: Group A, 23 wild-caught females maintained without males after feeding; 92% of all eggs hatched. Group B, 33 tenth-generation females maintained with males before, during and after feeding; 23% hatched. Group C, 19 tenth-generation females maintained without males after feeding; 15% hatched (as opposed to 92% in the case of eggs laid by wild-caught females maintained under similar conditions). Group D, 10 tenth-generation females maintained with males before but not during or after feeding; none of the eggs hatched. An interesting supplementary observation was that although the average number of eggs laid by Group B females was 43, Group C laid an average of 28 eggs, and Group D only 19 eggs.

Search for Index of Previous Blood Meals.—Some of the wild-caught females in our series probably were feeding for the second or third time and may have copulated after the first feeding with sperm being conserved in the spermathecae. At the present time we do not know what proportion of our wild-caught females have fed more than once. Preliminary investigations have failed to establish a satisfactory index of prior feeding. It has been considered that all sandflies which had granules in the accessory glands of the ovaries had taken a blood meal at some previous time (Adler and Theodor 1935). Lewis and Minter (1960), following extensive investigation and dissections of wild-caught African sandflies, stated that usually a fly may be considered unfed and nulliparous if there exists a combination of no remnant of a blood meal in the gut and no or few granules in the accessory glands of the ovaries. Unfortunately neither wild-caught nor laboratory-reared specimens of those Panamanian species for which we have the most information follow this pattern. The granules may be present or absent both before and after feeding; they may be in one gland and not the other; there may be active contraction and the granules are often evacuated. Lewis (1960) anticipated that this difficulty might exist with regard to the Central American species of *Phlebotomus* based on the finding by Garnham and Lewis (1959) that a surprisingly high proportion of wild-caught Honduran sandflies had granules in the accessory glands.

Longevity of Adults; Refeeding of the Females.—In the laboratory most females die after laying their first batch of eggs, with those that live past oviposition refusing a second blood meal. Refed females will live longer than females feeding only once. We have obtained refeeding, after egg laying, in *gomezi, sanguinarius, ylephiletor* and *trapidoi*; one *sanguinarius* female fed four times in all. The longest periods of survival (after the first feeding or offer of a blood meal, initial age unknown) for refed females and unfed females respectively, are: *gomezi* and *sanguinarius,* 19 and 14 days; *ylephiletor,* 17 and 12; *trapidoi,* 18 and 10. Males lived as long as the unfed females of their respective species.

In our laboratory, females in the presence of males both before and after refeeding laid eggs which were more often than not infertile and the larvae which did hatch had a low viability. Refed females maintained without males after the first feeding rarely laid fertile eggs. In only one such batch of eggs (from a refed *sanguinarius* female) did hatching occur. Dissections of females which had laid eggs previously in the laboratory rarely revealed sperm in the spermathecae. Whether this is an artificial phenomenon caused by laboratory conditions remains to be investigated.

Adler and Theodor (1935) stated that wild-caught *papatasi* may refeed several times with no relation to egg laying whereas *perniciosus* takes one blood meal and refuses to refeed until a batch of eggs has been laid. They also found that gravid *perniciosus* females need a higher humidity than do those of *papatasi* in order to lay eggs, and suggested that *papatasi* maintains its water balance by taking frequent blood meals.

There are indications that in nature certain Panamanian species may take additional blood meals between the primary meal and egg laying. In dissections of over 700 wild-caught females, taken from animal and human bait before or during feeding and dissected within 24 hours of capture, five females showed evidence of having fed within 3 or 4 days. In four cases, two *sanguinarius* and two *trapidoi*, females had what appeared to be the remains of an old blood meal without discrete red cells in the midgut, but there was no ovarian development. The fifth female, *sanguinarius*, had taken a small fresh blood meal but the ovaries had begun to develop previously. Further evidence is the following:

(i) Ten laboratory-fed females of *panamensis* all laid eggs beginning the fourth day after feeding. However, one or more of the females in 3 of 127 pools of wild-caught females laid eggs 1 and 2 days after feeding, while in the other 124 pools egg laying began after 3 to 5 days. The indication is that the females which laid eggs only 1 or 2 days after a blood meal probably had fed previously and the eggs were the result of the earlier blood meal.

(ii) Females of 2 of 64 pools of wild-caught *ylephiletor* laid eggs 2 days after feeding and the other 62 pools laid eggs after 3 to 7 days. Of 13 pools of laboratory-reared *ylephiletor*, only 2 laid eggs 3 days after feeding while the rest laid eggs after 4 to 8 days.

(iii) One wild-caught female of *gomezi* laid eggs 2 days after feeding. This female did not produce autogenous progeny and possibly she too had taken an additional blood meal before oviposition.

