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Evaluation of Methods for Sampling the Malaria Vector *Anopheles darlingi* (Diptera, Culicidae) in Suriname and the Relation With Its Biting Behavior

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ABSTRACT The effectiveness of CO_2-baited and human-baited mosquito traps for the sampling of *Anopheles darlingi* Root was evaluated and compared with human landing collections in Suriname. Biting preferences of this mosquito on a human host were studied and related to trapping data. Traps used were the Centers for Disease Control and Prevention Miniature Light trap, the BG Sentinel mosquito trap, the Mosquito Magnet Liberty Plus mosquito trap (MM-Plus), and a custom-designed trap. Carbon dioxide and humans protected by a bed net were used as bait in the studies. The number of *An. darlingi* collected was greater with human landing collections than with all other collection methods. *An. darlingi* did not show a preference for protected humans over CO_2 bait. The BG Sentinel mosquito trap with CO_2 or human odor as bait and the MM-Plus proved the best alternative sampling tools for *An. darlingi.* The BG Sentinel mosquito trap with CO_2 or human odor as bait was also very efficient at collecting *Culex* spp. In a field study on biting preferences of wild *An. darlingi*, the females showed directional biting behavior ($P < 0.001$), with a majority of females (93.3%) biting the lower legs and feet when approaching a seated human host. Higher efficiency of the closer-to-the-ground collecting MM-Plus and BG Sentinel mosquito trap when compared with the other trapping methods may be a result of a possible preference of this mosquito species for low-level biting. It is concluded that odor-baited sampling systems can reliably collect *An. darlingi*, but the odor bait needs to be improved, for instance, by including host-specific volatiles, to match live human baits.

KEY WORDS *Anopheles darlingi*, mosquito trapping, human odor, biting behavior, Suriname

In South America, *Anopheles darlingi* Root has been incriminated as a malaria vector since 1931 (Davis 1931, Deane 1948, Rachou 1958, Tadei et al. 1988) and is associated with severe malaria epidemics (Falavigna-Guilherme et al. 2005). Hudson (1984) and Rozendaal (1987) found *An. darlingi* to be the primary malaria vector in the interior of Suriname. After the free distribution of long lasting impregnated bed nets throughout the interior of the country in 2006, Suriname experienced a significant decrease in the annual number of malaria cases. However, malaria still occurs in specific parts of the interior, especially in an increasing number of remote gold mining areas (data Ministry of Health Malaria Program Suriname). These mining areas have hardly been explored concerning key characteristics of malaria transmission, such as mosquito population diversity and densities or human biting rates, and vector studies are urgently needed.

Sampling *An. darlingi* for epidemiological malaria studies is traditionally conducted by the use of human landing collections (HLC) (Turell et al. 2008, Rubio-Palis 1996) because existing traps proved inefficient. The HLC has risks for the collectors because of exposure to potentially infected vectors, and is thus less accepted on ethical grounds (Rubio-Palis and Curtis 1992). HLC is the most widely used method to estimate the human biting rate of malaria vectors, even though its reliability depends on dedication and personal attractiveness (Knols et al. 1995, Olanga et al. 2010) of the individual collectors. In addition, the method is costly and labor intensive (Rubio-Palis and Curtis 1992). As the degree of anthropophily of *An. darlingi* varies considerably (De Oliveira-Ferreira et al. 1992, Charlwood 1996, Zimmerman et al. 2006), the efficacy of HLC for this vector is unknown and adds a factor of uncertainty to the reliability of data on intensities of malaria transmission in South America. Finding an alternative method representing mosquito population structure and dynamics in a reliable and comparable way is a necessary, but time-consuming and difficult enterprise, particularly when the target mosquitoes exhibit variation in biting habits. The ideal alternative method holds no transmission risks for the collector and is not influenced by the collector's ability or interaction. It is cost effective and easy to use.

