Japan. J. Trop. Med. Hyg., Vol. 17, No. 2, 1989, pp. 149-155

OBSERVATIONS ON THE VALIDITY OF THE OVARIAN ACCESSORY GLANDS OF SEVEN ECUADORIAN SAND FLY SPECIES (DIPTERA: PSYCHODIDAE) IN DETERMINING THEIR PARITY

HIROYUKI TAKAOKA[1], EDUARDO A. GOMEZ L[2]., JOHN B. ALEXANDER[3] AND YOSHIHISA HASHIGUCHI[4]

Received December 13 1988/Accepted January 29 1989

Abstract: Females of seven sand fly species caught on man in several leishmaniasis-endemic foci in Ecuador were examined to assess the value of the accessory gland secretions as an indicator of parity. It was found that parous females could be distinguished from nullipars by the presence of granular secretions in the accessory glands in *Lutzomyia ayacuchensis,* probable vector of *Leishmania* in the Andean highlands of southern Ecuador. Examination of the female accessory glands was not a reliable method for determining parity in six other sand fly species caught in lowland areas, including *Lu. trapidoi, Lu. hartmanni,* and *Lu. gomezi,* three proven vectors of *Leishmania,* since glanular secretions were found in both parous and nulliparous females.

INTRODUCTION

Condition of the ovarioles has proved to be a useful characteristic for distinguishing parous from nulliparous females in several groups of Nematocera, including Phlebotominae (Detinova, 1962). This ovarian method has been thought of as impractical for routine work due to the small size of the ovarioles in sand flies. Examination of the accessory glands of the ovaries, which are large enough to examine quickly, is a useful method for distinguishing parous females in several African sand fly species of the genus *Phlebotomus* (Adler and Theodor, 1935; Lewis and Minter, 1960). Usefulness of this method seems to be dependent upon the sand fly species involved when applied to the New World genus *Lutzomyia.* Accessory gland secretions were reported as a reliable sign of parity in three species from northern California (Chaniotis and Anderson, 1967), and also in eight species from Brazil (Lewis *et al.,* 1970), but had no value for determining parity for many other *Lutzomyia* species (Johnson and Hertig, 1961; Johnson *et al.,* 1963; Lewis, 1965; Lewis *et al.,* 1970; Ward, 1974).

1 Division of Medical Zoology, Medical College of Oita, Hazama, Oita 879-56, Japan
2 Departamento de Parasitologia, Instituto Nacional de Higiene y Medicina Tropical, Apartado 3961, Guayaquil, Ecuador
3 Department of Entomology and Nematology, University of Florida, Gainesville, Florida 32611, U.S.A.
4 Department of Parasitology, Kochi Medical School, Nankoku City, Kochi 781-51, Japan

The present study was undertaken to determine if the accessory glands could be used to determine parity of several Ecuadorian sand fly species, including some proven vectors of *Leishmania*.

Materials and Methods

Sand fly collections were made at five localities, i.e., Challuabamba and Paute, Department of Azuay, both situated at altitudes of 2,300-2,500 m above sea level in the Andean plateau; Echeandia, Department of Bolivar (altitude ca. 500 m a.s.l.), and Puerto Quito, Department of Pichincha (altitude 450 m a.s.l.), both situated in the western foothills of the Andes; and Pajân, Department of Manabi, in the Pacific coastal region (altitude ca. 20 m a.s.l.). All of these localities lie in regions where *Leishmania* is endemic (Hashiguchi, 1987). At Challuabamba and Paute, collections were carried out for three and six nights respectively on rocky hillsides covered in grasses, small shrubs and *Agave* plants. Tree cover was confined to sparse groves of young *Eucalyptus* on the lower slopes.

At the other study sites, sand fly collections were made for one or two nights in secondary forest near human habitation except at Pajân, where insects were captured in a coffee plantation.

Flies that alighted on human volunteers between 18.30 and 21.00 were captured using a mouth aspirator (Hashiguchi, 1987) and maintained overnight in small plastic vials provided with sufficient moisture. Female sand flies captured were dissected in saline on a glass slide and the accessory glands checked microscopically for the presence of granular secretions. At the same time, ovaries were examined to determine their parous state, using the follicular relic method of Detinova (1962); the shapes of the spermatheca and cibarium were observed for sand fly identification, which was done using keys of Young (1979) in reference of Cáceres and Bianchi (1988).

