Journal of the American Mosquito Control Association, 20(4):390–394, 2004

RELATIONSHIPS BETWEEN BODY SIZE OF *ANOPHELES* MOSQUITOES AND *PLASMODIUM FALCIPARUM* SPOROZOITE RATES ALONG THE KENYA COAST

JOSEPH M. MWANGANGI,[1] CHARLES M. MBOGO,[1] JOSEPH G. NZOVU,[1] EPHANTUS W. KABIRU,[2] HENRY MWAMBI,[3] JOHN I. GITHURE,[4] AND JOHN C. BEIER[5]

ABSTRACT. The influence of body size of *Anopheles gambiae* s.s., *Anopheles arabiensis, Anopheles merus,* and *Anopheles funestus* on the transmission of *Plasmodium falciparum* was studied at 3 sites along the Kenyan coast. Adult mosquitoes were collected inside houses by pyrethrum spray collection (PSC) from April to September 2001. Wing length was measured microscopically to the nearest 0.01 mm as an index of mosquito body size. The *P. falciparum* circumsporozoite protein (CSP) was detected by enzyme-linked immunosorbent assay technique. A total of 1,715 anopheline mosquitoes were collected, of which 785 (45.77%) were *An. gambiae* s.s., 54 (3.15%) *An. arabiensis, An. merus* 27 (1.57%), and 849 (49.5%) were *An. funestus.* The mean wing length for *An. gambiae* s.s. was 2.94 mm and that of *An. funestus* was 2.50 mm. There was no site-to-site variation in the body size of *An. gambiae* or *An. funestus.* There was no significant difference in wing length between sporozoite-infected and uninfected *An. gambiae* s.s., and the same was found for *An. funestus.* At the 3 rural coastal sites in Kenya, the efficiency of malaria parasite transmission does not appear to be influenced by variation in vector body size.

KEY WORDS *Anopheles gambiae, An. funestus,* wing length, sporozoite rates, ELISA, malaria transmission, circumsporozoite protein

INTRODUCTION

In mosquitoes, body size has been shown to affect factors such as longevity (Nasci 1987, Packer and Corbert 1989), fecundity (Renshaw et al. 1994, Hurd et al. 1995), and blood meal volume (Xue et al. 1995, Takken et al. 1998). Among *Anopheles,* intraspecific variation in body size is inversely correlated with the need for multiple blood meals to complete the gonotrophic cycle, and this strongly influences vectorial capacity (Hurd et al. 1995, Hogg et al. 1996). Wing length is the most commonly used and readily available measure of body size of mosquitoes, showing good correlation with dry weight (Nasci 1987, Renshaw et al. 1994). Variability of body size within mosquito populations as a factor affecting malaria transmission has received only little attention, although some studies have shown that it affects patterns of transmission (Dye and Hasibeder 1986, Kingsolver 1987, Koella 1991).

Body-size variation within populations has been observed for many mosquito species (Fish 1985), including *Anopheles gambiae* s.l., the main vector of malaria in Africa (Gillies and Coetzee 1987). Kitthawee and colleagues (1990) artificially fed 4 size classes of laboratory-reared *Anopheles* with *Plasmodium falciparum* and showed that the largest size developed the highest number of oocysts, though the proportion of infected mosquitoes was independent of size. In the study of Lyimo and Koella (1992), intermediate-sized mosquitoes were found to harbor more sporozoites than the other sizes of *Anopheles* mosquitoes. They further found that the large number of oocysts in mosquitoes resulted in a higher mortality rate, which makes them less important for the transmission of malaria parasites. Earlier studies found no relationship between weight of *Aedes aegypti* and probability of infection by *Plasmodium gallinaceum* (Horanitz 1947) or between wing length of *Anopheles stephensi* and the number of oocysts of *Plasmodium yoelii nigeriensis* (Ichimori 1989).

