

## INNATE HOST SELECTION IN *ANOPHELES VESTITIPENNIS* FROM SOUTHERN MEXICO

ARMANDO ULLOA,<sup>1</sup> JUAN I. ARREDONDO-JIMÉNEZ,<sup>2</sup> MARIO H. RODRÍGUEZ,<sup>2</sup>  
ILDEFONSO FERNÁNDEZ-SALAS<sup>3</sup> AND LILIA GONZÁLEZ-CERÓN<sup>1</sup>

**ABSTRACT.** We assessed the degree of host specificity of the purported anthropophilic and zoophilic populations of *Anopheles vestitipennis*. A series of experiments were conducted in an experimental hut with 3 compartments lined with nylon netting. A central release compartment and 2 side compartments were each baited with equivalent surface area of human and animal baits. Wild *An. vestitipennis* collected on each host, as well as corresponding F<sub>1</sub> mosquitoes, were released in the central compartment. Overall, 22% (166/748) of all mosquitoes collected on humans were recaptured in the human compartment, whereas 23% of mosquitoes originally collected on animals were recaptured in this compartment. Experiments with F<sub>1</sub> females resulted in 59% human selection rates, a 2.6 times increase compared with wild anthropophilic females, while a 1.2 times decrease in human selection rates (from 24% to 20%) was observed in F<sub>1</sub> of wild zoophilic females. Host-selection experiments in the Lacandón Forest revealed the same trend. These findings suggested that the complex mode of inheritance that resulted in female mosquitoes showing a stronger tendency to return to their preferred host was obscured by the nature of the method of collection, i.e., wild parental females selecting a host either innately or opportunistically, the majority of which were likely innately attracted. This was revealed by F<sub>1</sub> females, of which, when given the choice to select a host, a higher proportion opted for the preferred one. The results presented here are in accordance with other studies that identified a subpopulation of *An. vestitipennis* in southern Mexico with higher anthropophily.

**KEY WORDS.** *Anopheles vestitipennis*, innate host selection, anthropophilic, zoophilic

### INTRODUCTION

*Anopheles (Anopheles) vestitipennis* Dyar and Knab is distributed throughout Central America (Vargas 1958, Belkin et al. 1970) and has been incriminated as a malaria vector in the Lacandón Forest, southern Mexico (Loyola et al. 1991, Arredondo-Jiménez 1995), Guatemala (Padilla et al. 1992), and Belize (Achee et al. 2000). Studies using isoenzyme analysis and biting behavior (Arredondo-Jiménez et al. 1996), egg ornamentation (Rodríguez et al. 1999), and gene markers (Martínez 2001, Murillo-Sánchez 2001) revealed the existence of two sympatric populations with different host preferences in Chiapas, Mexico.

Mark-recapture studies on the propensity of female *An. vestitipennis* to return to the same blood host, either human or animal, indicated more than 50% of mosquitoes returned to the original host type from which they were initially collected (Ulloa et al. 2002). Because it was not possible to identify previous blood meal hosts and the effect of host availability, it was not possible to establish the level of host specificity in these purported anthropophilic and zoophilic populations. In the current study, we attempted to diminish the effect of these 2 variables by carrying out a series of experiments in an experimental hut where the surface area of human and

animal baits were equivalent and the previous feeding history was controlled by using feral *An. vestitipennis* that initially selected human or animal baits and their F<sub>1</sub> descendants.