(iv) No wild-caught *sanguinarius* females have ever laid eggs within 2 days after feeding, nor have laboratory-reared females. Wild-caught females in only 2 of 258 pools laid eggs after 3 days, while all the others laid eggs 4 to 10 days after feeding. This may be an indication that *sanguinarius* normally does not feed more than once before laying eggs, even taking into account the fact that this species is often slow in ovarian development.

Diapause and Quiescence.—There are many reports of diapause in the fourth larval instar of Palaearctic species of *Phlebotomus*, with over-wintering achieved in this manner. This may be considered true diapause in which the larva enters a prolonged arrest of development which is genetically determined and independent of environmental factors. For example, laboratory-reared *P. chinensis* may go through two or three uninterrupted generations and then enter diapause in the third or fourth generation, during which the fourth-instar larvae may continue to eat but do not pupate. This diapause lasts at least 6 to 8 months in nature but in the laboratory the diapause is shortened by about 2 months. Of course, in nature diapause occurs during the cold winter months. In the laboratory, with a diapause of shorter duration, successive generations eventually get out of phase with the external environment and diapause will occur during summer, the most favorable natural period.

In the laboratory, adverse culture conditions such as too much moisture or a lowered atmospheric temperature may cause fourth-instar larvae of the Palaearctic species to enter a quiescent period (Whittingham and Rook 1923, Adler and Theodor 1957). Addis (1945), working in Texas with the Nearctic *P. anthophorus*, was unable to obtain pupation of fourth-instar larvae over a period of 2 months when using a mixture of dried rabbit feces and blood as larval food. He did not give the time of the year involved. When he added a few drops of all the known components of the vitamin B complex to the culture, the larvae pupated within 3 days. Later Addis used rich garden soil and rabbit blood and pupation occurred normally in his cultures. Possibly Addis was dealing with the same type of quiescence which is seen in Palaearctic species maintained under adverse conditions.

Barretto (1942) observed diapause in fourth-instar larvae of a Brazilian species, *P. whitmani*. While this phenomenon was rare in his laboratory, Barretto had certain cultures of *whitmani* in which as many as 47.6% of the larvae entered a diapause which lasted 96 to 203 days. According to Barretto's account this was a true diapause, since it was independent of environmental conditions of temperature, humidity, and food. It occurred in larvae reared from eggs laid by both wild-caught and laboratory-reared females.

Lindquist (1936) made an important and seemingly overlooked observation regarding diapause in Nearctic *P. diabolicus* from Texas. He found that eggs of *diabolicus* maintained in his laboratory diapaused 167 days, from 14 October 1933 to 29 March 1934. Lindquist suspected that the fourth-instar larvae of *diabolicus* might also overwinter.

In our tropical species we have indications that quiescence may involve not only the fourth-instar larvae but also the eggs. Quiescence in fourth-instar larvae of the Panamanian species probably takes place under adverse conditions only, with lack of moisture being the most important factor. In one culture of *panamensis*, which had produced adults and had then been overlooked and allowed to become too dry, fourth-instar larvae were still present in the absence of pupae and living adults 2 weeks after adults first appeared. This does not occur in a normally producing pot where adults and pupae are invariably present as long as there are still developing larvae in the pot. When the pot in question was again moistened, some of the larvae which had been motionless and desiccated in appearance, and with food visible in the gut, continued development, pupated, and produced normal adults. Others of these larvae died without pupating although they were alive at the time the culture was moistened.

In one culture of ninth laboratory generation *sanguinarius* the same situation as with the *panamensis* larvae was observed. Adults first emerged from this pot 55 days after oviposition. A month later (at 86 days after oviposition) the pot was very dry and contained one slowly moving fourth-instar larva with food in the gut, one pupa, and no living adults. The pot was watered and the pupa emerged at least 92 days after oviposition. The fourth-instar larva pupated but was not followed beyond this point.

There are extremely uneven rates of hatching time and larval development in some of the Panamanian species. *P. gomezi* may have all stages from the second larval instar through the pupa present in a culture established with eggs laid within a 2-day period. The same is true of *panamensis* although usually to a lesser degree. In several cultures of *panamensis* which were started from eggs laid by wild-caught females during the dry season, the first hatching took place within 9 to 10 days, but 3 or

4 weeks later, with most of the larvae in the fourth stage or already pupae, newly hatched larvae were discovered, i.e., at least 30 days after oviposition. Further, in a culture of *geniculatus*, a species related to *panamensis* and probably with the same habits in nature, a newly hatched first instar larva was observed 36 days after oviposition. Since other larvae in these cultures of *panamensis* and *geniculatus* had hatched at normal times culture conditions probably were not involved, and some eggs may be destined to be quiescent.

