Blood-feeding behavior of anopheles gambiae and anopheles melas in Ghana, Western Africa

doi: 10.1603/033.047.0104

Blood-Feeding Behavior of *Anopheles gambiae* and *Anopheles melas* in Ghana, Western Africa

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J. Med. Entomol. 47(1): 28–31 (2010)

ABSTRACT *Anopheles gambiae* is the predominant malaria vector species in Ghana, western Africa, with a strong local presence of *Anopheles melas* Theobald along the southern coast. We studied the biting behavior of these two species of the *Anopheles gambiae* complex inland and at the coast in Ghana, with special attention to the local peoples' preference for outdoor sleeping. We collected mosquitoes at two sites in 2007, representing the moist semideciduous forest zone and the strand and mangrove zone, and the sampling was repeated in the dry and rainy seasons. Sampled mosquitoes were examined for species, parity and size (wing length), and we identified the hosts of their bloodmeals. We interviewed 288 of the village people to determine where and when they slept outdoors. Our study confirmed that *An. gambiae* is the only species of the *An. gambiae* complex in the Ashanti region and revealed that *An. melas* is highly dominant on the western coast of Ghana. Both species showed high human blood rates in indoor resting mosquito samples. More people sleep outside on the coast than inland. *An. melas* demonstrated high exophily. *An. gambiae* bit people more frequently indoors and did so more often during the dry season than in the rainy season. We suggest that the degree of exophily in *An. melas* may be affected by humidity and the availability of human as well as by the mosquitoes' innate habits.

KEY WORDS exophily, endophily, malaria vector, host distribution

Appawu et al. (1994) studied the species composition of the *Anopheles gambiae* complex in different vegetation zones in Ghana. They found a predominance of *Anopheles gambiae* Giles with local presence of *Anopheles melas* Theobald in areas with brackish water along the southern coast. Bryan et al. (1987) studied the bionomics of sympatric populations of *An. melas* and *An. gambiae* in Gambia, western Africa. They reported that the distribution of *A. melas* was limited to the vicinity of breeding sites associated with mangrove swamps, and it was less anthropophilic and more exophilic than *A. gambiae*. However, Awolola et al. (2002) reported that both *An. gambiae* and *An. melas* were anthropophagic in southwestern Nigeria.

The rural people of Ghana include several ethnic groups whose settlement patterns tend to follow the vegetation zones. The localized character of lifestyles further includes variables such as the types of domestic animals people keep and where they sleep. The commonly found relationship between endophily of mosquitoes and their tendency toward anthropophily given that humans rest indoors is not universal. The human blood ratio of the *A. gambiae* complex has been showed to vary according to seasonal host changes in human and animal distributions in the Sahelian area (Lemasson et al. 1997). It has further been reported that both *A. gambiae* and *Anopheles arabiensis* Patton bite humans sleeping outdoors (Faye et al. 1997), and such human habits may influence the biting behavior of the mosquitoes.

Here, we address two main questions about anophelines in inland rainforest and coastal brackish habitats in Ghana: 1) What is the dominant malaria vector species in respective areas; and 2) how may human habits, in terms of host distribution, relate to the biting ecology of malaria vectors?

Materials and Methods

Study Areas. We performed preliminary surveys of anophelines in 2006 in several Ghanaian villages in the rain forest zone within 50 km from Kumasi and along 20 km of the Atlantic Ocean coastline (N.T., unpubl. data). Two villages with a variety of host animals were then chosen as representative villages for sampling *An. gambiae* and *An. melas*, respectively: Afamanaso outside Kumasi (6° 57′ 08 N, 1° 30′ 57 W; 280-m altitude) in the Ashanti Region is situated in the inland rainforest zone, whereas Ampain (4°0.57′ 33 N, 2° 24′ 09 W; 10-m altitude) in the Western Region is located on a sandy coast, vegetated by mangroves and palm trees and belongs to the strand and mangrove zone.