### MATERIALS AND METHODS

#### Study area

The study was carried out in 2 areas of Chiapas, Mexico: 1) the Pacific Ocean Coastal Plain located in southern Chiapas. The climate is hot subhumid (Aw<sub>1</sub> of Garcia 1973), with a wet season extending from May through October. The selected village, Nueva Independencia (14°37'30"N, 92°16'14"W; elevation 50 m), with a population of 112 living in 23 households, has a high prevalence of *An. vestitipennis*, where the vegetation surrounding the village are mostly crops (mango, banana, and corn) and cattle pastures, various forested, and flooded tall grass patches, favorable for *An. vestitipennis* populations (Rejmankova et al. 1998), remain; 2) the Lacandón Forest located in northeastern Chiapas, in an area of rolling hills. The climate is hot and humid with (Am(f) of Garcia 1973), an area of recent intense deforestation for cattle pastures (Arredondo-Jiménez 1995). Over the last 20 years, the area has been among the most highly malarious in Mexico. The test village, Benemérito de las Américas (16°31'08"N, 90°39'02"W; elevation 150 m), has a population of 2,570 living in 740 households and is situated next to the border with Guatemala. High prevalences of *An. vestitipennis* and other anophelines have been reported (Loyola et al. 1991, Arredondo-Jiménez 1995).

Both areas were clearly differentiated by the availability and location of domesticated animals.

<sup>1</sup> Centro de Investigación de Paludismo, Instituto Nacional de Salud Pública, Apartado Postal 537, Tapachula, Chiapas 30700, Mexico.

<sup>2</sup> Centro de Investigación sobre Enfermedades Infecciosas, Instituto Nacional de Salud Pública, Cuernavaca, Morelos, Mexico.

<sup>3</sup> Universidad Autónoma de Nuevo León, Laboratorio de Entomología Médica, San Nicolás de los Garza N. L.

Although fewer cows are raised in the coastal plain, they routinely were put overnight in animal shelters, normally located next to villages, compared with the Lacandón Forest, where animals remained in the open field overnight, far from human settlements. Horses and pigs, however, remained next to human dwellings in both areas.

### Host selection experiments

Three series of experiments were conducted in a 6 × 4 × 3-m experimental hut, built in both villages with split bamboo pole walls and a palm thatch roof and situated 800 m outside the village. Experiment 1 was carried out only in Nueva Independencia, while experiments 2 and 3 were carried out in both study villages (see below).

1. *Wild females, uncompartimentalized hut:* Unfed mosquitoes were collected on human volunteers and on horse bait between 1800 and 2200 h. Mosquitoes were dusted with fluorescent powder (Lumogen<sup>®</sup>, BASF, Holland, MI) of different color according to the bait used for collection. Marked mosquitoes were released at 2230 h inside the experimental hut containing 2 human volunteers and 1 tethered horse and recaptured the following morning at 0500 h. Blood-engorged mosquitoes were squashed onto Whatman No. 2 filter paper and later assayed in a standardized enzyme-linked immunosorbent assay (ELISA) test (Loyola et al. 1990) to identify the source of the blood meal.

2. *Wild females, compartmentalized hut:* Unfed mosquitoes were collected on human volunteers and horse, cow, or pig baits between 1800 and 2200 h. Females were taken to the experimental hut, dusted with fluorescent powder (different for each bait type), and released. This time, the hut was divided into 3 compartments, each lined inside with fine-mesh netting (Arredondo-Jiménez et al. 1992). The side compartments were connected to a central room, where mosquitoes were released by a funnel-like opening (2-cm diameter, from the floor to the upper lining of the net). The small size of the opening limited the return of mosquitoes to the central chamber after movement into a side room. In separate experiments, the side compartments were occupied by 2 human volunteers and either 1 horse, 1 cow, or 2 pigs, all with equivalent surface areas. Body surface area (BSA) was calculated by the formula:  $BSA (m^2) = ([height (cm) \times weight] / 3,600)^{0.725}$  (Mosteller 1987). The 2 human volunteers weighed 72 and 79 kg and had heights of 170 and 172 cm, respectively; resulting surface areas were 1.85 and 1.94 m<sup>2</sup>, totaling 3.79 m<sup>2</sup>. The same formula was used to approximate the body surface area for the test animals: calf, with a height to the cross of 142 cm and 363 kg of weight, resulted in 3.78 m<sup>2</sup> BSA; pigs, with a height of 110 cm and 90 kg and respective weights of 113 and 90 kg, resulted in surface areas of 1.85 m<sup>2</sup> and 1.5 m<sup>2</sup>, totaling 3.35 m<sup>2</sup>.

