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MARK–RECAPTURE STUDIES OF HOST SELECTION BY *ANOPHELES (ANOPHELES) VESTITIPENNIS*

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ABSTRACT. We present herein the results of a series of mark–recapture experiments with female *Anopheles vestitipennis*. Theses experiments used human and animal hosts to assess the degree of anthropophily of field-caught specimens, originally collected on either host, and of their offspring. Fidelity of mosquitoes to particular hosts was estimated by recapturing marked host-seeking mosquitoes returning for a 2nd blood meal. Results indicated that mosquitoes seeking animal hosts were more faithful (80.48%; 33 of 41) in returning to their original host than were those seeking human hosts (63%; 49 of 78).

KEY WORDS *Anopheles vestitipennis*, mark–recapture, host selection

INTRODUCTION

Anopheles (Anopheles) vestitipennis Dyar and Knab is a Neotropical anopheline, with a scattered distribution throughout Central America, the Greater Antilles, and northern South America (Vargas 1958, Belkin et al. 1970, Wilkerson and Strickman 1990). Its role as a secondary vector of *Plasmodium vivax* Grassi and Feletti and *P. falciparum* Welch has been confirmed in Guatemala (Padilla et al. 1992). In southern Mexico, *An. vestitipennis* was incriminated as a vector of malaria in the Lacandón Forest (Loyola et al. 1991, Arredondo-Jiménez 1995), but no specimens were found that were infected with malaria parasites on the Pacific Ocean Coastal Plain (Arredondo-Jiménez 1995).

Investigations to explore geographic dissimilarities in the role of *An. vestitipennis* as a malaria vector revealed the possible existence of 2 genetically distinct sympatric populations with specific preferences for humans or animals (Arredondo-Jiménez 1995). These findings were later supported by isoenzyme analysis (Arredondo-Jiménez et al. 1996), studies of egg ultrastructure (Rodríguez et al. 1999) and random amplified polymorphic DNA (RAPD) marker studies (Murillo et al., unpublished data). Nevertheless, more studies on the reputedly different populations within *An. vestitipennis* are necessary before any conclusion can be drawn regarding the taxonomic status of this species.

We present herein the results of a series of mark–recapture experiments with female *An. vestitipennis* that used human and animal hosts to assess the degree of anthropophily of field-caught specimens, originally collected on either host, and of their offspring.

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MATERIALS AND METHODS

Study area: The study was carried out in the Pacific Ocean coastal plain of Chiapas, México, in Nueva Independencia (14°37′30″N, 92°16′14″W, elevation 50 m), a village with a population of 112 living in 23 households. In general, houses in this village are well ventilated, with palm-thatch roofs and discontinuous walls made of split bamboo or palm poles. The climate in this area is hot subhumid (Garcia 1973), with a rainy season extending from May through October and an intervening dry season. Mean annual rainfall in the area is 2,100 mm, average monthly temperature is 27°C (range, 27–30°C), and relative humidity ranges from 61 to 100% (Verhoef 1986, Arredondo-Jiménez 1990). Village selection was based on mosquito abundance. Although the primary vegetation surrounding the village has been replaced mostly by crops (mango, banana, and corn) and cattle pastures, various forested patches and flooded tall grass formations that favor abundant populations of larval and adult *An. vestitipennis* (Rejmankova et al. 1998; Arredondo-Jiménez, et al., unpublished data) remain.

Mark–recapture experiments: Separate experiment series were undertaken with modified Magoon traps (Service 1993). During the 1st and 2nd experiment series, a set of 2 traps was placed at 1 corner of the village. The 2 traps were placed 5 m apart and were used to collect female *An. vestitipennis* during 12-h periods (1800–0600 h) for 20 consecutive nights. Traps were baited with 2 men in 1 trap and 1 horse in the other (1st experiment series), or by 2 men in 1 trap and 2 pigs in the other (2nd experiment series). The 3rd experiment series was also conducted during 20 consecutive nights, this time using 2 sets of 2 modified Magoon traps, with the sets of 2 traps placed at opposite ends (north and south) of the village. This time baits were 2 men in 1 trap and 1 cow in the other, and the baits were exposed only for 6-h periods (1800–2400 h).

In traps baited with humans, mosquitoes were collected during 45 min of each hour. Mosquitoes landing on human volunteers were captured with

Table 1. Anophelines (*Anopheles*) caught during 20 days in each experiment.