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Studies have been conducted worldwide to compare different mosquito sampling methods and find alternatives for HLC to obtain accurate estimates of the population density of malaria vectors. Among these, we find a variety of light traps (Faye et al. 1992, Rubio-Palis and Curtis 1992, Davis et al. 1995, Rubio-Palis 1996, Mboera et al. 2000, Burkett et al. 2001) and odor-baited entry traps (Duchemin et al. 2001, Schmied et al. 2008). Kline (2006) provides an overview of evaluations of mosquito traps, but few of these comparative studies have been conducted with *An. darlingi,* and the efficacy of the evaluated sampling methods varies greatly depending on local malaria vectors and local situations. The Centers for Disease Control and Prevention (CDC) Miniature Light trap (hereafter termed CDC trap) proved effective in the collection of indoor biting African anopheline mosquitoes (Garrett-Jones and Magayuka 1975, Lines et al. 1991) and became the standard sampling tool for the collection of malaria mosquitoes in many areas of the world. The CDC trap is often used in combination with protected human bait (Costantini et al. 1998), but the efficacy of this setup depends on the feeding preference of local vectors, which may vary from zoophilic to anthropophilic and from exophilic to endophilic.

Kline (2002) demonstrated that propane-powered traps, using CO_2 as bait, collect large numbers of mosquitoes in Florida and are easy in use. The Mosquito Magnet Liberty Plus mosquito trap (MM-Plus), for instance, is a propane-powered trap that has been evaluated under a variety of conditions and compared with light traps and HLC. Sithiprasasna et al. (2004) compared a number of traps with HLC. They found the performance of the MM-Plus in collecting large numbers of Thai *Anopheles* mosquitoes less effective than HLC, but among the best of the alternative methods tested.

The principle behind many mosquito traps is to combine a mosquito attractant (CO_2, human bait, or [human-derived] odors) with a suction mechanism that will trap the approaching mosquitoes in a holding chamber. Studies on biting behavior of anthropophilic anophelines show that they are attracted to skin temperature, exhaled CO_2, skin humidity, body odors, or a combination of these factors (De Jong and Knols 1995, Costantini et al. 1996, Takken et al. 1997, Dekker et al. 1998, Mukabana et al. 2004). Knowledge about the biting preferences of the target mosquitoes could be used to fine-tune sampling methods for this important malaria vector. The biting preferences of *An. darlingi* females were never determined and may vary locally (Charlwood 1996). *An. darlingi*, like *Anopheles gambiae s.s.*, may have preferential biting locations guided by odors or other factors on the human body (Dekker et al. 1998, Braks and Takken 1999, Olanga et al. 2010).

The first goal of the current study was to evaluate different sampling methods for their efficacy in collecting *An. darlingi* and to assess their ability to replace the conventional HLC as a tool to determine the malaria transmission risk in endemic areas of Suriname. Different trap models were selected, including the CDC trap, the BG Sentinel mosquito trap, the MM-Plus trap, and a custom-designed mosquito trap. Traps were baited with CO_2 or by the emanations of a human protected by a bed net. The second goal was to investigate whether *An. darlingi* females, under field conditions, show nonrandom biting behavior on human hosts. Directional biting may indicate certain preferences, which could explain or influence the efficiency of the evaluated trapping methods.

Fig. 1. Map of Suriname with study location (Palumeu village).

Materials and Methods

Study Site. The study was conducted between April and June 2009 in Palumeu (N 3.34376, W 55.44081), an Amerindian village along the Tapanahony River in the Interior of Suriname (Fig. 1). The villagers spent their time, when not away from their homes, mostly underneath their houses, which are built on stilts. The village has a relatively high density of *An. darlingi* mosquitoes (compared with other sites in Suriname), with peak biting between 01:00 and 02:00 h (H.H., unpublished data). Domestic animals in the village consist of dogs and chickens. Occasionally a monkey or forest bird is kept as pet.