The guts of all dissected sand flies were examined for the presence of blood meals. A number of sand flies caught at Challuabamba were allowed to take blood from an anesthetized golden hamster, and kept in a plaster-lined 120 m*l* plastic vial provided with a piece of sugar cane at a temperature of 15-20°C for three, seven or nine days. The accessory glands of these fed flies were then examined to determine whether granular material had been secreted in accordance with the gonotrophic cycle. The methods of collection of adult sand flies were detailed previously by Hashiguchi *et al.* (1985), and for ovarian dissection by Lewis (1965).

Results

A total of seven sand fly species taken in human bait collections in the five localities sampled were examined. The numbers of females of each species with or without accessory gland secretions, in relation to their parous state as demonstrated by the ovarian method, are shown in Table 1. In *Lu. ayacuchensis* Cáceres and Bianchi from Challuabamba and Paute, granular secretions in the accessory glands were seen in all 27 parous females but not in most nulliparous females.

The nine nulliparous flies with granular secretions had developing follicles of stage IIIa, but these were all among those that had fed on varying quantities of blood after being

Table 1 Granule secretions of accessory glands in relation to parity in seven anthropophilic sand fly species examined during July-August, 1988 in Ecuador

Lutzomyia spp.	Blood meal[1]	No. flies examined	Granule secretions present		absent	
			N[2]	P[2]	N	P
ayacuchensis						
Challuabamba	−	52	0	7	45	0
	+	12	6	2	4	0
Paute	−	41	0	15	26	0
	+	16	3	3	10	0
trapidoi						
Echeandia	−	84	38	16	28	2
	+	6	6	0	0	0
Puerto Quito	−	11	7	1	3	0
hartmanni						
Echeandia	−	35	13	13	9	0
	+	5	3	1	1	0
Puerto Quito	−	28	14	8	6	0
	+	4	3	0	1	0
carrerai thula						
Echeandia	−	2	1	1	0	0
panamensis						
Puerto Quito	−	12	4	5	3	0
gomezi						
Pajân	−	49	33	16	0	0
	+	14	10	4	0	0
shannoni						
Pajân	−	7	2	5	0	0
	+	1	1	0	0	0

1. Fed to a varying degree when collected on men
2. N: nulliparous; P: parous

Table 2 Changes in secretions of accessory glands of *Lutzomyia ayacuchensis* from Challuabamba, fully fed on blood from hamster and kept on sugar cane at a temperature of 14-20°C

Group	Day after blood feeding				
	1	3	5	7	9
fed	4/4[1]	4/4	5/5	5/5	16/18
	(IIIa)[2]	(IIIb)	(IV)	(V)[3]	(V)[3]
unfed	—	—	0/1	—	0/3
	(—)	(—)	(II)	(—)	(II)

1. No. positive for granule secretions/no. examined
2. Follicular stage
3. Eggs were already deposited in 1 of the 5 females dissected on day 7 and in 17 of the 18 females dissected on day 9.

collected. The granules found in these nulliparous females are therefore considered to have been secreted after the blood meal taken on the preceding day.

In contrast, *Lu. trapidoi* (Fairchild and Hertig), *Lu. hartmanni* (Fairchild and Hertig), *Lu. panamensis* (Shannon) and *Lu. carrerai thula* Young collected from Echeandia and Puerto Quito showed discordant relations between granular secretions and parity. Accessory gland secretions were found in more than half of the nulliparous females, as well as in most of the parous females. All the unfed nulliparous females with granular secretions had follicles of stage I or II.

Accessory gland secretions were seen in all females irrespective of parity, in two *Lutzomyia* species caught from Pajân. No developing follicles were seen except in blood-fed flies, which had stage IIIa follicles.

The results of dissections of female *Lu. ayacuchensis* kept for several days after blood feeding are shown in Table 2. This sand fly species was gonotrophically concordant. Egg maturation required approximately seven days, and eggs were laid shortly thereafter. The granules in the accessory glands began to be secreted soon after the blood meal, and were accumulated gradually as follicular development proceeded; the accessory glands enlarged and were filled with dark granules within five days of blood feeding. The accessory glands in 16 of 18 flies which had oviposited had remnants of dark granules in varying amounts, while those in the remaining two females had no residues and resembled those of nullipars. One and three unfed females, examined five and nine days after collection respectively, had no granule secretions and exhibited no follicular development beyond stage II.