P. sanguinarius has a uniform rate of larval development with usually no more than two stages of development present at any one time in a single culture, and probably either no quiescence occurs in the egg stage or our laboratory conditions have never induced such quiescence. As noted earlier, *panamensis* and other surface feeders in culture, including *gomezi*, have eggs with thick, black shells, and *sanguinarius* eggs have thin, light-colored, easily collapsible shells. As well as furnishing protection against drying during the normal prehatching time of 5 to 12 days, depending on the species, the resistant shell probably serves as protection during periods of egg quiescence.

In Panama, unless the year is exceptional, occasional rain falls during the dry season, especially on the Atlantic side of the Isthmus. Because periods of drying would not necessarily be extensive, eggs of our species might not be geared to a lengthy and true diapause, a possibility which has not been tested in the laboratory. *P. panamensis* is not very abundant during the dry season, but in June, i.e., a month after the rains begin, there is a rapid increase followed by a decline at the end of June. The related species *pessoana* has a similar but lower June curve.

We routinely discard batches of eggs which do not hatch within 21 days, and longer periods of quiescence involving all the eggs of a given female or pool of females might occur without our knowledge. However, at the end of the 3-week period most unhatched eggs are either shrivelled or moldy and obviously not viable. Furthermore, eggs of *sanguinarius, gomezi, ylephiletor* and *trapidoi* have not been observed to hatch between days 17 and 21 after oviposition.

Autogeny.—One Panamanian species, *gomezi*, has occasional autogenous individuals in nature, and several autogenous strains have been maintained in our laboratory, with one of them attaining the 11th laboratory generation (Johnson 1961). Autogeny has not been demonstrated in the other species we have reared in successive laboratory generations.

OBSERVATIONS ON INDIVIDUAL SPECIES

We have reared large numbers of *sanguinarius, gomezi, trapidoi, ylephiletor* and *panamensis* and our observations on these species are extensive. The other Panamanian species were reared only as fed or gravid females became available. The number of such rearings is relatively small and observations on the laboratory development are correspondingly fragmentary. Although we have as yet made no attempt at taxonomy of the immature stages, the larvae of our common species or species groups can be identified from both morphological characters and behavior in cultures. Therefore in the following accounts, appearance of the larvae and pupae is sometimes mentioned. Conspicuous features of the larvae of all species are the caudal bristles. They may be longer than the body and project fanlike behind or are held erect. In general there is a single pair in the first instar but two pairs thereafter. The *Brumptomyia* group, however, has only a single pair in all four instars.

The average numbers of eggs per female as given in table 2 for the various species are probably low since figures from our early rearing efforts are included. Those cases in which only one or two eggs were laid probably represent the dying efforts of females in extremis and bear no relation to the number which would otherwise have been laid. With the improved methods of the past 2 years for maintaining wild-caught females the number of eggs per female has increased. For example, in one group of 23 *sanguinarius* collected in late 1960, the average number of eggs per female was 39, compared with 27 as given in the table.

Table 2.—Number of eggs laid by individual wild-caught females; principal man-biting species.

	sanguinarius	*gomezi*	*trapidoi*	*ylephiletor*	*panamensis*	*pessoana*
Number of ♀♀	2,268	377	1,103	449	1,215	147
Average no. eggs per ♀	27	29	21	27	28	20
Extremes	1 - 80	1 - 72	1 - 75	1 - 92	1 - 90	2 - 55
Extremes of middle 90%	3 - 55	5 - 53	2 - 45	2 - 63	3 - 57	5 - 42
Extremes of middle 50%	16 - 38	18 - 32	9 - 31	13 - 37	16 - 42	11 - 28

PRINCIPAL MAN-BITING SPECIES

P. sanguinarius F. & H.—This is the most common species in our biting collections from man and horse. In the laboratory, wild-caught females laid an average of 27 eggs, which closely approximates the averages for *gomezi, panamensis* and *ylephiletor* (table 2). The extremes and the curve of number of eggs per female are also approximately the same for the above species. The period of development of *sanguinarius* from oviposition to adult emergence averages about 50 days and is the longest of the common species we have dealt with. (Tables 3 and 4.)

Table 3.—Shortest number of days, oviposition to adult emergence, in successive laboratory generations: principal man-biting species.

Laboratory generation	*sanguinarius* No. cultures	*sanguinarius* Extremes	*sanguinarius* Avg. days	*gomezi* No. cultures	*gomezi* Extremes	*gomezi* Avg. days	*trapidoi* No. cultures	*trapidoi* Extremes	*trapidoi* Avg. days	*ylephiletor* No. cultures	*ylephiletor* Extremes	*ylephiletor* Avg. days	*panamensis* No. cultures	*panamensis* Extremes	*panamensis* Avg. days	*pessoana* No. cultures	*pessoana* Extremes
1st	40	44–53	49.3	106	28–43	33.8	39	38–54	42.9	21	34–48	40.3	27	30–45	36.4	4	32–38[a]
2nd	21	45–59	49.0	9	29–45	36.0	4	44–49	46.0	7	37–49	41.3	3	32–48	40.7		
3rd	22	45–63	51.0	10	29–39	32.5				3	40–47	43.0					
4th	23	47–55	51.1	9	28–36	32.7											
5th	20	47–54	50.0	10	31–42	34.8											
6th	9	47–56	51.4	13	31–38	36.0											
7th				10	30–41	35.3											
8th				8	34–41	37.0											
9th	4	44–50	47.5														
10th	5	48–55	49.0														
13th				4	31–36	34.3											
14th				3	36–40	38.3											

[a] Exact number of days known for only one of the four cultures.