Laboratory studies have shown that small adult female *An. stephensi* mosquitoes from larvae stressed during development may not develop eggs after their first blood meal (Reisen 1975). Small females take longer to achieve reproduction and produce fewer offspring; however, they have a high frequency of blood feeding during the first gonotrophic cycle and have greater chances of picking up and transmitting malaria parasites at an early age. Consequently, they also run a greater risk of being killed by any domestic mosquito control measures, including personal-protection activities (Curtis 1992). There is a lack of information on the body size of *Anopheles* mosquitoes along the Kenyan coast and how it influences malaria parasite transmission. There are different larval habitats at coastal Kenya (Mwangangi 2002), which may con-

[1] Kenya Medical Research Institute, Centre for Geographic Medicine Research—Coast, PO Box 428, Kilifi 80108, Kenya.

[2] Department of Zoology, Kenyatta University, PO Box 43844, Nairobi GPO 00100, Kenya.

[3] School of Mathematics, Statistics and Information Technology, University of Natal, Private Bag X01, Scottsville, 3209 Pietermaritzburg, South Africa.

[4] Human Health Division, International Center of Insect Physiology and Ecology, PO Box 30772, Nairobi GPO 00100, Kenya.

[5] University of Miami, School of Medicine, Department of Epidemiology and Public Health, Highland Professional Building, 1801 NW 9th Avenue, Suite 300(D-93), Miami, FL 33136.

tribute to different sizes of *Anopheles* mosquitoes. The objective of this study was to determine the relationship between body size of wild-caught *An. gambiae* s.s., *An. arabiensis, An. merus,* and *An. funestus* on the *P. falciparum* sporozoite rates along the Kenyan coast.

MATERIALS AND METHOD

Study sites

Three collection sites in Kilifi District were selected for this study. These sites are a subset of the 30 sites in the study area described by Mbogo et al. (2003) and Mwangangi et al. (2003). The criteria for selection were the presence of known aquatic habitats of anopheline mosquitoes, malaria vector species composition, and accessibility. The 3 sites lie in the foot plateau, but differ in the terrain and seasonal rivers that transect them. Each site covers approximately 4 km^2 and has a school at the center. Jaribuni is located at 03°37.3″S and 039°44.6″E. River Jaribuni traverses the site with several small pools of water and vegetation along both sides. Majajani, located at 03°39.5″S and 039°47.2″E, has an almost flat terrain. A stream passes through the area with several swampy pools and water-logged man-made depressions. Mtepeni lies at 03°54.5″S and 039°43.6″E and has a seasonal stream passing through the area, which has a hilly terrain compared with the other 2 sites. The houses are mainly mud walled and roofs are thatched with palm leaves. The vegetation consists mainly of shrubs and bushes. Inhabitants are mainly farmers, growing maize and cassava for subsistence and cashew nuts, mangoes, and coconuts as cash crops. Domestic animals kept include cows, goats, and sheep.

Weekly entomological sampling was conducted at each site from April to October 2001. At each site, 10 houses randomly selected were sampled for indoor resting mosquitoes by the pyrethrum spray collection (PSC), (WHO 1975). The mosquitoes were placed in Petri dishes, labeled, and then placed in a cool box for transportation to the laboratory for analysis.

Laboratory processing

Anopheline mosquitoes were identified morphologically as *An. gambiae* complex, *An. funestus,* and other *Anopheles* using the key of Gillies and Coetzee (1987). The *An. gambiae* complex were further identified to sibling species by polymerase chain reaction (PCR) (Scott et al. 1993). Briefly, rDNA was extracted from single mosquito triturates using the method described by Collins and colleagues (1987). Eight microliters of the sample DNA were then used as template for PCR amplification. Each amplified sample was run in 1.8% horizontal agarose-Tris-Boric acid-EDTA gels and visualized by a UV transilluminator.

The mosquitoes were then placed in vials according to the house number, date of collection, and site, and were dried on calcium sulphate (driarates) for at least 1 wk before the next procedure. The *Anopheles* mosquitoes were cut transversely between the abdomen and the thorax. The wings were removed gently with forceps and mounted on a microscope glass slide using Distyrene Plasticizer Xylene mountant and measured to the nearest 0.01 mm with an ocular micrometer from the distal end of the alula to the wing tip, excluding the fringe scales. The vials with head and thorax region were given a number corresponding with the abdomen and the wings. The vials with head and thorax region were kept in the freezer at −20°C until testing for *P. falciparum* sporozoites.