In each experiment, hosts were placed in the experimental hut at 1900 h, and mosquitoes were released in the central compartment at 1930 h. Mosquitoes were recaptured in all compartments at 0500 h. Host selection was assessed by recording the number of mosquitoes selecting each compartment. This was possible because, in a sample of mosquitoes assayed for ELISA bloodmeal identification, only 2% (3/150) moved among compartments.

3. *F<sub>1</sub> females, compartmentalized hut:* Mosquitoes that selected either host in the experimental hut were fed to repletion on the same host type they selected and taken to an insectary, where they were allowed (about 90% were already blood engorged) on the same host type they selected and allowed to develop eggs and oviposit. The resulting F<sub>1</sub> larvae were reared to adults at 28°C and 70% relative humidity. F<sub>1</sub> females were dusted with fluorescent powder, one color per each host type, and released in the compartmentalized experimental hut. Experiments were further conducted as in experiment 2.

### Data analysis

Differences between the proportions of mosquitoes attracted to and fed upon human or animal baits and between those recaptured in each compartment were analyzed using chi-square tests with continuity correction (Zar 1999).

## RESULTS

For the experiment using wild-caught females in the hut without compartments, a total of 247 and 158 mosquitoes were collected on human and horse baits, respectively, and were released in the hut without compartments. According to the bloodmeal analysis, 30% (73/247) of mosquitoes originally collected on human bait contained human blood. Similarly, 27% (42/158) of mosquitoes originally collected on horse bait contained human blood, the comparison being not statistically significant (Table 1).

Similar not statistically significant results were obtained in experiments using the compartmentalized hut. In the first experiment, 25% (65/259) of mosquitoes originally collected on human bait and 31% (84/273) of mosquitoes originally collected on horse bait selected human (Table 2). In the second experiment, 15% (35/228) of mosquitoes originally collected on humans and 16% (77/476) of mosquitoes originally collected on cows were recaptured in the human compartment ( $\chi^2 = 0.029$ ,  $df = 1$ ,  $P = 0.86$ ). In the third experiment, 25% (66/261) and 32% (63/194) of mosquitoes originally collected on humans and pigs, respectively, were recaptured in the human compartment. Overall, 22% (166/748) of all mosquitoes collected on humans were recaptured in the human compartment, whereas 24% of

Table 1. Host fidelity of wild-caught females of *Anopheles vestitipennis* released in the noncompartmentalized hut, as determined by bloodmeal ELISA tests in Nueva Independencia, Chiapas.

| Original host | Number released | Recaptured on Human | Recaptured on Animal | <i>P</i>   |
|---------------|-----------------|---------------------|----------------------|--|
| Human         | 247             | 73 (30%)            | 174 (70%)            | $\chi^2 = 0.286$ , <i>df</i> = 1, <i>P</i> = 0.593 |
| Horse         | 158             | 42 (27%)            | 116 (73%)            |  |

mosquitoes originally collected on animals were recaptured in this compartment (Table 2).

In contrast, for the test using  $F_1$  females, in the first experiment, human vs. horse, the proportion of  $F_1$  mosquitoes from wild anthropophilic females that selected the human compartment significantly increased, 2.7 times, i.e., from 25% to 67% (174/260), while the proportion of  $F_1$  mosquitoes from wild zoophilic (horse) female mosquitoes that selected the human compartment was 14% (25/179), a 2.2 times decrease with respect to that of parental females (31%) (Table 2). In the second experiment, human vs. cow, 55% (176/320) of  $F_1$  mosquitoes from wild anthropophilic females chose the human compartment, corresponding to a 3.7 times increase with respect to that of parental female mosquitoes (15%). In contrast, 21% (50/240) of  $F_1$  mosquitoes from wild zoophilic (cow) females collected in the cow compartment chose human, corresponding to a 1.3 times increase in relation to parental females (16%), but this was not statistically significant. In the third experiment, human vs. pig, 54% (104/193) of  $F_1$  mosquitoes from wild anthropophilic females chose the human compartment, corresponding to a statistically significant 29% increase in relation to parental females (25%), while 28% (29/102) of  $F_1$  mosquitoes from wild zoophilic (pig) females collected in the pig compartment chose the human, corresponding to a decrease of only 4% in relation

to the parental females (32%). Finally, pooled data, i.e., human vs. animal, indicated that human selection rates of  $F_1$  mosquitoes from wild anthropophilic females significantly increased 2.6 times (from 22% to 59%), while a 1.2 times decrease in human selection rates (from 24% to 20%) was observed in  $F_1$  of wild zoophilic females.

