OBSERVATIONS ON MULTIPLE FEEDING BY *LUTZOMYIA LONGIPALPIS* IN THE LABORATORY (DIPTERA: PSYCHODIDAE)[1]

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An important factor governing the vector potential of an arthropod is its propensity toward taking subsequent blood meals. Smith et al. (1940), Barretto (1942) (quoted by Ward 1977), Johnson and Hertig (1961), and Ward (1977) studied the refeeding behavior of selected phlebotomine species including *Lutzomyia sanguinaria* (Fairchild and Hertig), *Lutzomyia flaviscutellata* (Mangabeira) and *Phlebotomus argentipes* Annandale and Brunetti. The results of these investigations indicated considerable differences in the multiple feeding ability of these species based largely on oviposition mortality. Killick-Kendrick et al. (1977a) and Ready (1978) have described the life cycle, blood feeding habits and laboratory rearing techniques for *Lutzomyia longipalpis* (Lutz and Neiva); however, neither of these studies addressed the question of multiple feeding in this species. Killick-Kendrick et al. (1977a) reported that the majority of *L. longipalpis* observed in their study died during or within 24 hr of the first oviposition. Endris et al. (1982) reported that individuals of certain *Lutzomyia* species in colonization survived the first oviposition and were subsequently given the opportunity to refeed, but the authors did not elaborate or quantify this information.

The acquisition of a colony of *L. longipalpis* (a known vector of neotropical human leishmaniasis) for repellent testing allows us to report some observations on the multiple feeding aptitude of this species (Deane and Deane 1955, Buescher et al. 1982). Such information may be useful to investigators planning disease transmission studies with this sand fly and in understanding the epidemiology of sand fly borne diseases.

The *L. longipalpis* used in this study were colonized from eggs supplied by Dr. R. Killick-Kendrick, Imperial College, London, England. Sand flies were reared and maintained at 24°C and 65% RH under a 12:12-hr photoperiod incorporating 1 hr of simulated sunrise and 1 hr of simulated sunset. Daytime illumination was held at 30 fc. Larvae were reared on a diet of desiccated liver powder, and adults were maintained on a 30% sucrose solution prior to blood feeding. Forty to fifty nulliparous females of 2–7 days postpupal eclosion were aspirated into a 30 cm^3 plastic cage and allowed to feed for 30 min on a guinea pig anesthetized with 0.5 ml of acepromazine and ketamine. Blood fed females were transferred to 7.6 cm diam cardboard cups with an equal number of male flies for mating and held for 3 days on a 30% sucrose solution. Females were subsequently aspirated into 7.6 cm diam sections of Plexiglas® tubing lined with plaster of paris and filled approximately 1/3 with commercial potting soil. Flies were allowed to oviposit for 4 days and were maintained on 30% sucrose. Surviving females were transferred to the feeding cage as before and the process of feeding, mating and oviposition was repeated until all sand flies were dead or moribund. Seven groups of 40–50 sand flies were sampled in this manner over a 3-mo. period.

Table 1 illustrates the vitality and mortality of 332 *L. longipalpis* from time of the first blood meal until death. Approximately 88.5% of the females sampled survived the interval between the first blood meal and oviposition. Less than half (46.9%) of the total females sampled survived the first oviposition. Forty-nine *L. longipalpis* accepted a second blood meal in this study. This represents approximately 14.7% of the group sampled. All remaining *L. longipalpis* were dead or moribund following the second oviposition.

Since approximately 1/3 of the sand flies surviving the first oviposition accepted a second blood meal, the major limiting factor for multi-

Table 1. Multiple feeding aptitude and oviposition vitality for 7 groups of *Lutzomyia longipalpis* in the laboratory.

	Test group							
No. of females	1	2	3	4	5	6	7	% total
1st blood meal	50	50	50	50	50	42	40	100
1st oviposition	47	48	48	44	38	36	33	88.5
Oviposition survival	34	34	10	11	18	31	18	46.9
2nd blood meal	2	—	3	8	9	17	10	14.7
2nd oviposition	—	—	—	—	—	13	—	3.9
Oviposition survival	—	—	—	—	—	—	—	

[1] The opinions and assertions contained herein are the private views of the authors and are not to be construed as official or as reflecting the views of the Department of the Army or the Department of Defense. Citation of trade names in this report does not constitute an official endorsement or approval of the use of such items.

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ple feeding by *L. longipalpis* in this study would appear to be oviposition mortality. We attribute our success in lowering mortality of this species to keeping all adult flies in an essentially quiescent state whenever possible. During the course of rearing procedures it was observed that under the standard lighting conditions used at the time, adult agitation and general activity were marked. By placing all adult colony and holding cages under sheets of semitranslucent polyethylene black plastic, the general level of activity in the cages was noticeably reduced and daily mortality decreased. When left unhandled or undisturbed in this manner for several hours, adult flies would appear moribund with considerable agitation being necessary to induce a taxis.