Plasmodium falciparum sporozoite ELISA

The head and thorax of each individual mosquito was ground using 50 μl of boiled casein blocking buffer with Nonidet P-40; 200 μl of blocking buffer (BB) were then added, bringing the final volume to 250 μl. Fifty microliter-aliquots were tested by ELISA using monoclonal antibodies to detect circumsporozoite (CS) proteins of *Plasmodium falciparum* (Wirtz et al. 1987). The results were read visually (Beier and Koros 1991).

Statistical analysis

The statistical analyses were done using SPSS software (Version 11 for Windows; SPSS Inc., Chicago, IL). One-way analysis of variance (ANOVA) was used to test for site-to-site variation of the wing length of *An. gambiae* s.s. and *An. funestus* and also to test the influence of *P. falciparum* sporozoite rates and month. Two-way ANOVA was used to compare wing length with sporozoite rates and site. Multiple ANOVA was used to compare the *Anopheles* species wing length, and Tukey's honestly significantly different post-hoc test was performed to separate the means.

RESULTS

A total of 1,715 anopheline mosquitoes were collected, of which 785 (45.77%) were *An. gambiae* s.s., 54 (3.15%) *An. arabiensis,* 27 (1.57%) *An. merus,* and 849 (49.5%) *An. funestus* (Table 1). The overall mean wing length for *An. gambiae* s.s. was 2.95 mm; (95% confidence interval [CI] 2.93–2.97); for *An. arabiensis,* 2.95 mm (95% CI 2.86–3.03); for *An. merus,* 3.03 mm (95% CI 2.90–3.15); and for *An. funestus,* 2.50 mm; (95% CI 2.48–2.51). There was significant wing length variation ($F_{(3,1,711)} = 363.61$, $P < 0.001$) in the *Anopheles* mosquitoes. The wing length for *An. gambiae, An. arabiensis,* and *An. merus* were significantly longer than those for *An. funestus.* There was no significant site-to-site variation in the wing length of *An. gambiae* s.s.

Table 1. The mean wing length of *Anopheles gambiae* s.s., *Anopheles arabiensis, Anopheles merus*, and *Anopheles funestus* collected from the 3 collection sites, along the Kenyan Coast.

	Jaribuni			Majajani			Mtepeni		
	n[1]	MWL[2]	95% CI[3]	n[1]	MWL[2]	95% CI[3]	n[1]	MWL[2]	95% CI[3]
An. gambiae s.s	417	2.96	2.93–2.99	138	2.97	2.92–3.02	230	2.93	2.89–2.97
An. arabiensis	52	2.95	2.87–3.04	0	—	—	2	2.80	0.26–5.34
An. merus	23	3.08	2.97–3.20	4	2.70	1.95–3.45	—	—	—
An. funestus	846	2.52	2.52–2.53	1	2.60	—	2	2.75	2.11–3.39

[1] Number of observations.
[2] Mean wing length.
[3] 95% confidence interval.

($P = 0.408$), *An. arabiensis* ($P = 0.492$) and *An. funestus* ($P = 0.342$); however, *An. merus* showed site variation (0.026).

The mean monthly wing length variation and *P. falciparum* sporozoite rates for *An. gambiae* s.s., *An. arabiensis, An. merus,* and *An. funestus* at the 3 collection sites is shown in Table 2. The overall sporozoite infection rates were 12.3% for *An. gambiae* s.s., 9.3% for *An. arabiensis,* 18.5% for *An. merus,* and 6.2% for *An. funestus. Anopheles gambiae* had a significantly higher sporozoite rate than *An. funestus* at the 3 sites ($\chi^2 = 17.31$, df = 1, $P < 0.001$).

The sporozoite infection rates for *An. gambiae* s.s. were not significantly different ($F_{(1,782)} = 0.90$, $P = 0.408$) in the 3 sites. The results further indicate that wing length is independent of *P. falciparum* sporozoite infection for *An. gambiae* s.s. ($P = 0.641$), *An. arabiensis* ($P = 0.152$), *An. merus* ($P = 0.344$), and *An. funestus* ($P = 0.855$). Two-way ANOVA showed that body size is independent of *P. falciparum* sporozoite infection ($P = 0.804$) and site ($P = 0.924$), and the interaction between site and sporozoite infection is not significant ($P = 0.750$).

