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Behaviour and population dynamics of the major anopheline vectors in a malaria endemic area in southern Nigeria

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Abstract

Background & objectives: Anopheline mosquitoes consist of a large number of species each of which differs from another in population, resting and feeding behaviour in relation to the prevailing conditions in the locality. A longitudinal study was carried out to investigate the population dynamics, resting and feeding behaviour of the major anopheline species found in a rain forest zone of Nigeria.

Methods: Mosquitoes resting and biting indoors were collected using WHO standard techniques and supplemented with outdoor-biting collections in the study areas between January and December 2004. Samples were sorted and identified microscopically for morphological features while molecular identification was carried out using polymerase chain reaction (PCR) techniques.

Results: PCR-based tests showed that both indoor and outdoor collections constitute three groups of *Anopheles* mosquitoes, *An. gambiae s.l.* Giles (68.6%), *An. funestus* Giles (30.7%) and *An. moucheti* Evans (0.7%). Of the 1,342 female *Anopheles* mosquitoes collected indoors, 799 were caught resting and 543 were caught biting. The outdoor-biting population accounted for 28.8% of the total collections (n =1885). There was no significant difference ($p > 0.05$) in the biting activities (indoors and outdoors) of these species in four villages. However, *An. arabiensis* and *An. moucheti* were more exophagic with >60% of their biting occurring outdoors while *An. gambiae* and *An. funestus* were more endophagic with >55% of their biting occurring indoors. The human-vector contact with *An. gambiae* and *An. funestus* (indoors) was about 73.3 and 66.7%, respectively as against 27.7% in *An. arabiensis* and 25.3% in *An. moucheti.* This gave the corresponding man-biting rates (MBR) of 17.5 bites/man/night for *An. gambiae*, 14.6 bites/man/night for *An. funestus*, 6.7 bites/man/night for *An. arabiensis* and 4.3 bites/man/night for *An. moucheti*. Moreover, the number of *An. gambiae* and *An. funestus* caught resting indoors was significantly higher than the other two species ($p < 0.05$). The wet season collections showed that *An. gambiae* caught were more than 67% of the total catch while *An. arabiensis* was predominant in the dry season ($\chi^2 = 75.44$, df = 3, $p < 0.01$).

Interpretation & conclusion: The present study highlights some aspects of the behaviour of anopheline mosquitoes in southern Nigeria which is an important component of epidemiological study of malaria. This information provides basis to the understanding of the role played by the identified anopheline species in malaria transmission and a baseline to formulate malaria control programme.

Key words *An. funestus* – *An. gambiae s.l.* – *An. moucheti* – behaviour – Nigeria – population dynamics

Introduction

Anopheles mosquitoes constitute various species with peculiar behaviour associated with their biting activities and malaria transmission. A large number of *Anopheles* species have been reported in Nigeria, but the main vectors of malaria belong to members of *Anopheles gambiae* and *An. funestus* Complexes while the secondary vectors include *An. moucheti* and *An. nili*[1–4]. The *An. gambiae* group consists of at least seven species including *An. gambiae* Giles and *An. arabiensis* Patton which are good vectors of malaria and are known to coexist in most part of West Africa[5–8]. The *An. funestus* group too consists of nine morphologically similar species which often display ecological and behavioural differences that affect their vectorial capacities. The major malaria vector in this group is *An. funestus* due to its close association with humans. Previous studies have documented the occurrence of this species and others regarded as minor species in the group in other parts of Africa[9–11].

The sympatric occurrence of *An. funestus* with *An. rivulorum* Leeson and *An. leesoni* Evans (minor vectors) have been reported in Nigeria[12–14]. Reports also showed that morphologically indistinguishable species are usually characterised with marked variations in blood-feeding patterns, biting-resting behaviour and species abundance[15–18]. Most of the previous works on malaria transmission in Nigeria were concentrated on the species of *Anopheles* mosquitoes in the northern region[2,3,19–21], meanwhile, there is little information on the behaviour and population dynamics of the major vectors of malaria in the southern part of Nigeria[12, 22–24]. The ecological pattern of Nigeria shows that as one moves from south to north, the humid forest from south turns to arid savannah, mean annual rainfall decreases, the number of dry season months increases and vegetation becomes shorter and sparser[13]. These climatic conditions cause the breeding sites to dry up more quickly leading to a rapid numerical decline in densities of mosquitoes. Moreso, the northern region is characterised with large cattle herd which are often kept around human habitation. Hence, the initial attraction emanated from cattle kept inside or around human houses may increase the risk of human being bitten by mosquito species that are both zoophilic and anthropophilic. This may indicate a difference in the abundance and behaviour of host-seeking mosquitoes and malaria transmission between northern and southern parts of Nigeria.