Mosquito Traps. During a first round of evaluation (14–26 April 2009), five trapping methods and HLC were compared in a 6 × 6 Latin square that was performed twice. During a second round (26 May–7 June 2009), one method tested in the previous round was excluded from further testing and replaced by another collection method. This new method and the remaining methods (some in adapted form; see trap details below) were tested in a 6 × 6 Latin square, again performed twice. Testing of the traps was done between 21:00 and 03:00 h. The trap evaluations were done outdoors, mostly underneath the houses in which the local people were sleeping. Locations of houses with traps were at least 50 m apart to prevent interference. Outdoor temperature and relative humidity were recorded for each hour of collection with a digital temperature and humidity measuring device.

The day after each nightly collection, the catches of the night before were, for each collection method, sorted and the mosquitoes were counted. *Culex* spp. and *Aedes* spp. mosquitoes were identified to genus level. *Anopheles* mosquitoes were sexed and identified to species level. Identification was done using the keys of Faran and Linthicum (1981), Linthicum (1988), and Gorham et al. (1967).

The Trapping Methods Used

CDC Miniature Light Trap (CDC Trap) Baited With Carbon Dioxide (500 cc/min) Provided From a Gas Cylinder (see below). Carbon dioxide was led to the trap by a polyethylene hose of 5 mm diameter, and released at 1 cm from the trap entrance. The CDC trap was placed with the trap entrance at a height of ≈150 cm. During the first evaluation round, the CDC trap was used with light. During the second round, the trap was operated without light because of the large number of insects of no interest in the trap. By removing the light, most of this bulk of nontarget insects will not enter, which makes sorting and counting of the mosquitoes easier. Leaving the light off could actually improve the collection by the CDC trap (Carestia and Savage 1967, Takken and Kline 1989).

CDC Miniature Trap (CDC Trap) Baited With a Protected Person. The CDC trap was placed at ≈150 cm height next to a mosquito net baited with a person (local male, age 21) sleeping in a hammock. During the first round of evaluation, the light bulb of the CDC trap was switched on; during the second round, the trap was operated without light, for reasons explained for the CDC trap.

BG Sentinel Mosquito Trap (BG Sentinel), Baited With Carbon Dioxide (500 cc/min) From a Gas Cylinder, Was Released Near the Trap Entrance From a 5-mm Polyethylene Hose. The BG Sentinel is normally provided with BG Lure, containing components of human skin odor, as a mosquito attractant. This bait was not used in the current setup, to make the BG Sentinel comparable to the other trapping methods that were used in combination with only carbon dioxide or a protected human as attractant.

BG Sentinel Mosquito Trap (BG Sentinel) Baited With a Protected Person (as With CDC Trap). In this experiment, the BG lure was also not used as bait, as natural human odor from a person sleeping under a bed net served as bait. Two BG Sentinel traps were placed at ground level next to a mosquito net with a person sleeping under it: one near the head and one near the feet (local male, age 29) sleeping in a hammock. The mosquitoes collected with both BG Sentinels were summed, and a mean per trap was calculated to account for differential attractiveness of different body parts. This collection method was tested during the second round only.

Mosquito Magnet Liberty Plus (MM-Plus). MM-Plus converts propane into CO_2 (flow 500 cc/min), which is used as bait (Kline 2002). The MM-Plus is generally provided with 1-octen-3-ol (octenol) as bait, but in this experiment the octenol was not used and CO_2 was the only chemical stimulus. For technical reasons (connecting parts for the cylinder damaged), the MM-Plus could not be tested in the first round of evaluation and was only tested during the second round.

Mosquito Net Trap (MNT) Designed by the Authors and Made of Cotton Cloth. The trap section at the bottom of the mosquito net was made of gauze. The MNT was baited with a person (local male, age 29) sleeping in a hammock. This method proved very inefficient in the first round of evaluation and was replaced by the BG Sentinel baited with a protected person in the second round.

Human Landing Collection (HLC). HLC to collect *An. darlingi* was performed by eight persons, working as a pair in different combinations. Mosquitoes landing on their exposed lower legs were aspirated. The number of mosquitoes that was obtained with HLC was divided by 2 to obtain the number of biting female mosquitoes per person, thereby decreasing the influence of differences in personal attractiveness.