Discussion

The present study clearly demonstrates that the accessory glands are a reliable organ for distinguishing parous from nulliparous females of *Lu. ayacuchensis* from Challuabamba and Paute. Granular secretions in the glands of this sand fly were apparently produced only after a blood meal was taken. The changes in the quantity of granular secretions during follicular development and after oviposition are the same as those observed in three Californian sand fly species by Chaniotis and Anderson (1967). It should be remembered that not all females with granular secretions can be judged as parous, since some of the blood-fed nulliparous females might have secreted granules shortly after feeding. In future studies it would therefore be better to discard blood-fed females or to also check the ovarian follicles when examination of these females is required. There is also a possibility that some parous females are mistaken for nulliparous when granules are entirely expelled from the accessory glands after oviposition, as demonstrated in two of the 18 wild-caught females that laid eggs in the laboratory during the present study.

At present little is known about the biology of *Lu. ayacuchensis* or its role in the transmission of *Leishmania* in the Andes of southern Ecuador. This species was first recorded from Ecuador during the present study but originally misidentified as its close relative, *Lu. peruensis* (Shannon). Closer examination revealed that the sand fly fauna of the Paute area consisted of two closely related species, i.e., *Lu. ayacuchensis* and *Lu. osornoi* (Ristorcelli and Van Ty), both of which are anthropophilic and may be involved in transmission of *Leishmania* in the area. Our findings will facilitate future studies on bionomics and dynamics of wild populations of *Lu. ayacuchensis,* and may also applied in the future to *Lu. osornoi.*

By contrast, the accessory gland secretions did not prove to be useful in distinguishing between parous and nulliparous females for *Lu. trapidoi, Lu. hartmanni, Lu. panamensis* and *Lu. carrerai thula* collected from Echeandia and Puerto Quito. The first two species have been reported as probable vectors of leishmaniasis in Ecuador (Hashiguchi *et al.*, 1985), while *Lu. panamensis* is a proven vector of *Leishmania* in Panama (WHO, 1984). In each of these four species, granular secretions were seen in most parous females, but more than half of the unfed nulliparous females dissected also had granular secretions. It may be that accessory gland secretions are produced a few days after eclosion, irrespective of whether or not a blood meal is taken, as has been observed in *Lu. longipalpis* (Lutz and Neiva) from Brazil (Ward, 1974).

Accessory gland secretions were observed in all dissected females of *Lu. gomezi* (Nitzulescu), another suspected vector of *Leishmania* in Ecuador (Hashiguchi, 1987), and *Lu. shannoni* (Dyar) from Pajân. This indicates that the glands are of no value in determining parity. Lewis *et al.* (1970) reported that some females of *Lu. shannoni* in Belize and *Lu. gomezi,* and three other species in Brazil were probably autogenous, based on the high proportion of flies with granular secretions showing discordant ovarian development. No sign of autogeny was seen in females of the two species collected from human bait in Pajân. None of the ovarian follicles in unfed nulliparous females had developed beyond stage II, and accessory gland secretions were found in all these females.

It appears that female accessory gland secretions are not associated with autogeny, and they are probably produced shortly after adult emergence. Autogenous strains have been reported in *Lu. shannoni* from Florida (Perkins, 1982) and in *Lu. gomezi* from Panama (Johnson, 1961). It is possible that autogenous strains of these species also exist in Ecuador. A somewhat high proportion of parous females (five of eight dissected, or 62.5%), among those collected suggests that an autogenous population may be present. Further studies would be required to determine whether this is the case.

At present, there are no reliable methods other than the ovarian relic method in determining reproductive parity of vector sand flies in lowland areas endemic for *Leishmania.* Further efforts are needed to find out reliable methods of distinguishing parous from nulliparous females.

ACKNOWLEDGEMENTS

We thank Drs. R. F. Lazo S. and V. V. de Coronel for their encouragement, and express our appreciation to our collaborators in the Instituto Nacional de Higiene y Medicina Tropical, Guayaquil, Ecuador. Thanks are also due to our research team members, Drs. T. Mimori, M. Furuya and S. Nonaka for their support. This study was supported by the Overseas Research Program of the Ministry of Education, Science and Culture, Japan (No. 63041096).