In the present study, we report a longitudinal study on the behaviour and population dynamics of the major vector species in a malaria endemic rain forest area of Nigeria.

Material & Methods

Study area: Anopheles mosquitoes were collected from the forest zone in the southern part of Nigeria. The ecological features of Nigeria show that it consists mainly of six zones—mangrove, forest, forest-savanna mosaic (transition zone) in the south and Guinea-savannah zone in the central region while the northern part consists of open woodland (Sudan) and arid (Sahel) savannah. The prevailing climatic feature in the south consists of two distinct seasons: wet season that normally occur from March/April to October/November (with a short break in July) with mean annual rainfall of 200 cm and dry season usually between November and March. The northern part experiences short period of rains (mean annual rainfall <50 cm) while dry season covers 6–8 months[23,25,26]. However, the ecological conditions in each zone reflect the existence of *Anopheles* species associated with malaria transmission; hence, the country is endemic for the disease.

The present study was carried out in the villages of Ilara-Remo (06°55′54ˢN 03°43′33ˢE), Akaka-Remo (06°57′45ˢN 03°43′12ˢE), Ijesa-Ijebu (06°54′43ˢN 03°46′14ˢE) and Idagolu (06°55′13ˢN 03°47′29ˢE) in Remo-North and Odogbolu areas of Ogun state. These areas come under tropical rain forest and are located about 150 km away from Lagos, the most

populous commercial city in Nigeria.

The inhabitants are mainly engaged in subsistent farming and hunting. Dwellings are either traditional houses partially or totally plastered with mud or cement or houses constructed with woven palm leaves or corrugated iron sheets. Domestic animals are either sheltered inside the dwellings or nearby cattlesheds. The inhabitants were approached through their community leaders and their consent was obtained before the commencement of the project. Ethical approval was granted by the Ethical Committee of the Nigerian Institute of Medical Research, Lagos, Nigeria.

Mosquito collections: Mosquitoes resting and biting indoors were collected using WHO standard techniques[27] and supplemented with outdoor-biting collections in study villages twice a month between January and December 2004 using human bait at night. Collection was carried out in five randomly selected dwellings where indoor catches were made between 1800 and 0600 hrs and outdoor catches between 1800 and 2200 hrs in order to understand the feeding habits of the vectors. Indoor resting mosquitoes were collected from 0600 to 0900 hrs in houses with mixed human/cattle habitations using mechanical aspirator and spray sheet collection methods in houses which were different from those used for the human-bait collection. The number of mosquitoes caught per hour and the hour biting rate (HBR) were recorded. Samples were stored individually over desiccated silica in Eppendorf tubes prior to identification and ELISA test.

Species identification: Mosquitoes were sorted and identified using morphological features with the aid of identification manual[1,4]. PCR identification was carried out using DNA extracted from legs or wings of each morphologically identified specimen following the method of Scott *et al*[16] with minor modifications as detailed in Van Rensburg *et al*[28]. Amplification was done with a Hybrid thermal cycler (Ashford, U.K.) which gave an initial denaturation at 94ºC for 2 min and 30 cycles of denaturation 94ºC for 30 sec, 72ºC for 30 sec and final extension at 72ºC for 8 min 10 µl of the amplified product was visualised on 2.5% agarose gel stained with ethidium bromide.

ELISA tests: ELISA tests for circumsporozoite antigens of *Plasmodium falciparum* were carried out as described by Wirtz *et al*[29], since *P. falciparum* is the only prevalent species in Nigeria. Absorbance values were determined photometrically at 405 nm using ELISA plate reader (Anthos 2010, Anthos Labtec GmbH, 5017 Wals/Salzburg, Austria). Samples were considered positive if the absorbance values were greater than twice the mean of negative controls.

Statistical analysis: The relative abundance of the species was expressed as the percentage of the total number of *Anopheles* collected. Chi-square and student's t-tests were used to analyse differences in indoor and outdoor biting activities, resting behaviour and sporozoite infection rates for each mosquito species.