One-way ANOVA results showed that there was a significant monthly variation in wing length for *An. gambiae* s.s. ($P < 0.001$), *An. arabiensis* ($P = 0.029$), and *An. funestus* ($P = 0.006$) at the 3 sites; however, *An. merus* had no monthly variation ($P = 0.451$). The largest mean wing length for *An. gambiae* s.s. was observed in April and May in Jaribuni and Majajani, while in Mtepeni it was in August and September. *An. funestus* were biggest in the months of June and July and were smallest in April.

DISCUSSION

The results indicated that there were no significant difference between the wing length of the sporozoite-infected and uninfected anopheline mosquitoes. Thus, wing length does not appear to be a predetermining factor for the infectivity of *An. gambiae* and *An. funestus* mosquitoes in the 3 study sites along the Kenyan coast. These findings are unlike the results of Lyimo and Koella (1992), who found that the intermediate-sized *Anopheles* mosquitoes had a higher sporozoites infection rate. Although the 3 study sites were ecologically different, the *An. gambiae* wing length did not differ significantly. However, the wing length of *An. gambiae* was significantly larger than that of *An. funestus* in the study areas. *Anopheles funestus* were collected mostly in Jaribuni, while a few were from Majajani and Mtepeni. The larval habitats in Jaribuni are different from those in Majajani and Mtepeni. In Jaribuni, most habitats are stream pools (Mwangangi, 2002), which are long-lived, shaded, with clear water and are covered with vegetation most of the year, which are mostly preferred by *An. funestus.* It appears the aquatic habitats utilized by the mosquitoes for their oviposition are similar in their nutritional richness (Mwangangi 2002); thus, this may be a contributing factor to their body size similarities.

The coastal strip on Kenya experiences a bimodal rainfall pattern with long rains falling between April and July and short rains in December. There was a significant monthly variation in the wing length of mosquitoes. This is because the month of the year might be wet or dry. The wing length of *An. gambiae* s.s. was biggest at the beginning of the rain season in the months of April and May but *An. funestus* body size was biggest in June and July, which is when most of the habitats were more permanent and covered with vegetation. Studies by Mbogo and colleagues (2003) found that the *An. gambiae* and *An. funestus* show temporal variation in their abundance and follow the rainfall pattern characterized by 2 peaks along the Kenyan coast. This seasonal variation in vector densities and the corresponding variations in body size depend on larval habitat availability and conditions of the habitat and the nutritional level. This implies that there is seasonal variation in vector densities and size depending on the availability of larval habitats, the conditions at the habitat, and the environmental temperature, which are important for larval development.

Temperature varies between the wet and dry seasons. At the dry season, temperatures are high (above 30°C), whereas during the wet season, the temperatures are lower (28°C). Although during the dry season, the temperatures are more favorable for

Table 2. The mean monthly wing length variation and the *P. falciparum* sporozoite rates of *Anopheles gambiae* s.s., *Anopheles arabiensis, Anopheles merus*, and *Anopheles funestus* in the 3 collection sites.