As noted in the introduction, significant differences appear to exist in the multiple feeding aptitude of different sand fly species. Guilvard et al. (1980) reported that *Phlebotomus ariasi* Tonnoir undergoes at least 3 anautogenous gonotrophic cycles in nature, and Ward (1977) has demonstrated that individuals of *L. flaviscutellata* may refeed and oviposit up to 4 times. Conversely, Johnson and Hertig (1961) showed that the majority of *L. sanguinaria* examined in their study died at the first oviposition or refused a second feed while Chaniotis (1967) reported similar findings with *Phlebotomus vexator occidentis* Fairchild and Hertig. The results of our study suggest *L. longipalpis* may refeed and complete up to 2 gonotrophic cycles in the laboratory and indicate the feasibility of using this species for cyclical disease transmission studies (Killick-Kendrick et al. 1977a).

Although the ability to survive oviposition and refeed represent an important aspect of sand fly vector potential, the role of interrupted partial feeding or probing may also be noteworthy. Dhanda and Gill (1982) noted mixed bloodmeals of human, bovine and rodent origin due to partial feeding in *Phlebotomus papatasi* Scopoli and *P. argentipes*. Killick-Kendrick et al. (1977b) reported that leishmania-infected *L. longipalpis* tend to probe more frequently than uninfected flies. Our own observations with *L. longipalpis* indicate this species will bite and probe repeatedly even after totally replete with blood. These facts might indicate that interrupted feeding or probing constitute as likely a manner of disease transmission for some species as complete gonotrophic cycles.

References Cited

Barretto, M. P. 1942. Contribuiçao par o estudo da biologia dos *Flebotomos* em condicoes experimentais (Diptera: Psychodidae). Tese de Doutoramento, Fac. Med. Univ. Sao Paulo, Mangione, Sao Paulo. 162 p.

Buescher, M. D., L. C. Rutledge, R. A. Wirtz, K. B. Glackin and M. A. Moussa. 1982. Laboratory tests of repellents against *Lutzomyia longipalpis* (Diptera: Psychodidae). J. Med. Entomol. 19:176–180.

Chaniotis, B. N. 1967. The biology of California *Phlebotomus* (Diptera:Psychodidae) under laboratory conditions. J. Med. Entomol. 2:221–233.

Deane, M. P. and L. M. Deane. 1955. Observacoes sobre a transmissao de leishmaniose visceral no Ceara. Hospital, Rio de J. 48:95–118.

Dhanda, V. and G. S. Gill. 1982. Double blood meals by *Phlebotomus argentipes* and *P. papatasi* in two villages of Maharashtra. Indian J. Med. Res. 76:840–842.

Endris, R. G., P. V. Perkins, D. G. Young and R. N. Johnson. 1982. Techniques for laboratory rearing of sand flies (Diptera: Psychodidae). Mosq. News 42:400–407.

Guilvard, E., T. J. Wilkes, R. Killick-Kendrick and J. A. Rioux. 1980. Ecology of leishmaniasis in the south of France: 15. Gonotrophic cycles in nature of *Phlebotomus ariasi* and *Phlebotomus mascittii* in the Cevennes: Epidemiological significance. Ann. Parasitol. Hum. Comp. 55:659–664.

Johnson, P. T. and M. Hertig. 1961. The rearing of *Phlebotomus* sand flies (Diptera: Psychodidae). I. Technique. Ann. Entomol. Soc. Am. 54:764–776.

Killick-Kendrick, R., A. J. Leaney and P. D. Ready. 1977a. The establishment, maintenance and productivity of a laboratory colony of *Lutzomyia longipalpis* (Diptera: Psychodidae). J. Med. Entomol. 13:429–440.

Killick-Kendrick, R., A. J. Leaney, P. D. Ready and D. H. Molyneux. 1977b. *Leishmania* in phlebotomid sand flies. Part 4. The transmission of *Leishmania mexicana amazonensis* to hamsters by the bite of experimentally infected *Lutzomyia longipalpis*. Proc. R. Soc. Lond. B. Biol. Sci. 196:105–114.

Ready, P. D. 1978. The feeding habits of laboratory-bred *Lutzomyia longipalpis* (Diptera: Psychodidae). J. Med. Entomol. 14:545–552.

Smith, R. O. A., K. C. Halder and I. Ahmed. 1940. Further investigations on the transmission of Kala-Azar. Part III. The transmission of Kala-Azar by the bite of the sand fly *P. argentipes*. Indian J. Med. Res. 28:585–591.

Ward, R. D. 1977. The colonization of *Lutzomyia flaviscutellata* (Diptera: Psychodidae), a vector of *Leishmania mexicana amazonensis* in Brazil. J. Med. Entomol. 14:469–476.

THE PRODUCTION OF *AEDES AEGYPTI* BY A WEEKLY OVITRAP SURVEY

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The presence of dengue in the Caribbean nations has necessitated surveillance of local *Aedes aegypti* (Linn.) populations in the southern