The egg of *sanguinarius* is oval, brown in color and the shell is thin, often collapsing after hatching. The larvae have black heads and pale-buff bodies. The caudal bristles are short and the larvae are found with equal frequency burrowing in the food or on its surface. The larvae are slow-moving and are not noticeably affected by a change in light intensity although the pupa at times performs a bowing movement when exposed to a sudden increase in light. Although there may be a great many larvae in a rearing pot, they usually are not disturbed by each other and flourish if enough food is present. As the time of pupation approaches, the larvae climb the sides of the pot. In a crowded pot they often crawl onto the cover of the pot and almost immediately become desiccated. It has been noticed that sometimes the mass migration will occur only on a certain area of the pot wall, which may be covered thickly with larvae all slowly moving upward toward the rim, while the other parts of the wall have relatively few. In an aquarium culture consisting of crumbled soil not mixed with leaves, *sanguinarius* usually pupated at high points of the surface although some pupae were found at lower levels of the soil surface. Larvae in the aquarium culture were seldom seen and presumably were below the soil surface.

Tables 3 and 4 show that the successive laboratory generations of *sanguinarius* do not differ significantly in total number of days from oviposition to adult emergence nor in number of days per individual stage. Experiments suggest that the average number of eggs laid by individual females of the 10th laboratory generation, 43 eggs per female in one experiment, is significantly higher than for wild-caught females, which have laid an overall average of 27 eggs (39 under later improved conditions) although the fertility of the eggs from the 10th generation may be very low. Despite this difficulty, our 10th and succeeding generations are well adapted to the laboratory and females take blood meals in satisfactory numbers.

P. gomezi Nitz.—This is our most rapidly developing man-biting species. Total time from oviposition to adult emergence averages about 34 days (table 3). All stages of the life cycle take less time than in *sanguinarius*, especially the egg, the fourth-instar larva and the pupa (table 4). The average number of eggs laid by individual wild-caught females, 29, is about the same as in *sanguinarius* (table 2).

Table 4.—Shortest number of days per stage, principal man-biting species.

Species, laboratory generation	Blood meal to egg No. cultures	Blood meal to egg Extremes	Blood meal to egg Avg. days	Egg No. cultures	Egg Extremes	Egg Avg. days	Larval instar I No. cultures	I Extremes	I Avg. days	Larval instar II No. cultures	II Extremes	II Avg. days	Larval instar III No. cultures	III Extremes	III Avg. days	Larval instar IV No. cultures	IV Extremes	IV Avg. days	Pupa No. cultures	Pupa Extremes	Pupa Avg. days
sanguinarius																					
1st	44	3–10	4.1	45	9–13	11.0	55	5–9	6.0	55	2–5	3.6	54	3–7	4.8	52	7–12	9.7	50	12–16	14.2
5th	18	4–7	4.7	20	10–14	11.5	20	4–7	6.0	20	3–6	4.3	20	3–7	5.0	20	8–14	11.0	20	11–13	12.2
10th	4	4		3	10–11	10.3	3	4–7	5.7	4	3		5	2–6	3.4	5	10–16	13.8	5	11–14	12.0
gomezi																					
1st	121	3–10	5.1	82	6–14	8.2	105	3–8	4.8	106	2–6	3.3	94	2–8	4.1	87	4–10	6.4	60	5–9	7.0
5th	9	3–5	3.4	10	7–10	7.7	10	4–7	5.4	10	2–6	4.0	10	3–6	4.0	10	5–9	6.5	10	6–9	7.1
14th	4	3–4	3.8	4	8–10	8.8	4	6–8	6.8	4	4–8	5.3	4	1–4	3.0	3	6–8	6.7	3	7–10	8.7
trapidoi																					
1st	41	3–5	4.0	47	8–14	10.8	50	4–9	5.9	49	2–7	3.9	48	3–8	4.7	48	5–15	8.8	38	7–14	8.5
2nd	4	4–5	4.3	4	8–15	12.0	4	4–9	5.5	4	4–5	4.3	4	5–7	5.8	4	9–15	11.5	4	7	
ylephiletor																					
1st	24	2–7	4.6	24	7–13	9.5	22	5–8	5.7	24	3–6	3.8	22	3–6	4.9	20	4–12	7.9	20	7–10	8.4
2nd	6	3–6	4.7	7	7–10	8.9	7	4–7	5.6	7	3–4	3.7	7	3–6	4.9	6	7–15	9.8	7	7–9	7.7
3rd	3	4–8	5.3	3	9–10	9.3	3	5–8	6.3	3	3–6	4.0	3	5–6	4.3	3	8–12	10.0	3	7–9	7.7
panamensis																					
1st	35	1–5	3.6	35	8–12	9.8	35	3–7	4.9	34	2–5	3.2	28	2–6	3.8	27	4–12	7.6	25	3–9	6.6
2nd	1	4		3	9		3	6–8	6.7	3	2–5	3.0	3	5–8	6.0	3	3–19	7.3	3	4–7	4.3
pessoana																					
1st	20	3–7	4.1	20	8–12	9.6	5	5–8	6.0	4	4–6	5.3	1	3		1	9				