		An. gambiae s.s.				*An. arabiensis*				*An. merus*				*An. funestus*			
Site	Month	n[1]	MWL[2]	95% CI[3]	SR[4]	n[1]	MWL[2]	95% CI[3]	SR[4]	n[1]	MWL[2]	95% CI[3]	SR[4]	n[1]	MWL[2]	95% CI[3]	SR[4]
Jaribuni	April	64	3.15	3.33–3.21	10.9	4	3.10	2.76–3.44	0.0	2	2.95	2.31–3.59	50.0	34	2.37	2.31–2.42	2.9
	May	84	3.12	3.05–3.17	11.9	8	3.23	2.99–3.46	12.5	6	3.25	3.16–3.34	16.7	159	2.51	2.48–2.56	5.0
	June	177	2.82	2.78–2.86	22.6	23	2.85	2.71–2.98	17.4	9	3.01	2.75–3.27	22.2	307	2.52	2.50–2.55	5.5
	July	51	2.86	2.76–2.96	7.8	9	2.89	2.67–3.11	0.0	3	3.07	2.19–3.94	0.0	304	2.53	2.50–2.56	8.9
	Aug	29	3.12	3.03–3.22	10.3	6	3.05	2.80–3.30	0.0	2	2.95	1.04–4.86	0.0	0	0	—	0.0
	Sept	12	2.79	2.58–3.00	0.0	2	2.80	0.26–5.34	0.0	1	3.30	—	0.0	42	2.48	2.43–2.53	0.0
Majajani	April	16	3.15	3.01–3.29	6.3	0	0	—	0.0	0	0	—	0.0	0	0	—	0.0
	May	1	3.10	—	0.0	0	0	—	0.0	0	0	—	0.0	0	0	—	0.0
	June	75	2.86	2.81–2.92	10.7	0	0	—	0.0	1	2.30	—	0.0	0	0	—	0.0
	July	35	3.02	2.91–3.13	5.7	0	0	—	0.0	2	3.10	18.3–4.37	0.0	0	0	—	0.0
	Aug	7	3.31	3.10–3.53	28.6	0	0	—	0.0	0	0	—	0.0	0	0	—	0.0
	Sept	4	3.23	2.92–3.53	0.0	0	0	—	0.0	1	1	—	0.0	1	2.6	—	0.0
Mtepeni	April	3	2.90	2.24–3.58	0.0	0	0	—	0.0	0	0	—	0.0	0	0	—	0.0
	May	33	3.24	3.16–3.32	6.1	0	0	—	0.0	0	0	—	0.0	0	0	—	0.0
	June	140	2.83	2.78–2.88	11.4	2	2.8	0.26–5.34	0.0	0	0	—	0.0	0	0	—	0.0
	July	37	2.97	2.87–3.08	2.7	0	0	—	0.0	0	0	—	0.0	0	0	—	0.0
	Aug	14	3.01	2.79–3.24	0.0	0	0	—	0.0	0	0	—	0.0	0	0	—	0.0
	Sept	3	3.02	2.70–3.70	0.0	0	0	—	0.0	0	0	—	0.0	1	2.8	—	0.0
Overall		785	2.95	2.93–2.97	12.3	54	2.95	2.86–3.03	9.3	27	3.03	2.90–3.15	18.5	849	2.52	2.50–2.53	

[1] Number of observations.
[2] Mean wing length.
[3] 95% confidence interval.
[4] Sporozoite rate.

faster larval development, there is the disadvantage of habitats drying up. The diminishing habitat results in inter- and intraspecific competition for resources within the aquatic habitat. Even though the development is faster, small-sized mosquitoes are produced due to the stressful conditions.

In conclusion, there is evidence that, at the 3 rural sites in coastal Kenya, the efficiency of malaria parasite transmission does not appear to be influenced by variation in vector body size. The vectorial system, *An. gambiae* s.s., *An. arabiensis, An. merus,* and *An. funestus* along the Kenya coast are known to be anthropophagic and endophilic (Mwangangi et al. 2003b) and are efficient vectors of malaria parasites, indicating that differences in body size do not affect their transmission dynamics.

ACKNOWLEDGMENTS

We are grateful to Gabriel Nzai, Festus Yaa, John Masa, and Shida David for help in field collections. This work was supported by NIH grants U19 AI45511, D43 TW01142, and D43 TW00920. This article has been published with the permission of the Director of the Kenya Medical Research Institute (KEMRI).

REFERENCES CITED

Beier JC, Koros J. 1991. Visual assessment of sporozoite and blood meal ELISA samples in malaria field studies. *J Med Entomol* 28:805–808.

Collins FH, Mendez MA, Rasmussen M, Mehaffey PC, Besansky NJ, Finnerty V. 1987. A ribosomal RNA gene probe differentiates members of the *Anopheles gambiae* complex. *Am J Trop Med Hyg* 37:37–41.

Curtis CF. 1992. Personal protection methods against vectors of disease. *Rev Med Vet Entomol* 80:543–553.

Dye C, Hasibeder G. 1986. Population dynamics of mosquito borne disease: effects of flies which bite some people more frequently than others. *Trans R Soc Trop Med Hyg* 80:69–77.

Fish D. 1985. An analysis of adult size variation within natural mosquito populations. In: Lounibos LP, Rey JR, Frank JH, eds. *Ecology of Mosquitoes. Proceedings of a workshop.* p 419–429.