The CO_2 used in combination with the CDC trap and the BG Sentinel was obtained from a gas cylinder. Using a pressure regulator (Concoa, model CGA 320) and a flow meter (Brooks, model 1355), a constant flow of ≈500 cc/min CO_2 was obtained. This was similar to the CO_2 production of the propane-driven MM-Plus trap, which is calibrated to produce a 500 cc/min flow.

Biting Location. The experimental design used was an in-the-field design similar to the method used by Self et al. (1969). A human bait (one of the researchers) sat outdoors on a stool wearing only shorts, whereas a second person walked around him with a headlight to check exposed body surfaces for probing or biting *Anopheles* females. At regular intervals, the human bait would turn 180 degrees to account for position effects. For each *Anopheles* female discovered on the bait, the biting location was determined and the mosquito was collected and stored for identification. The biting tests took place between 21:15 and 22:00 h and were repeated on eight nights between 29 May and 7 June 2009. Temperature and relative humidity were recorded at onset of the collections with a handheld temperature and humidity meter, and considered stable for the 45-min test period.

Statistical Analysis. Numbers of *An. darlingi* were $\text{Ln}(x + 1)$ transformed and imported in a General Linear model (SPSS version 17) to locate differences in the total number of *An. darlingi* mosquitoes taken between collection methods. Tukey's honestly significant difference (post hoc) tests were used to find differences per collection method. A comparison was made between the two Latin square designs within each period to determine whether the data per period could be summed, and a comparison was made between the two evaluation periods. Tukey's honestly significant difference (post hoc) tests were also used to determine the influence of temperature, relative humidity, and locations. Ranking of the data and

Table 1. Mean number + SEM of *An. darlingi* and *Culex* spp. mosquitoes and collection days per collection method in evaluation round 1

Collection method	No. days	Mean no. *An. darlingi*	Mean no. *Culex* spp.
HLC (per person)	11	7.82 ± 8.42a	46.59 ± 23.14a
CDC trap[a] + protected person	10	0.90 ± 1.26b	6.70 ± 9.36b
CDC trap[a] + CO_2	9	1.11 ± 1.36b	5.44 ± 5.43b
MM-Plus	—	—	—
BG Sentinel + CO_2	9	2.56 ± 4.00b	46.00 ± 35.26a
MNT	11	0.00b	0.00c

Means within a column, followed by the same letter, are not significantly different ($P > 0.05$).
[a] With light.

Table 2. Mean number + SEM of *An. darlingi* and *Culex* spp. mosquitoes and collection days per collection method in evaluation round 2

Collection method	No. days	Mean no. *An. darlingi*	Mean no. *Culex* spp.
HLC (per person)	12	15.08 ± 10.60a	22.71 ± 15.01a
CDC trap[a] + protected person	12	1.33 ± 2.06b	8.75 ± 4.83ab
CDC trap[a] + CO_2	11	0.73 ± 0.65b	10.82 ± 8.04ab
MM-Plus	12	3.42 ± 4.85bc	3.58 ± 3.23b
BG Sentinel + CO_2	12	6.50 ± 8.27c	39.33 ± 28.33a
BG Sentinel(2) + protected person	10	6.00 ± 6.09c	38.50 ± 42.92a

Means within a column, followed by the same letter, are not significantly different ($P > 0.05$).
[a] Without light.

Spearman's rho tests was used to determine the correlation between alternative collection methods and the conventional HLC over time.

To determine biting preferences in the biting study, we compared the observed and expected number of bites per body part in relation to their relative skin surface areas (Mitchell and Wyndham 1969) with a χ^2 test and based on the null hypothesis that mosquito bites are distributed in proportion to the exposed skin surface areas per body part. Average temperature and humidity over the testing days were determined.