The eggs are oval, black and thick-shelled. The larva has short caudal bristles, a pale brown head, and a very pale, almost translucent body. The larvae are extremely active and apparently are not affected by changes in light intensity. They remain on the surface of the food, never burrowing under or into it. Like *sanguinarius* and other species, the larvae tend to climb the wall of the pot in order to pupate.

Successive laboratory generations take a variable number of days for development (tables 3 and 4). One strain which is now in the 16th laboratory generation is vigorous and well adapted to laboratory conditions.

In a comparison of the number and fertility of eggs of early and advanced generations of *gomezi*, 11 second-generation females laid an average of 49 eggs, 85% of which hatched, while 10 14th-generation females laid an average of 55 eggs, only 49% of which hatched. In an earlier section we discussed the possibility that in the older laboratory generations copulation is not so frequent, for unknown reasons. The increase in number of eggs per female with succeeding generations probably reflects their adaptation to laboratory conditions.

P. panamensis Dyar.—This is a litter- and leaf-dwelling species in nature. In length of life cycle it is intermediate between *sanguinarius* and *gomezi*, averaging about 38 days from oviposition to adult emergence (table 3). Wild-caught females lay an average of 28 eggs, closely approximating the number laid by *sanguinarius*, *gomezi* and *ylephiletor* (table 2).

The eggs are long, slim, black and thick-shelled, with an adhesive substance on the shell. The larvae at all stages have very long caudal bristles. After the first instar the larvae are dark gray in color and flattened dorsoventrally. In the first two instars the head is dark but in the third and fourth there is a striking change in gross appearance. The head becomes much lighter than the body and the greatly enlarged conical bases of the antennae suggest the dorsal profile of a cat's head.

Almost all larvae while on leaves in a culture constantly manipulate the mandibles, apparently scraping the leaf surface and presumably ingesting fungi or other organic material. They also consume visible fungus growth. As a rule, larvae pupate high on the sides of the pot. In an aquarium culture which had a top layer of large leaves, the pupae were all on the upper leaf surfaces. The pupae are slender and very dark.

The larval mortality in cultures is high. Although a pot started with 200 or 300 eggs may yield 30 to 50 adults, the reluctance of *panamensis* to feed on any animal in the laboratory has prevented us from carrying this species beyond a second laboratory generation.

P. pessoana Barr.—This species is the least common of the six regularly taken in biting collections from man and horse. It is a close relative of *panamensis* and like this species is a litter- and leaf-dwelling species in nature. Possibly because conditions in the laboratory are not optimal, females lay an average of only 20 eggs, with 55 the highest number (table 2). In the laboratory the time from oviposition to adult emergence, based on incomplete and limited data, varies from 32 to 38 days (table 3), a period comparable to that of *gomezi*.

Eggs and larvae are morphologically similar to those of *panamensis* and larval behavior in cultures is the same.

Cultures of *pessoana* in rearing pots have been on the whole unsuccessful. This species is quite irregular in its distribution and in its seasonal occurrence. In one patch of dense forest, one of the few places where Mr. Hanson found both larvae and adults in dead leaves, with adults also on the green leaves of low vegetation, the temperature under the leaves (25.0° C.) was at least a degree lower than in another patch of more open woodland where the related *panamensis* was abundant and *pessoana* rare. Because of this temperature difference in nature we tried keeping pots of *pessoana* larvae in an air-conditioned room slightly cooler than the rearing room, but with no better results. However, a culture in a large aquarium kept in the cooler room produced a good number of adults. Larvae were found mainly on the upper leaf surfaces although some crawled onto the glass sides and a few were found on leaves below the upper layer but not where the leaves were closely appressed. Pupation occurred on the upper leaf surfaces and on the lower parts of the glass walls.