Gillies MT, Coetzee M. 1987. A supplement to Anophelinae of Africa south of Sahara (Afro-tropical region). *Publ South Africa Instit Med Res* 55:1–143.

Hogg JC, Thomson MC, Hurd H. 1996. Comparative fecundity and associated factors for two sibling species of the *Anopheles gambiae* complex occurring sympatrically in the Gambia. *Med Vet Entomol* 10:385–391.

Horanitz W. 1947. Physiological factors which influence the infection of *Aedes aegypti* with *Plasmodium gallinaceum. Am J Hyg* 45:67–81.

Hurd H, Hogg JC, Renshaw M. 1995. Interaction between blood feeding, fecundity and infection in mosquitoes. *Parasitol Today* 11:411–416.

Ichimori K. 1989. Correlations of mosquito size, blood meal size and malaria oocyst production. *Japanese J Sanit Zool* 2:81–85.

Kingsolver JG. 1987. Mosquito host choice and epidemiology of malaria. *Am Nat* 130:811–827.

Kitthawee S, Ehman JD, Sattabongkot J. 1990. Evaluation of survival potential and malaria susceptibility among different classes of laboratory reared *Anopheles dirus. Am J Trop Med Hyg* 43:328–332.

Koella JC. 1991. On the use of mathematical models of malaria transmission. *Acta Tropica* 49:1–25.

Lyimo EO, Koella JC. 1992. Relationship between body size of adult *Anopheles gambiae* and infection with the malaria parasite of *Plasmodium falciparum. Parasitology* 104:233–237.

Mbogo CM, Mwangangi JM, Nzovu J, Gu W, Yan G, Gunter J, Swalm C, Keating J, Regens JL, Shililu JI, Githure JI, Beier JC. 2003. Spatial and temporal heterogeneity of *Anopheles* mosquitoes and *Plasmodium falciparum* transmission along the Kenyan coast. *Am J Trop Med Hyg* 68:734–742.

Mwangangi JM. 2002. The influence of nutritional status of a larval habitat on the body size of Anopheles mosquito. M.Sc. thesis. Kenyatta University, Nairobi, Kenya.

Mwangangi JM, Mbogo CM, Nzovu JG, Githure JI, Yan G, Beier JC. 2003. Blood meal analysis for anopheline mosquitoes sampled along the Kenyan coast. *J Am Mosq Control Assoc* 19:371–375.

Nasci RS. 1987. Adult body size and parity in field populations of the mosquitoes *Anopheles crucians, Aedes taeniorhynchus* and *Aedes sollicitans. J Am Mosq Control Assoc* 3:636–637.

Packer MJ, Corbet PS. 1989. Seasonal emergence, host seeking activity, age composition and reproductive biology of the mosquito *Aedes punctor. Ecol Entomol* 14: 433–442.

Reisen WK. 1975. Intra specific competition in *Anopheles stephensi* Liston. *Mosq News* 35:473–483.

Renshaw M, Service MW, Birley MH. 1994. Size variation and reproductive success in the mosquito *Aedes cantans. Med Vet Entomol* 8:179–186.

Scott JA, Brodgon WG, Collins FH. 1993. Identification of single specimens of *Anopheles gambiae* complex by polymerase chain reaction. *Am J Trop Med Hyg* 49: 520–529.

Takken W, Klowden MJ, Chambers GM. 1998. Effect of body size on host seeking and blood meal utilization in *Anopheles gambiae* sensu stricto (Diptera: Culicidae): the disadvantage of being small. *J Med Entomol* 35: 639–645.

WHO. 1975. Manual on practical entomology in Malaria. Part II. Methods and techniques. *Wrld Hlth Org Offset Publ.* 13. Geneva.

Wirtz RA, Zavala F, Charoenvit Y, Campbell GH, Burkot TR, Schneider I, Esser KM, Beaudoin RL, Andre RG. 1987. Comparative testing of *Plasmodium falciparum* circumsporozoite antibody. *Bull Wrld Hlth Org* 65:39–45.

Xue R, Edman JD, Scott TW. 1995. Age and body size effects on blood meal size and multiple blood feeding by *Aedes aegypti* (Diptera: Culicidae). *J Med Entomol* 32:471–474.