Host	*An. vestitipennis*	*An. albimanus*	*An. punctimacula*	Total
Humans vs.	2,088 (77.73%)	557 (20.73%)	41 (1.52%)	2,686
Horse	1,712 (49.73%)	1,577 (45.81%)	153 (4.44%)	3,442
Humans vs.	6,634 (87.10%)	869 (11.41%)	113 (1.48%)	7,616
Pigs	1,709 (70.82%)	692 (28.67%)	12 (0.49%)	2,413
Humans vs.	4,332 (74.48%)	1,351 (23.22%)	133 (2.28%)	5,816
Cow (south)	4,840 (43.06%)	5,719 (50.88%)	680 (6.05%)	11,239
Humans vs.	1,779 (55.23%)	1,393 (43.24%)	49 (1.52%)	3,221
Cow (north)	1,514 (31.37%)	2,974 (61.62%)	338 (7.00%)	4,826
Total humans	14,833 (76.69%)	4,170 (21.56%)	336 (1.73%)	19,339
Total animals	9,775 (44.59%)	10,962 (50.00%)	1,183 (5.39%)	21,920
Total	24,608	15,132	1,519	41,259

mouth aspirators (World Health Organization 1975, Bown et al. 1987). In traps with animal baits, collections of blood-engorged female mosquitoes were made during the last 15 min of each hour. Mosquitoes were placed in plastic containers lined with towel papers dusted profusely with fluorescent powder (1 color for each bait type; Lumogen®, Basf, Holland, MI). This allowed self-marking of the mosquitoes.

Marked bloodfed and unfed females next were released to breeding sites located approximately 300 m from the bait stations on 16 consecutive nights and recaptured until day 20. Host fidelity was estimated by the number of marked mosquitoes returning to feed on the same host type. The 3rd experiment was designed to assess the dispersal of mosquitoes and to determine whether fidelity could be observed in mosquitoes returning to the same host type, but in a trap set on the other side of the village.

Data analysis: Differences between collections of mosquitoes in each experiment were analyzed with Wilcoxon's t-tests for paired data. Differences in proportions of mosquitoes returning to the original host were examined with chi-square tests with continuity correction (Zar 1984).

RESULTS

Mosquito collections

Overall, in the 3 experiment series, *An. vestitipennis* was the predominant anopheline caught on human hosts (76.7%, $n = 19{,}339$), followed by *Anopheles albimanus* Wiedemann (21.6%) and *An. punctimacula* Dyar and Knab (1.7%), whereas more *An. albimanus* mosquitoes were collected on animal hosts (50.0%, $n = 10{,}962$), followed by *An. vestitipennis* (44.6%) and *An. punctimacula* (5.4%; Table 1). Ratios of human to animal hosts were 1.51:1 ($n = 24{,}608$), 1:2.62 ($n = 15{,}132$), and 1: 3.52 ($n = 1{,}519$), respectively, for *An. vestitipennis*, *An. albimanus*, and *An. punctimacula*. Hereafter, we only report data on mark–recapture studies with *An. vestitipennis*.

In the 1st experiment series, 1,663 *An. vestitipennis* mosquitoes selected humans hosts, whereas 1,351 selected horse bait. The resulting human to horse ratio was 1.23:1, with no differences found in the preference for either host ($\chi^2 = -1.88$, df = 1, $P = 0.0591$; Table 2). In the 2nd experiment series, 5,170 mosquitoes were collected in the human-baited trap, and 1,487 mosquitoes selected the pig-baited trap, yielding a human to pig ratio

Table 2. Host fidelity in *Anopheles vestitipennis*.

Host	Number released	Recapture rates (%)	Host fidelity (%)	P
Human	1,663	1.38	66 (23/35)	
vs.				0.1722
Horse	1,351	1.62	85 (22/26)	
Human	5,170	0.29	60 (15/25)	
vs.				0.7913
Pigs	1,487	0.06	33 (1/3)	
Human	1,160	0.34	100 (4/4)	
vs.				0.1258
Cow (north)	1,005	0	0 (0/2)	
Human	4,034	0.17	50 (7/14)	
vs.				0.0277
Cow (south)	3,045	0.32	100 (10/10)	
Human	12,027	0.40	63 (49/78)	
vs.				0.0269
Animal	6,888	0.47	80.5 (33/41)	

of 3.48:1 (χ^2 = −3.5185, df = 1, P = 0.0004). During the 3rd experiment series, 1,160 mosquitoes selected humans and 1,005 selected the cow in the north trap set (human to cow ratio = 1.15:1; χ^2 = −1.411, df = 1, P = 0.1579), whereas in the south trap set, 4,034 mosquitoes selected humans and 3,045 selected the cow (human to cow ratio = 1.32:1; χ^2 = −0.568, df = 1, P = 0.5701).

After pooling, more mosquitoes were collected in the human-host traps (12,027) than in the animal-baited traps (6,888), with a ratio of 1.74:1 (χ^2 = 0.6040, P = 0.003).