Similar results were obtained in the host-selection experiments in the Lacandón Forest using wild females released in the compartmentalized experimental hut (Table 3). Thirty-eight percent (154/405) of mosquitoes originally collected on human bait and 47% (275/586) of mosquitoes first collected on horse bait, respectively, preferred the human compartment, a figure statistically significant. When  $F_1$  progeny from the human compartment females were released, 66% (172/261) selected human, corresponding to a significant 28% increase in relation to parental females (38%), while a 29% of  $F_1$  descendants of females from the horse compartment selected human, corresponding to a significant decrease of 18% in relation to parental females (47%).

## DISCUSSION

The experiments conducted in the experimental hut with feral *An. vestitipennis* gave no indication of any host preference of either mosquito groups originally collected on human or animal baits. This

Table 2. Faithfulness of wild-caught female *Anopheles vestitipennis* and resulting  $F_1$  progenies released in the experimental hut where human or animal host were set in the side compartments of an experimental hut in Nueva Independencia, Chiapas.

| Experiment | Original host | Number released | Recaptured on human | Recaptured on animal | Statistics   |
|------------|---------------|-----------------|---------------------|----------------------|--|
| 2          | Human         | 259             | 65 (25%)            | 194 (75%)            | $\chi^2 = 1.84$ , <i>df</i> = 1, <i>P</i> = 0.173    |
|            | Horse         | 273             | 84 (31%)            | 189 (69%)            |  |
|            | Human         | 228             | 35 (15%)            | 193 (85%)            | $\chi^2 = 0.029$ , <i>df</i> = 1, <i>P</i> = 0.86    |
|            | Cows          | 476             | 77 (16%)            | 399 (84%)            |  |
|            | Human         | 261             | 66 (25%)            | 195 (75%)            |  |
|            | Pig           | 194             | 63 (32%)            | 131 (68%)            |  |
| Overall    | Human         | 748             | 166 (22%)           | 582 (78%)            | $\chi^2 = 0.489$ , <i>df</i> = 1, <i>P</i> = 0.48    |
|            | Animal        | 943             | 224 (24%)           | 719 (76%)            |  |
| 3          | $F_1$ -human  | 320             | 176 (55%)           | 144 (45%)            | $\chi^2 = 86.72$ , <i>df</i> = 1, <i>P</i> < 0.0001  |
|            | $F_1$ -cows   | 240             | 50 (21%)            | 190 (79%)            |  |
|            | $F_1$ -human  | 260             | 174 (67%)           | 86 (33%)             | $\chi^2 = 89.69$ , <i>df</i> = 1, <i>P</i> < 0.0001  |
|            | $F_1$ -horse  | 179             | 25 (14%)            | 154 (86%)            |  |
|            | $F_1$ -human  | 193             | 104 (54%)           | 89 (46%)             | $\chi^2 = 37.33$ , <i>df</i> = 1, <i>P</i> = 0.0001  |
|            | $F_1$ -pig    | 102             | 29 (28%)            | 73 (72%)             |  |
| Overall    | $F_1$ -human  | 773             | 454 (59%)           | 319 (41%)            | $\chi^2 = 208.68$ , <i>df</i> = 1, <i>P</i> < 0.0001 |
|            | $F_1$ -animal | 521             | 104 (20%)           | 417 (80%)            |  |

Comparisons made with respect to human host selection, either  $P$  vs.  $F_1$  or  $F_1$  vs.  $F_1$  mosquitoes.