Ethical Considerations. The use of HLC for this study was approved by the ethical committee of the Ministry of Health (MoH; Project VG2006-006, MoH Letter Reference 406, August 2006). The collectors were two researchers assisted by three entomology technicians of the Bureau of Public Health (MoH), a trained nonlocal collector (MoH) and two trained local collectors. All collectors operated under informed consent. Free malaria diagnosis and treatment were available at all times. Malaria transmission risk in Palumeu was considered very low. One confirmed malaria case had been reported from this village in the previous year (2008; *Plasmodium vivax*, imported from Kawemhakan area), and one case had been reported in week 9 of 2009 before onset of the study (also *P. vivax*, imported from Puleowime area) (data Medical Mission Malaria Program Suriname).

Results

Trap Evaluation. Combined over the two trap evaluation periods, a total of 4,027 mosquitoes was collected, consisting of 790 *An. darlingi*, 10 unidentified *Anopheles* spp., 3,222 *Culex* spp., and five *Aedes* spp. All anophelines were females, except for one. No significant differences in the total number of collected *An. darlingi* mosquitoes were found per collection method within the evaluation rounds (all $P > 0.05$), which allowed for pooling of the results within the evaluation rounds.

Trap Evaluation Round 1. Table 1 shows the number of *An. darlingi* collected and the number of collection days per method. The average temperature and average relative humidity were 24.8°C and 74.5% for the first Latin square and 25.3°C and 69.8% for the second Latin square, respectively. Significant differences in total collected *An. darlingi* mosquitoes were found only between the HLC and the three other collecting methods (for all three combinations: $P < 0.001$). Between CDC trap plus protected person, CDC trap plus CO_2, and BG Sentinel plus CO_2, no significant differences in the number of *An. darlingi* collected were found (all combinations: $P > 0.05$).

Trap Evaluation Round 2. The mean number of female *An. darlingi* and *Culex* spp. collected and the number of collection days per method are shown in Table 2. The average temperature and average relative humidity were 24.5°C and 88.3% for the first Latin square and 24.3°C and 91.8% for the second Latin square, respectively. Significant differences in total number of collected *An. darlingi* mosquitoes were found between the HLC and all other collection methods (all combinations $P < 0.05$). The BG Sentinel plus CO_2 and the BG Sentinel(2) plus protected person each collected significantly more *An. darlingi* than either CDC traps ($P < 0.05$). The number of *An. darlingi* collected in the two BG Sentinel trap setups (with CO_2 or a protected person) was not significantly different ($P > 0.05$). The number of *An. darlingi* collected in the two CDC trap setups was not significantly different ($P > 0.05$). By ranking the data and using Spearman's rho test, we found that the total number of *An. darlingi* mosquitoes collected per day with the alternative methods showed no correlation with the total number of *An. darlingi* mosquitoes collected per day using the HLC. This was found for the CDC plus CO_2 traps (both periods), the CDC trap plus protected person (both periods), the BG Sentinel plus CO_2 (both periods), the BG Sentinel(2) plus protected person, as well as the MM-Plus. In other words, none of the traps compared with HLC when monitoring population dynamics over time. Temperature, relative humidity, and location were not influencing the results obtained in either of the study periods (all $P > 0.05$).

Of the mosquitoes found besides the anophelines, some were *Aedes* spp., but most turned out to be *Culex* spp. (Tables 1 and 2). The BG Sentinel traps, both with CO_2 and with a protected person as bait, collected

Table 3. Expected and observed distribution of *Anopheles darlingi* bites ($N = 105$) on the various body parts of the human host in relation to the skin surface area

	Head[a]	Trunk	Arms	Lower body			
				Total	Upper legs	Lower legs	Feet
Skin surface (% total)[b]	7	35	19	39	19	13	7
Expected no. bites	7	37	20	41	20	14	7
Received no. bites	0	6	1	98	11	31	56

[a] Head includes neck region.
[b] Mitchell and Wyndham (1969).

large numbers of *Culex* spp. mosquitoes, comparable to the numbers collected by HLC.