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0022-2585/10/0028–0031$04.00/0

Table 1. Number of anophelines collected by HLC per human per night and by resting indoors (PSC) per room in the two villages

Collection method	Dry season (Feb.)				Rainy season (June)			
	HLC		PSC		HLC		PSC	
	Per person	Total catch	Per room	Total catch	Per person	Total catch	Per room	Total catch
Afamanaso								
An. funestus	1.5	6	0.19	7	2.0	16	0.3	10
An. gambiae	13.5	54	3.06	110	24.1	193	4.9	186
Ampain								
An. funestus					0.1	1		
An. gambiae					0.8	6		
An. melas	35.75	143	1.42	44	252.8	2,022	0.1	3

Mosquito Collections. Mosquitoes were collected by means of human landing catches (HLC) and pyrethrum spray catches (PSC) and nearly identical sampling programs were conducted in both villages and repeated in February (dry season) and June (rainy season) in 2007. To collect blood-fed mosquitoes, morning PSC (7–11 a.m.) was carried out indoors in a number of representative households, in total 37 houses at Afamanaso and 31 houses at Ampain. HLC involving two volunteers were then conducted throughout a full night, one indoors and one outdoors in two of the houses where PSC had previously been undertaken, one in the center of the village and the other in the outskirts. The mosquitoes were sampled individually and kept separate according to time and place of collection.

Interviews. We surveyed the households where PSC was performed to determine host distributions and the magnitude of human malaria cases within the last month. The number of people, as well as the kind and number of domestic animals were recorded for each household. We then interviewed people at random to determine where humans of different ages and sexes spent the night. For those who slept outdoors, we made further queries to determine when and how long they slept outdoors.

Mosquito Identification and Processing. All mosquitoes were morphologically identified under a stereomicroscope. Polymerase chain reaction (PCR) analysis by using the protocol described by Scott et al. (1993) or Koekemoer et al. (2002), was then performed on all specimens identified as the *An. gambiae* complex and the *Anopheles funestus* complex to identify their species. We further identified hosts of blood meals of engorged females caught by PSC through PCR (Kent and Norris 2005). The ovaries of unfed specimens were dissected to determine the parity ratio, and their wing size was measured (length between incision to tip of wing without fringe). To avoid collection-time bias the mosquitoes chosen for dissection were selected subequally among the early night (8–12 p.m.) and the late night (0–4 a.m.) HLC samples.

Data Analysis. For the dominant malaria vectors, we analyzed their size (wing lengths) and tendency for exophagy (outdoor biting) versus endophagy (indoor biting) in relation to the sampling location, season, and mosquito ovary status (parity). Analysis of variance (ANOVA) and chi-square tests were performed using JMP version 5.0.1 (SAS Institute, Cary, NC).

Results and Discussion

Village Descriptions and Human Sleeping Habits. At Afamanaso, the sprayed households housed 193 people >5 yr, 34 young children (≤5 yr), 13 domestic birds, 37 goats or sheep, 12 dogs, one pig, and six cats. The sprayed households at Ampain housed 99 people >5 yr, 22 young children, 115 domestic birds, 30 sheep, and 10 cats. The numbers of malaria cases per person per month in the two villages were estimated to 0.15 in January and 0.10 in May at Afamanaso, compared with 0.21 in both January and May at Ampain. Altogether, 69 men and 59 women at Afamanaso and from 89 men and 71 women at Ampain were interviewed regard-

Table 2. Number of *An. gambiae* and *An. melas* biting outdoors and indoors per hour in the two villages in two seasons

Time	Dry season (Feb. 2007)				Time	Rainy season (June 2007)			
	Afamanaso		Ampain			Afamanaso		Ampain	
	An. gambiae		*An. melas*			*An. gambiae*		*An. melas*	
	Indoor	Outdoor	Indoor	Outdoor		Indoor	Outdoor	Indoor	Outdoor
7–8 p.m.	0	1	2	3					
8–9 p.m.	3	1	2	4	8–9 p.m.	2	1	82	45
9–10 p.m.	4	0	3	17	9–1 p.m.	9	7	122	40
10–11 p.m.	9	0	16	17	10–1 p.m.	13	5	93	25
11–12 p.m.	9	0	15	21	11–1 p.m.	14	11	108	72
0–1 a.m.	7	3	13	10	0–1 a.m.	25	10	164	175
1–2 a.m.	7	2	13	No data	1–2 a.m.	29	2	272	192
2–3 a.m.	4	1	6	No data	2–3 a.m.	32	6	150	99
3–4 a.m.	2	0	1	No data	3–4 a.m.	24	3	144	239
No. of mosquitoes	45	8	71	72		148	45	1,135	887

Table 3. Wing sizes of malaria vectors by gonotrophic age (parity) and season, with a summary of ANOVA