Table 3. Faithfulness of wild-collected female *Anopheles vestitipennis* resulting F<sub>1</sub> progenies released in the experimental hut where human or animal hosts were set in the side compartments in the Lacandón Forest, Chiapas.

| Experiment | Original host | Number released | Recaptured on human | Recaptured on animal | Statistics <sup>1</sup>                    |
|------------|---------------|-----------------|---------------------|----------------------|--|
| 2          | Human         | 405             | 154 (38%)           | 251 (62%)            | $\chi^2 = 7.375$ , $df = 1$ , $P = 0.006$  |
|            | Horse         | 586             | 275 (47%)           | 311 (53%)            |  |
| 3          | F1-human      | 261             | 172 (66%)           | 89 (34%)             | $\chi^2 = 48.24$ , $df = 1$ , $P < 0.0001$ |
|            | F1-horse      | 156             | 45 (29%)            | 111 (71%)            |  |

<sup>1</sup>Comparisons made with respect to human host selection, either P vs. F, or F<sub>1</sub> vs. F<sub>1</sub> mosquitoes.

was in opposition to the results we obtained in previous mark-recapture studies, where feral *An. vestitipennis* preferentially returned to the same host type on which they were collected after releasing (Ulloa et al. 2002). In the hut experiments, we attempted to balance the host attraction by offering to the mosquitoes equivalent body surface area of human and animal baits. However, it is possible that the amount and type of chemical attractants differed among hosts (Constantini et al. 2001) and that attraction equivalency was not achieved (Gillies 1988).

On the other hand, the mosquitoes released in the experimental hut had a purported host preference identified by the host on which they were originally collected in the field, but host availability could not be controlled, i.e., wild parental females selecting a host either innately or opportunistically. This resulted in a mixture of individuals with different previous feeding history, but perhaps the majority of which were more likely to be innately attracted. This was revealed by F<sub>1</sub> females, showing an increased tendency to return to the same host type on which their mother was recaptured, and that these preferences were inherited. Further selection studies are warranted to investigate the possibility of obtaining an *An. vestitipennis* population with high preference for humans, as has been done after a few generations with *An. gambiae* Giles (Gillies 1964).

These findings suggested that the complex mode of inheritance that resulted in female mosquitoes showing a stronger tendency to return to their preferred host was obscured by the nature of the method of collection. Previous generations of *An. vestitipennis* mosquitoes were exposed, first at random to new hosts, such as humans in the new world (about 10,000 years ago). When humans became sedentary (ca. 8,000 years ago), mosquitoes were exposed to a blood source that did not move and were selected after repeated and sustained contact with humans, perhaps because they were almost the only large mammal (sedentary) host available until the Spaniards arrived in Central America, with cows and horses only about 500 years ago. Nevertheless, those mosquitoes not in close contact with humans remained mainly zoophilic. Also, *An. vestitipennis*, as most anophelines, display opportunistic blood feeding behavior and, when given the

chance, they readily contact alternative animal hosts (Arredondo-Jiménez 1995).

The results presented here are in accordance with others (Arredondo-Jiménez et al. 1996, Rodríguez et al. 1999, Murillo-Sánchez 2001) that could identify a subpopulation of *An. vestitipennis* in southern Mexico with higher anthropophily. Theoretical models indicate that nonrandom host choice by mosquitoes in contrast with opportunist behavior can have important quantitative and qualitative effects on the dynamics of malaria infection (Kingsolver 1987). Although *An. vestitipennis* has been incriminated as a secondary malaria vector in southern Mexico (Loyola et al. 1991, Arredondo-Jiménez 1995) and Guatemala (Padilla et al. 1992), it would be expected that the existence of a subset of the population with better taste for humans (i.e., anthropophilic) would make this species a more important vector in the region. This apparent contradiction could be explained by the opportunistic behavior of the species, along with other factors (vectors susceptibility, seasonality, human migration) conditioning unstable malaria transmission in the region (Rodríguez and Loyola 1989).

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