Results

Population density and species composition: Overall, 1,885 adult female *Anopheles* mosquitoes were collected, 1,342 were caught indoors (biting/resting), 799 (59.5%) of these were indoor-resting while 543 (40.5%) were indoor-biting samples. The outdoor-biting samples account for 28.8% of the total collections (n =1,885). All the species collected fall into three major groups of *Anopheles* mosquitoes: *An. gambiae s.l.* (68.8%), *An. funestus* Giles (30.7%) and *An. moucheti* (0.7%). The products of the PCR analysis showed that, of the 920 mosquitoes of the *An. gambiae s.l.* collected, 490 (53.3%) were *An. gambiae s.s.* while 430 (46.7%) were *An. arabiensis*. Other species of *Anopheles* (n = 965), that is 51.2% of the total species analysed consisted of *An. funestus* Giles and *An. moucheti* population. The density of the *Anopheles* mosquitoes collected varied according to the season of the year. For instance, most *An. gambiae*

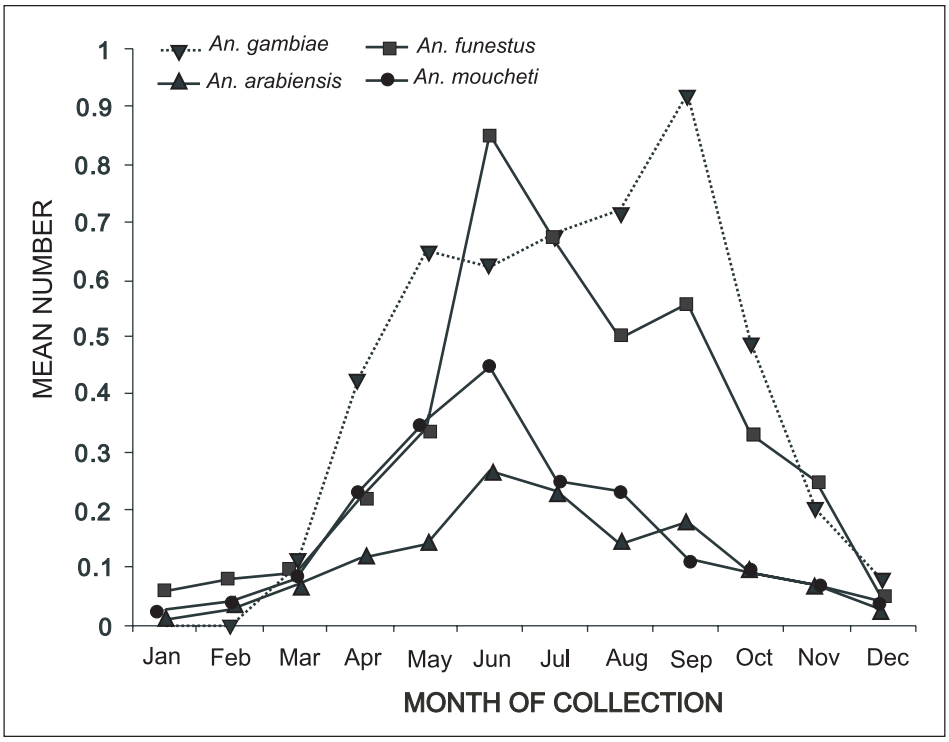

Fig. 1: Mean number of *Anopheles* mosquitoes collected resting indoors from January to December 2004

collected resting indoors were from May to September (with a peak in September) which coincided with period of rains and none was collected in January and February (dry season period). Meanwhile, the wet season collections showed that *An. gambiae* was >67% of the total catch. However, species of *An. arabiensis* and *An. moucheti* were caught throughout the year while *An. arabiensis* was predominant in the dry season ($\chi^2 = 75.44$, df =3, $p < 0.01$). Generally, more collections were made in June (the first peak of rains) than in the other months of the year (Fig.1). The overall indoor collections indicated a level of significant difference ($p < 0.05$) compared with that recorded outdoors.

Feeding and resting habits: The indoor and outdoor biting activities of the four species were not statistically different ($p > 0.05$) in four villages. However, *An. arabiensis* and *An. moucheti* were more exophagic with >60% of their biting population occurring outdoors while *An. gambiae* and *An. funestus* were more endophagic with >55% of their biting population occurring indoors. However, the population of *An. gambiae* and *An. funestus* biting indoors was significantly higher than those biting outdoors ($p < 0.05$) (Table 1). The monthly biting activity of all the species collected at human baits indoors and outdoors is shown in Fig. 2. Most biting mosquitoes of *Anopheles* species were collected in June which co-

Table 1. Relative abundance of *Anopheles* mosquitoes collected on human baits indoors and outdoors in the study sites (2004)

Species	Indoors	Outdoors	Total	(%)
An. gambiae	218	97	315	29.0
An. funestus	180	103	283	26.1
An. arabiensis	88	211	299	27.5
An. moucheti	57	132	189	17.4
Total	543	543	1086	100

($p < 0.05$).