Biting Study. In the biting behavior study, the biting location of 105 *An. darlingi* females was determined. The expected and observed distributions of the biting sites in relation to skin surface areas are shown in Table 3. Fig. 2 shows the location of the individual bites. The mean temperature during the tests for the eight test days was 24.3°C and the mean relative humidity 81%.

The observed distribution of biting sites differed significantly ($P < 0.001$) from the expected distribution in relation to the skin surface area. A total of 93.3% (98 of 105) of the bites was located on the legs and feet. More than half of the total number of bites were located on the feet (56 of 105).

Discussion

None of the alternative sampling methods tested in this study collected as many *An. darlingi* mosquitoes as the HLC, but some showed potential as a surveillance trap for this malaria vector species. Data from the second evaluation period suggested that when the objective of collection would be to collect as many *An. darlingi* mosquitoes as possible, the BG Sentinel baited with CO_2 or a protected person is a better alternative method than the CDC trap baited with CO_2 or a protected person and was comparable to the MM-Plus. Both the BG Sentinel and the MM-Plus need much less manpower, and are therefore less expensive methods, than the HLC. As CO_2 can be difficult to obtain and transport in the field, the BG Sentinel baited with a protected person or the MM-Plus (if propane is available) may be the most workable alternative. Recently, a cheap and effective method for the production of CO_2 has been developed, which does not rely on gas cylinders, but produces CO_2 locally from a sugar-yeast solution (Smallegange et al. 2010). Using this method in combination with the BG Sentinel may be preferable. It allows the traps to be operated in the absence of a human host. Addition of human odor components to the CO_2 bait could possibly increase the catch, depending on the mosquitoes' preferences.

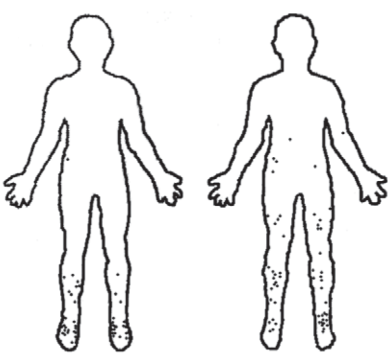

Fig. 2. Distribution of observed biting sites of *An. darlingi* ($N = 105$) on the human body.

For both the CDC and the BG Sentinel traps, we found no significant difference in *An. darlingi* numbers between CO_2-baited or human-baited traps. This suggests that a person (with natural host emanations) does not provide an added attractive effect over CO_2 alone. This implies that CO_2 is the main host attractant for *An. darlingi* in Palumeu, and that other hosts, exhaling equal amounts of CO_2, could be as attractive as humans to the mosquitoes. The degree of anthropophily of *An. darlingi* may thus be low, but a thorough evaluation with other host species remains to be executed. Given the recent discovery of alternative odor blends for *An. gambiae* (Okumu et al. 2010, Olanga et al. 2010), it is possible that studies on odor quality can significantly improve the catch of *An. darlingi*, compared with that of CO_2 alone.

The traps with CO_2 or with a protected person as bait yielded equal amounts of *Culex* spp. mosquitoes, but contrary to *An. darlingi* collections, the yield of the traps (except for the MM-Plus) was comparable to the HLC. In other words, both the CDC traps and the BG Sentinel traps can collect as many *Culex* spp. as HLC when CO_2 or a protected person is used as bait. The BG sentinel trap collected significantly more than the MM-Plus, which makes it a better option as sampling tool for these mosquito species.

None of the tested methods proved a good alternative to the HLC to monitor the population dynamics (especially density over time and feeding behavior) of *An. darlingi.* Studies conducted elsewhere with other *Anopheles* species indicate that CDC traps baited with a protected person may have the potential to provide population dynamics data that are comparable to HLC (Davis et al. 1995, Mathenge et al. 2004, Sithiprasasna et al. 2004, Sadanandane et al. 2004).