P. ylephiletor F. & H.—This close relative of *trapidoi* is one of the litter- and leaf-dwelling species (although unique among our principal man-biters in that the adults may also be found resting in buttresses). The time from oviposition to adult emergence averages about 40 days, with second- and third-generation cultures taking slightly longer (table 3). Wild-caught females lay an average of 27 eggs (table 2), comparable to that for *sanguinarius*.

The eggs of *ylephiletor* are slim, oval, and black, coated with an adhesive substance. Larvae have long caudal bristles, have black heads and pale bodies, are slow-moving and occur only on the surface of the food. If a particularly attractive colony of fungus is present, the larvae may remain more or less stationary for 24 hours or more, and by swinging the head, eat a semicircular hole in the mold colony. With a sudden increase in light the larvae flick the caudal bristles but do not make other movements. Pupation usually occurs on the wall of the rearing pot. In aquarium cultures the pupae are usually found on the upper side of the leaves, chiefly of the top layer but also on those beneath if the leaves are not closely appressed. Mortality in cultures is high and females are reluctant to feed in the laboratory, with the result that our attempts to produce colonies adapted to the laboratory have so far been unsuccessful beyond the third generation because of the progressive attrition in numbers.

P. trapidoi F. & H.—This species, like *ylephiletor*, *panamensis* and *pessoana*, is a litter- and leaf-dweller in nature. Unlike the other common man-biters we have reared (except *pessoana*), *trapidoi* lays in the laboratory an average of only 21 eggs, although the extremes, 1 to 75, are comparable to those of the other species (table 2). The time from oviposition to adult emergence averages about 43 days in the first laboratory generation and 46 in the second (table 3).

In morphology of the egg and larva, and in larval habits in the rearing pots and in aquarium culture, *trapidoi* is similar to its close relative, *ylephiletor*, but it is definitely a more difficult species to work with in the laboratory. We have not succeeded in producing more than two consecutive laboratory generations, since larval mortality is high and females are very reluctant to take a blood meal.

SPECIES OTHER THAN THE PRINCIPAL MAN-BITERS

This group includes a number of species characteristically found in buttresses, hollow trees and animal burrows, as well as in various other habitats. Some of these species are known to bite man, e.g., *shannoni, cruciatus*, although the natural hosts of most of them are unknown; some are relatively rare.

A number of the failures in rearing occurred before certain of the difficulties with regard to food and molds were overcome. Further, in a number of cases the progeny of individual females were reared in the original plaster-lined vials in which the eggs were laid. While adequate in general, these vials do not provide quite so favorable an environment for the larvae as the rearing pots.

The number of days per stage and total time from oviposition to adult emergence for the following species are shown in table 5.

P. aclydiferus F. & H.—Number of eggs laid by an individual female: 69. Total days, oviposition to adult emergence: 33 (one observation).

P. apicalis F. & A.—Number of eggs laid by individual females: 10, 12, 12, 24, 26. Total days, oviposition to adult: 36 (one observation).

The larva has very long caudal bristles, is slow-moving and remains on the surface of the food. The larva is deeply pigmented (dark gray) except for the head, prothorax and the last abdominal segment. The pupa is long and slim, resembling *panamensis* in shape, and is pale centrally and dark laterally.

P. arborealis F. & A.—Number of eggs laid by individual females: 2, 9, 47, 50. Total days, oviposition to adult: 38, 40, 40, 44. The larvae occur both beneath and on the surface of the food.

P. barrettoi Mang.—Number of eggs laid by individual females: 7, 8, 53. None were carried beyond the first instar.

P. camposi Rodr.—Number of eggs laid by individual females: 12, 15 (95 by two females). Total days, oviposition to adult: 42 (one observation). The eggs are small and pale. The larvae have short caudal bristles and occur only on the surface of the food.

P. carpenteri F. & H.—Number of eggs laid by individual females: ca.12, ca.27, 56, 62, 71, 75, 77. None were carried beyond the first instar.

P. cayennensis F. & A.—Number of eggs laid by individual females: >53, 95, 95. Total days, oviposition to adult: 26, 27. The larvae have short caudal bristles, are extremely active and crawl both under and on the surface of the food.

P. cruciatus Coq.—Number of eggs laid by an individual female: 41. Total days, oviposition to adult, >45.

P. dysponetus F. & H.—Number of eggs laid by an individual female: 75. The eggs did not hatch.

P. galindoi F. & H.—Number of eggs laid by individual females: 37, 44, 50, 59, 63, 98, 113, 127. Total days, oviposition to adult: 76 (one observation). The eggs are large, oval and dark. The larva has but one pair of rather short caudal bristles during the entire cycle (a characteristic of the *Brumptomyia* group). Larvae are slow-moving and tend to hide under masses of food.