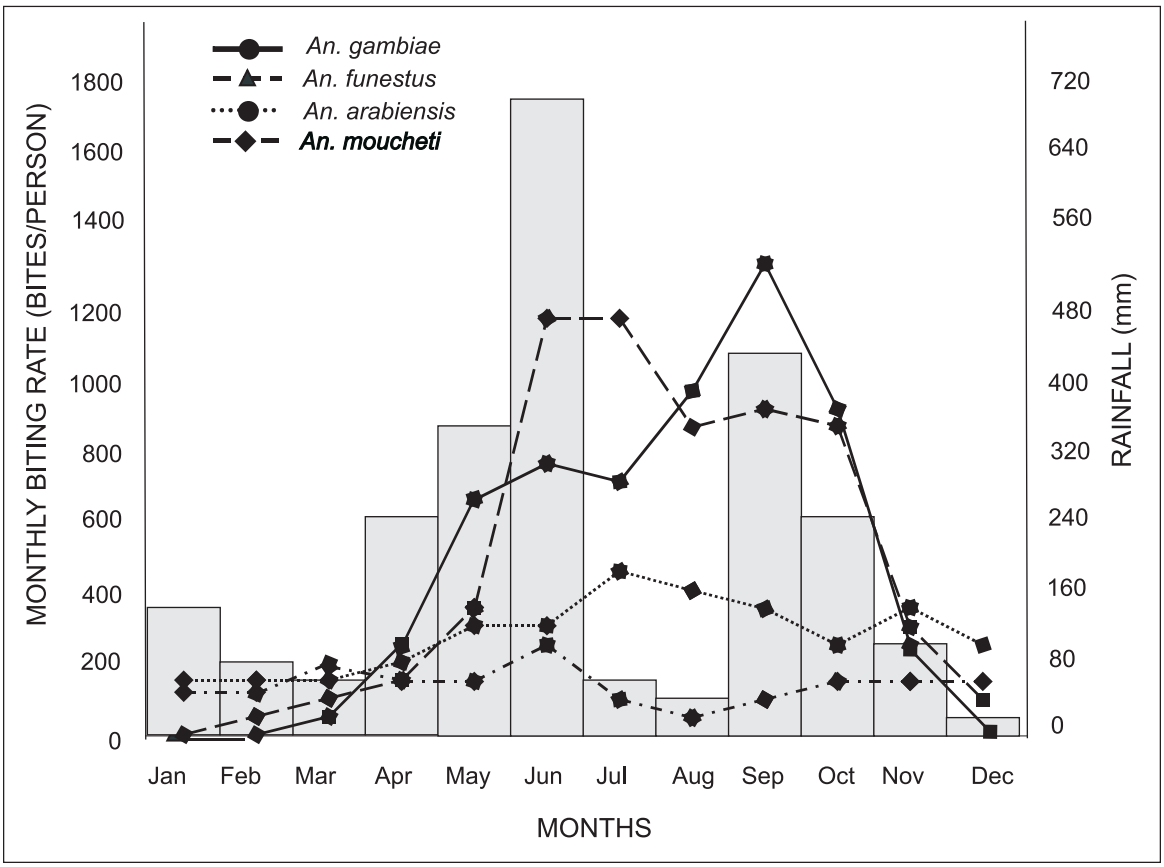

Fig. 2: Monthly biting rates for *Anopheles* mosquitoes and monthly rainfall for the year 2004.

incided with the peak of the rain, however, the biting activity of female *An. gambiae* and *An. funestus* was more pronounced during the wet season. Meanwhile, *An. arabiensis* and *An. moucheti* were attracted to human bait throughout the year. Although, biting *An. arabiensis* was mainly collected in the dry season through November to March.

Biting generally commences at 1800 hrs while the biting activities of *An. gambiae* in indoor and outdoor peaked at 0130 and 2200 hrs and that for *An. funestus* at 0300 and 2000 hrs respectively. More than 86% of *An. funestus* were collected resting indoors, which is an indication that this species is more endophagic in behaviour than the remaining species. However, the number of *An. gambiae* and *An. funestus* caught resting indoors were significantly higher than the other two species ($p < 0.05$). Generally, the overall indoor collection was significantly higher ($p < 0.05$) than that recorded outdoors.

Man biting rates: The human-vector contact with *An. gambiae* and *An. funestus* (indoors) was 73.3 and 66.7%, respectively as against 27.7% in *An. arabiensis* and 25.3% in *An. moucheti.* This gave the corresponding man biting rates (MBR) of 17.5 bites/man/night for *An. gambiae*, 14.6 bites/man/night for *An. funestus*, 6.7 bites/man/night for *An. arabiensis* and 4.3 bites/man/night for *An. moucheti*. MBR for *An. funestus* and *An. moucheti* reached maximum in June while that for *An. arabiensis* was in July and for *An. gambiae* in September, which corresponds to the second peak of the rain (Fig. 2).