REFERENCES

1) Adler, S. and Theodor, O. (1935): Investigations on Mediterranean kala azar VIII, Proc. Trop. S. Paulo, 3, 230-238

2) Cáceres, A. and Bianchi, G.E.A. (1988): Descriçâo de *Lutzomyia ayacuchensis* sp. n. (Diptera,

Psychodidae, Phlebotominae) dos Andes peruanos, Revta Bras. Ent., 32, 55-61
3) Chaniotis, B.N. and Anderson, J.R. (1967): Age structure, population dynamics and vector potential of Phlebotomus in northern California. Part I, J. Med. Entomol., 4, 251-254
4) Detinova, T.S. (1962): Age-grouping methods in Diptera of medical importance, Monograph Ser. W.H.O. no. 47, pp. 216
5) Hashiguchi, Y. (1987): Studies on New World leishmaniasis and its transmission, with particular reference to Ecuador, Kyowa Printing & Co. Ltd., Kochi, pp. 174
6) Hashiguchi, Y., Gomez L. E.A., De Coronel, V.V., Mimori, T. and Kawabata, M. (1985): Natural infections with promastigotes in man-biting species of sand flies in leishmaniasis-endemic areas of Ecuador, Am. J. Trop. Med. Hyg., 34, 440-446
7) Johnson, P.T. (1961): Autogeny in Panamanian *Phlebotomus* sandflies, Ann. Ent. Soc. Am., 54, 116-118
8) Johnson, P.T. and Hertig, M. (1961): The rearing of *Phlebotomus* sandflies, Ann. Ent. Soc. Am., 54, 764-776
9) Johnson, P.T., McConnell, E. and Hertig, M. (1963): Natural infections of leptomonad flagellates in Panamanian *Phlebotomus* sandflies, Exp. Parasitol., 14, 107-122
10) Lewis, D.J. (1965): Internal structural features of some Central American phlebotomine sandflies, Ann. Trop. Med. Parasitol., 59, 375-385
11) Lewis, D.J., Lainson, R. and Shaw, J.J. (1970): Determination of parous rates in Phlebotomine sandflies with special reference to Amazonian species, Bull. Ent. Res., 60, 209-219
12) Lewis, D.J. and Minter, D.M. (1960): Internal structural changes in some African Phlebotominae, Ann. Trop. Med. Parasitol., 54, 351-365
13) Perkins, P.V. (1982): The identification and distribution of phlebotomine sand flies in the United States with notes on the biology of two species from Florida (Diptera: Psychodidae), Ph.D. theses, University of Florida, Gainesville, Florida, pp. 196
14) Ward, R.D. (1974): Granule formation in the accessory glands of a laboratory strain of *Lu. longipalpis* (Diptera: Phlebotominae) from Ceará state, Brazil, Trans. R. Soc. trop. Med. Hyg., 68, 171
15) WHO Expert Committee (1984): The Leishmaniases, Tech. Rep. Ser. 701, WHO, Geneva, pp. 140
16) Young, D.G. (1979): A review of the bloodsucking psychodid flies of Colombia (Diptera: Phlebotominae and Sycoracinae), University of Florida Agricultural Experimental Station Technical Bulletin 806, pp. 266

エクアドルにおける人吸血性サシチョウバエ7種の卵巣付属腺による顆粒分泌と経産歴との関係

高岡　宏行[1]・Eduardo A. Gomez L[2].・
John B. Alexander[3]・橋口　義久[4]

旧大陸のサシチョウバエでは卵巣付属腺の分泌する顆粒の有無が経産，未経産雌の区別に用いられているが，新大陸のサシチョウバエではこの方法の応用は難しいとされている。今回，エクアドルにおいて人囮法で採集した7種のサシチョウバエを解剖し，ovarian relic 法による経産歴と付属腺での顆粒の有無との関係を検討した。低地のリーシュマニア症媒介種，*Lutzomyia trapidoi*，*Lu. hartmanni* および *Lu. gomezi* を含む6種では未経産雌，経産雌いずれにも付属腺の分泌顆粒が見られるなど顆粒の有無だけでは経産歴を判定できないという結果を得た。一方，アンデス高地の *Lu. ayacuchensis* では，分泌顆粒は経産雌のみに見られ，経産個体の判別に用いられ得ることが分かった。

1）大分医科大学医動物学教室
2）エクアドル国立衛生学熱帯医学研究所寄生虫部門
3）フロリダ大学昆虫学線虫学部門
4）高知医科大学寄生虫学教室