P. geniculatus Mang.—Number of eggs laid by individual females: 3, 4, 5, 6, 9, 12, 19, 34. None were carried through from egg to adult. In the one successful culture the only larva reaching the fourth instar was preserved for study. The eggs are elongate and black. The larvae are slow-moving and occur on the surface of the food. The caudal bristles are long and the larvae are dark in hue, resembling the closely related *panamensis*. One egg hatched 36 days after oviposition and two other eggs hatched after more than 36 days.

P. hamatus F. & H.—Number of eggs laid by an individual female: 80. Total days, oviposition to adult: >39. Like other members of the *Brumptomyia* group, there is only a single pair of caudal bristles throughout larval development.

While *hamatus* is rather rare in adult collections, Hanson (1961) found it by far the most abundant species recovered as larvae in nature. Out of a total of 2,258 immature stages, he reared 600 to the adult stage and of the 15 species thus identified (see table 1) 437 were *hamatus*. Nearly all of the latter were from the top inch or two of the soil between buttresses.

P. hartmanni F. & H.—Number of eggs laid by individual females: 12, 27. Total days, oviposition to adult: 53 (one observation). The egg is oval and black. The larva has short caudal bristles, a dark head and pale body. Larvae occur both below and on the surface of the food and are slow-moving.

P. isovespertilionis F. & H.—Number of eggs laid by individual females: 6 to 44; average for 20 females: 28. Total days, oviposition to adult; 36 to 45; average for 17 females: 41. The eggs are large, oval and dark. Larvae have black heads, occur below and on the surface of the food, and the caudal bristles are rather long. See further comment under *vespertilionis*.

P. nordestinus Mang.—Number of eggs laid by an individual female: 59 (one observation). Total days, oviposition to adult: 38.

This single culture is the one referred to in Part I (Hertig and Johnson 1961) which provided our first demonstration of the potential value of leaf litter as an ingredient of the larval food. The newly hatched larvae were crawling away from the feces-blood mixture, climbing the bare walls of the pot, and would certainly have perished. Within a matter of minutes after adding a small pile of leaf litter some larvae had reversed direction and soon there were a number at the leaf debris. It appeared that the mere presence of this material provided an attractive environment since the larvae seemed not to eat it but took the food they had at first refused. Later they moved about actively beneath the food and web of fungi, pushing the material aside to form tunnels large enough to accommodate the nearly erect caudal bristles. The original 59 eggs produced 56 adults.

P. ovallesi Ortiz.—Number of eggs laid by individual females: 6, 14, 15, 15, 28. Total days, oviposition to adult: 42, 42, 44, 53. The eggs are oval, dark and thin shelled. The caudal bristles are not particularly long and the larvae occur both beneath and on the surface of the food.

P. pinealis F. & A.—Number of eggs laid by an individual female: 26. The larvae were not carried beyond the first instar.

P. rubidulus F. & H.—Number of eggs laid by individual females: 2, 5, 6. Of the two successful rearings, the fourth-instar larvae or the pupae were all preserved. The larvae remain on the surface of the food.

P. runoides F. & H.—Number of eggs laid by an individual female: 19. The two larvae which survived to the third and fourth instar were preserved.

Table 5.—Shortest number of days per stage, species other than principal man-biting sandflies.[a]

Species	Blood meal to egg	Egg	Larval instars				Pupa
			I	II	III	IV	
apicalis	8	10-11	5-6	3	8	preserved	
"	7	11	5	2	preserved		
"	7	11	4	6	4	preserved	
"	8	9	5	3	4	10	5
"	>2(gravid)	9	6	3	5	preserved	
arborealis	>4(gravid)	9	6	5	4	9	7
"	>3(")	11	6	2	5	8	8
"	>2(")	9	6	3	3	10	7
"	>3(")	9	6	5	3	14	7
camposi	>5(gravid)	10	5	3	5	preserved	
"	>4(")	8	5				11
cayennensis	>1(gravid)			2	2	preserved	
"	6	3	2	3	4	8	
galindoi	>2 to >6(gravid)	17	9	9	12	17	12
"	>3 to >6(")	13	9	9	9	preserved	
"	5	16	12	7	preserved		
geniculatus	4	16	7	5	5	preserved	
hartmanni	6	12	8	5	8	12	preserved
"	>5(gravid)	12	6	5	5	14	11
isovespertilionis (17 cultures)	(gravid)	9-12 av:10.9	4-6 av:5.0	3-4 av:3.5	4-5 av:4.3	6-10 av:7.4	9-11 av:10.0
ovallesi	5	10	6	4	4	9	9
"	>4(gravid)	7	7	4	4	8	10
"	9	9	5	5	5	9	9
"	4					11	13
rubidulus	>4(gravid)	9	6	4	5	9	preserved
"	>6(")	12	6	4	4	preserved	
runoides	>4(")	15	8	6	8	preserved	
serranus	4	9	5	3	4	7	9
"	7	7	6	3	4	7	preserved
"	7	6	5	3	4	6	11
" (2nd lab. gen.)	6	7	5	3	4	7	7
shannoni	4	11	5	3			8
"	>4(gravid)		7	4			
"	>2 to >4(")	11	5	5	5	10	8
"	6	14	7	4	4	8	preserved
"	>5(gravid)	12	7	9	6	9	8
"	3			8	3	7	10
spinosus			7	7	6	12	10
trinidadensis	>3(gravid)	8	4	4	2	7	13
"	>4(")	10	4	3	3	8	10
triramulus	>2(gravid)	8	6	3	7	8	14
"	>6(")	8	6	4	6	7	11
vespertilionis	>0(gravid)	15	4	4	4	7	11
vexillarius	4	8	8	7	7	16	9