Species	Parity	Season	Wing size (mm)			ANOVA			
			Mean	SE	*n*	Parameter	*F* ratio	df	*P*
An. funestus	All	Dry	3.01	0.04	13	Season	32.34	1, 27	<0.001
		Rain	3.35	0.03	16				
	Nulliparous	Dry	3.17	0.15	2				
		Rain	3.23	0.05	4				
	Parous	Dry	2.98	0.15	2				
		Rain	3.39	0.04	12				
An. gambiae	All	Dry	3.62	0.03	49	Whole model	14.51	3, 238	<0.001
		Rain	3.84	0.02	193	Season	32.31	1	<0.001
	Nulliparous	Dry	3.47	0.07	7	Parity	3.73	1	0.055
		Rain	3.83	0.03	57	Parity × season	1.92	1	ns
	Parous	Dry	3.64	0.04	42				
		Rain	3.85	0.02	136				
An. melas	All	Dry	3.38	0.02	82	Whole model	32.04	3, 224	<0.001
		Rain	3.67	0.02	146	Season	54.48	1	<0.001
	Nulliparous	Dry	3.45	0.07	15	Parity	2.28	1	ns
		Rain	3.68	0.02	61	Parity × season	0.70	1	ns
	Parous	Dry	3.37	0.03	67				
		Rain	3.66	0.02	85				

ing sleeping habits. Both sexes slept outdoors more frequently at Ampain (56.3% of women, 82% of men) compared with Afamanaso (15.9% of women, 37.3% of men) (χ^2 test, $P < 0.001$ for both sexes), and outdoor sleeping hours were significantly shorter at Afamanaso (mean ± SE: women, 0.2 ± 0.11 h; men, 0.7 ± 0.28 h) compared with Ampain (women, 2.0 ± 0.42 h; men, 3.8 ± 0.56 h); ANOVA results were as follows: women, $F_{1, 128} = 41.5$, $P < 0.001$; men, $F_{1, 156} = 44.8$, $P < 0.001$).

Mosquito Collection. Overall, 2795 female *Anopheles* mosquitoes were collected: 2,435 by HLC and 360 by PSC. All were either the *A. gambiae* complex (98.8%) or *Anopheles funestus* Giles (1.2%). PCR confirmed that 100% of the specimens collected at Afamanaso were *A. gambiae*, whereas 99.6% of the specimens collected at Ampain were *A. melas* (Table 1).

Sampling efficacy changed seasonally and significantly more *A. gambiae* were caught by HLC in the rainy season at Afamanaso (χ^2 test, $P < 0.001$). Similarly at Ampain, significantly more *A. melas* were caught in the rainy season (χ^2 test on HLC catches between 8 p.m. and 1 a.m., $P < 0.001$).

The indoor/outdoor ratio of *A. gambiae* collected by HLC was 0.85 in the dry season and 0.77 in the rainy season, whereas that of *A. melas* was 0.50 in the dry season and 0.56 in the rainy season (Table 2). *An. gambiae* bit people indoors more frequently than did *A. melas* (χ^2 test, $P < 0.001$) and did so more often in the dry season than in the rainy season (χ^2 test, $P < 0.001$).

Despite a variety of hosts available at the households, bloodmeal identification revealed a high human blood ratio in the indoor resting mosquitoes collected by PSC. Among 42 bloodmeals collected in the dry season at Afamanaso, 92.9% were from humans and 1.1% were from cattle; all 96 examined bloodmeals collected in the rainy season were from humans. All examined bloodmeals from *An. melas* were from humans ($n = 24$ in the dry and three in the rainy season).

Parity and Wing Size. The parity ratios of *A. gambiae* at Afamanaso were 0.857 ($n = 49$) in the dry season and 0.705 ($n = 193$) in the rainy season, showing no seasonal difference (χ^2 test, $P > 0.05$). The parity ratios of *An. melas* at Ampain were higher in the dry season (0.807, $n = 83$) than in the rainy season (0.567, $n = 254$; χ^2 test, $P = 0.016$). In both villages, we found no differences in parity ratios between mosquitoes caught before and after midnight (χ^2 test, $P > 0.05$).

The average wing sizes of, *An. funestus*, *An. gambiae*, and *An. melas*, grouped according to parity and sampling season are presented in Table 3. All of them were larger in the rainy season than in the dry season.

Conclusions. Our study confirmed that *An. gambiae* is the only species of the *An. gambiae* complex in the Ashanti region, and revealed that *An. melas* is highly dominant on the western coast of Ghana. Both species showed high human blood rates in indoor resting mosquitoes samples despite availability of a range of hosts. Our results indicate that the high exophily of *An. melas*, also reported by Bryan et al. (1987), could both reflect the year-around higher humidity in coastal areas and peoples' habit of more frequent outdoor sleeping along the coast. We emphasize that blood feeding behavior by mosquitoes, and their successive resting behaviors, may be largely influenced by environmental factors in addition to their innate features.

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Received 20 February 2009; accepted 11 September 2009.