Mark–recapture studies

Host fidelity of mosquitoes was estimated by recapturing marked mosquitoes returning for a 2nd blood meal. During the 1st and 2nd experiment, recapture rates were 1.38 and 0.29% on humans and 1.62 and 0.06% on horse or pig, respectively. In the 3rd experiment, recaptures from the north and south trap sets, respectively, included 0.34 and 0.40% mosquitoes from humans and 0 and 0.47% from cows.

During the 1st experiment, 66% (23 of 35) of mosquitoes collected on humans returned to humans, whereas 85% (22 of 26) of mosquitoes collected on horse returned to horse. Both rates of fidelity were similar (χ^2_{corr} = 1.86, P = 0.172). In the 2nd experiment, 60% (15 of 25) of mosquitoes showed fidelity to human hosts, whereas 33% (1 of 3) were faithful to pig. Also, the rates of fidelity were similar (χ^2_{corr} = 0.07, P = 0.791). During the 3rd experiment, in the north trap set, 100% (4 of 4) of mosquitoes showed fidelity to humans, and 0% (0 of 2) showed fidelity to cow (χ^2_{corr} = 2.34, P = 0.126), whereas in the south trap set, 50% (7 of 14) were faithful to humans and 100% (10 of 10) were faithful to cow (χ^2_{corr} = 4.85, P = 0.027). After pooling, mosquitoes seeking animal hosts were more faithful (80.48%; 33 of 41) in returning to their original host than were mosquitoes seeking human hosts (63%; 49 of 78; χ^2_{corr} = 4.90, P = 0.027; Table 2).

Movements between north and south trap sets were observed in only 12 mosquitoes, of which 10 returned to the same host (6 and 4 to animal and human host, respectively, that is, were faithful). Parallel collections in the surroundings of the village revealed that 2 marked mosquitoes that were originally collected on human hosts were caught landing on humans at 0.8 and 2 km, respectively.

DISCUSSION

The overall mosquito collection in our experiments confirms previous studies carried out in the region (Breeland 1972, Loyola et al. 1993, Arredondo-Jiménez 1996, Ulloa et al. 1999). *Anopheles vestitipennis* was the predominant anopheline caught on human baits, whereas more *An. albimanus* were caught on animal baits. The ratios of human to animal host preferences for *An. vestitipennis, An. albimanus*, and *An. punctimacula* indicated that *An. vestitipennis* was relatively more anthropophagic, whereas *An. albimanus* and *An. punctimacula* were more zoophagic.

The results of the mark–recapture experiments support the possibility of 2 subpopulations of *An. vestitipennis* with higher preference for either human or animal hosts. The segregation of feeding behavior, with >50% (80 and 60% on animal and human bait, respectively) of mosquitoes returning for a 2nd blood meal on the same host from which they were 1st captured, indicates host-specific tendencies. Although recapture of mosquitoes on the same host type may result from a genetically imprinted feeding behavior, host location can also respond to memorized home range, as reported for *Anopheles farauti* Laveran in Papua New Guinea (Charlwood et al. 1988) and *An. balabacensis* Baisas in Sabah, Malaysia (Hii et al. 1991). A 3rd possibility is that mosquitoes may learn to identify their hosts, based on previous feeding experiences. This question was explored in a study that assessed the host-selection behavior of *An. albimanus*. That study found that, when given the choice, learning was not involved in the selection of human or animal hosts, but rather that mosquitoes had an inherent preference for host essence (Arredondo-Jiménez et al. 1992). Nevertheless, the recapture of *An. vestitipennis* on the same bait type in traps located in different places is indicative of the 1st possibility.

Differences in host preferences have been observed in other anopheline species (Hackett 1937, Joshi et al. 1988) as a result of the existence of morphologically indistinguishable subspecies. Thus, *Anopheles gambiae* Giles sensu stricto and *An. arabiensis* Patton, 2 members of the *An. gambiae* complex, have differences in their anthropophagic index, with *An. gambiae* s.s. being more anthropophagic than *An. arabiensis* (Coluzzi et al. 1975, Mollineaux and Gramiccia 1980). The same differences were reported between *Anopheles culicifacies* Giles species A and B, where species A is more anthropophagic (Sugana et al. 1983). Our results provide the 1st behavioral evidence of the existence of 2 populations with different host preferences in *An. vestitipennis*. These findings, along with our previous observations on differences on biting behavior (Arredondo-Jiménez 1995), isozyme pattern (Arredondo-Jiménez et al. 1996), egg ultrastructure (Rodríguez et al. 1999), and DNA sequence (evidenced by RAPD marker analysis; Murillo et al., unpublished data), support the possible existence of 2 subspecies of *An. vestitipennis* in southern Mexico.

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