[a] Except as noted, each line represents a single culture, the progeny of one individual or a pool of wild-caught females.

The eggs are large, oval and dark. The larva has long caudal bristles and remains on the surface of the food.

P. serranus D. & A.—Number of eggs laid by individual females: 10, 14, 33. Total days, oviposition to adult: 35, 37 (two observations).

The eggs are oval and dark. The larvae are vigorous and active, occurring both on the surface and beneath the food. This seems to be a "hardy" species; 6♂♂ and 2♀♀ accidentally left in the refrigerator for 16 hours at 4.5° C. revived and remained alive more than 24 hours after removal to room temperature.

P. shannoni Dyar.—Number of eggs laid by individual females: 5 to 92; average for 17 females: 34. Total days, oviposition to adult: 42, 44, 51 (three observations). The larva is pale in hue and has very long caudal bristles. Larvae occur only on the surface of the food.

P. spinosus F. & A.—Number of eggs laid by an individual female: 31. Total days, oviposition to adult: 52±3 days (one observation). The larvae are in constant but slow movement and are always found on the surface of the food. The caudal bristles, about as long as the body, are held erect.

P. trinidadensis Newst.—Number of eggs laid by individual females: 39 to 100; average for 12 females: 73. Total days, oviposition to adult: 38 (two observations). The eggs are large and dark. Larvae occur both beneath and on the surface of the food. The caudal bristles are not especially long. This species is one of the most abundant buttress-inhabiting species and is known to feed on geckos in nature.

P. triramulus F. & H.—Number of eggs laid by an individual female: 100 (194 by 2 females). Total days, oviposition to adult: 41, 46.

The eggs are light in hue with thin shells and the larvae are pale with light brown heads and short caudal bristles. The larvae are slow-moving and occur both under the food and on the surface. The fourth-instar larva climbs as high as possible up the walls of the pot to pupate. Two females fed on a volunteer in the laboratory but the eggs they produced did not hatch. In nature *triramulus* is often found in burrows and has never been taken by us while biting either horse or man.

P. vespertilionis F. & H.—Number of eggs laid by individual females: 13 to 64; average for 21 females: 38. Total days, oviposition to adult: 45 (one observation).

The eggs and larvae are indistinguishable in appearance and behavior from those of *isovespertilionis*. The average number of eggs per female may be lower than the number given above. Since these two species are distinguishable only in the adult male, females producing eggs which did not complete development or which yielded only females could not be included in the data given above.

While both these species are commonly found in hollow trees if bats are also present, several attempts to feed laboratory-reared *vespertilionis* on bats failed.

P. vexillarius F. & H.—Number of eggs laid by individual females: 4, 10, 16. Total days, oviposition to adult: 55 (one observation). The eggs are oval and dark. The larva has a pale head and long caudal bristles. The larvae are slow-moving and occur only on the surface of the food.

Warileya rotundipennis F. & H.—Number of eggs laid by individual females: 29, 40, 66, 66, 79.

W. rotundipennis is an avid man-biter in the areas where it occurs. All the fed females involved in our rearing attempts, taken while biting man, came from an area at an altitude of 1,200 feet, where both day and night temperatures are lower than near the coast. The fed females brought to the laboratory all died without ovipositing. It was found that if the females were kept in their normal environment, in a rearing pot partially buried in the earth, they would live long enough to produce eggs. Of the seven females which laid eggs, five took 8 days from feeding to oviposition and one laid eggs within a day, probably the result of a previous blood meal. The pots were then brought to the laboratory and until hatching were kept in either of two of the coolest available rooms at 23° or 27° C. The hatching times for those pots for which we had accurate data were 15, 15 and 16 days, a period longer than normal for *Phlebotomus* and doubtless a reflection of the lower temperature.

We were not successful in carrying the larvae beyond the first instar even though different attempts were made at room temperatures from 23° to 27° C., and the food included, in two cases, soil from the area of capture.

The egg is very small, rounded-oval in shape, and black. The first instar larva is correspondingly short and relatively much broader than *Phlebotomus*. It has very fine caudal bristles.

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