The sampling methods evaluated in the second period of the present studies showed differences in efficiency. The BG Sentinel baited with CO_2 or with a protected person collected significantly more *An. darlingi* mosquitoes than the two CDC traps (without light) and as many as the MM-Plus. This trend in mosquito yield was also observed during the first evaluation round, but the differences in number of *An. darlingi* mosquitoes collected proved not significant. This may have been as a result of the lower number of trapping nights for the BG Sentinel trap because of technical difficulties.

Removal of the light from the CDC light trap eliminated most of the unwanted insects from the collections, and at the same time made the method more comparable to the other methods with CO_2 or a protected person as bait (Carestia and Savage 1967, Takken and Kline 1989). Whether or not the light would have had an effect on *An. darlingi* catches is questionable. A similar study with the mostly anthropophilic *An. gambiae* s.l. in West Africa, for instance, showed that light increased collections indoors, but not outdoors (Costantini et al. 1998). *An. darlingi* is in fact more attracted to black light (ultraviolet light) than to white light (Laubach et al. 2001). Using the baited CDC trap in combination with a black light may improve its performance.

An advantage of the BG Sentinel over the MM-Plus is its compactness. A disadvantage of the BG Sentinel is its constant need of an energy source (batteries or electric grid). This applies also for the CDC trap. The MM-Plus is difficult to transport, but can last up to 3 wk without renewal of its propane supply or batteries.

Both the BG Sentinel trap and the MM-Plus trap collect mosquitoes closer to the ground than the CDC light trap. They thus select for mosquitoes flying at ground level at the time of capture. In the biting preference study, a significant deviation from the expected distribution of bites was found, with almost all of the bites occurring on the leg and foot regions. This was thus, because the feet were down, at ground level. Whether the higher number of *An. darlingi* mosquitoes collected by the BG Sentinel traps when compared with the CDC traps is related to this biting behavior needs further study.

The biting behavior of *An. darlingi* compares to that of *An. gambiae s.s.* in preference for biting the foot regions. De Jong and Knols (1995) studied the biting preferences of *Anopheles atroparvus* and *An. gambiae s.s.* and found that the anthropophilic *An. gambiae s.s.* preferred to bite the foot regions and the more opportunistic *An. atroparvus* preferred biting the head region. The selection of biting sites of these two mosquito species correlated with particular combinations of skin temperature and eccrine sweat gland densities on the human skin. Other factors influencing their biting site selection were the presence of CO_2 for *An. atroparvus* and the presence of skin bacteria for *An. gambiae s.s.* Similar to *An. atroparvus*, the Central American malaria vector *Anopheles albimanus* prefers the head region above other parts of the human body (Knols et al. 1994). Dekker et al. (1998) compared the preferences of the anthropophilic *An. gambiae s.s.* with those of *Anopheles arabiensis* Patton (an opportunistic species) and *Anopheles quadriannulatus* Theobald, a zoophilic species. The results showed that *An. gambiae s.s.* is attracted to the feet, but that the females may also base their preference on factors like the position of body parts relative to the ground (preference for ground-level biting), or the convection currents created by body heat, which lead them toward these body parts. Both *An. darlingi* and *An. gambiae s.s.* bite at the lower extremities of a human host, and both seem to be attracted to foot odors and humidity, or find their way across the host along convection currents. They may even use a combination of these traits to reach the preferred biting site. Considering our previous discussion on the degree of anthropophily of *An. darlingi*, biting of lower legs and feet appears to be a short-range preference of the vector or may be guided by a preference for ground-level biting. For instance, Braack et al. (1994) found, in a comparable setup, that ground-level biting *An. arabiensis* would not shift to higher body parts if the feet were covered. For this species, ground-level biting seems a behavioral trait that is independent of the availability of host body parts. Additional testing using different body positions and different persons (to eliminate individual differences in attractiveness) (Kahn et al. 1965, Braks and Takken 1999, Dekker et al. 1998) or using a dual-port olfactometer will provide additional information on the host-related cues that attract *An. darlingi*. Knowing what drives its preference makes it possible to ultimately develop specialized traps needed for entomological surveillance and evaluation systems. Also, evaluating the traps at different heights may reveal the impact of height on trap efficiency.

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