Sporozoite ELISA: Analysis of the ELISA tests for circumsporozoite antigens showed that 23 (1.9%) of

Table 2. Number of *An. gambiae*, *An. funestus* and *An. moucheti* groups tested and percentage found with *P. falciparum* circumsporozoite antigens

Period	*An. gambiae*			*An. arabiensis*			*An. funestus*			*An. moucheti*			All species		
	N	+ve	SPR	N	+ve	SPR	N	+ve	SPR	N	+ve	SPR	N	+ve	SPR
Wet season (April–October)	232	10	4.3	220	5	2.3	112	2	1.8	5	0	0	569	17	3.0
Dry season (November–March)	170	4	2.3	420	11	2.6	63	1	1.6	2	0	0	655	16	2.4
Total	402	14	3.5	640	16	2.5	175	3	1.7	7	0	0	1224	23	1.9

N—Total No. tested; +ve—No. tested positive; SPR—Sporozoite rates.

tested population were positive for *P. falciparum*. The sporozoite rates for *An. gambiae* were 4.3% (wet season), which dropped to 2.3% (dry season); this showed a level of comparative significant difference with the dry season rates for *An. arabiensis* (2.6%; $p < 0.01$). The overall sporozoite rates for *An. funestus* was significantly less (1.7%, $p < 0.05$) than that those for *An. gambiae* and *An. arabiensis*. However, the overall 'rates' for all the species dropped from 3% in wet season to 2.4% in the dry season (Table 2). There was no significant difference ($p > 0.05$) in the sporozoite rates in both the indoor and outdoor biting collections in all the study villages.

Discussion

The rain forest zone of Nigeria has been described as a malaria endemic area because of the high level of human-vector contact, which has been attributed to high density of anopheline vectors in this area[12,14]. Reports have shown that *An. gambiae s.l.* and *An. funestus* group constitute the largest proportion of malaria vectors in Nigeria[3,13,14,17,22–24]. In the present study, three out of the four species reported (*An. gambiae*, *An. funestus* and *An. arabiensis*) were abundant in the study areas and this may associate with malaria transmission dynamics here. In addition, *An. gambiae* and *An. arabiensis* were found to occur in sympatric and were the predominant species in all the communities sampled. Elsewhere in Africa, sympatric occurrence of these species has been reported as well[23,30–32]. Previous studies also showed that these two species often predominate the total collections especially in the forest zone where they may occur in ratio 1 : 2[5,17,30]. In this study, we found that *An. arabiensis* and *An. moucheti* were most abundant in the early and late dry seasons and *An. gambiae* was completely absent during these seasons. Also, the occurrence of *An. funestus* persist in the early and late dry seasons until the slow moving streams and cool, shady places where they breed disappeared. In the present study, the collections were generally most abundant in June which coincided with the peak of rain, however, *An. gambiae* was most abundant in September after which collections gradually declined.

In addition, *An. gambiae* was largely responsible for the indoor biting activity and was found to be highly endophilous compared to other species. This indicates that this species preferred to rest indoors after feeding. Similar behaviour was recorded in *An. funestus*, but with less indoor resting population. These two species also recorded very high MBR and may be largely responsible for malaria transmission in the study area. On the other hand, *An. arabiensis* and *An. moucheti* were more exophagic with >60% of their biting population occurring outdoors. Previ-

ous findings also reported similarity in behaviour of these species[3,24,33]. Although this study showed that *An. arabiensis* was more anthropophilic with their large feeding population occurring outdoors. Ordinarily, *An. moucheti* would have been expected to occur outdoor but few of them (27.5%) were still found resting indoor after feeding. The indoor and outdoor biting activities of species sampled in this study were found to extend throughout the night and year round and this may explain the reason for the perennial malaria transmission in the area. Biting activity of the anophelines in the study area commences effectively at the early part of the night before the inhabitants retire to bed. This suggests that the use of measures such as protective clothing, mosquito coil and repellents to prevent mosquito bite would be effective in reducing man-vector contact. Furthermore, the extended biting activity of *An. gambiae* and *An. funestus* into the larger part of the night could be confronted by the use of insecticide-treated bednets.

The MBR recorded for *An. gambiae* was significantly high compared to other species ($p < 0.05$), although increase in MBR in each of the species coincides with the level of rainfall, reaching maximum at different months of the year (Fig. 2). The results also indicate that wet season transmission in the study area was largely maintained by *An. gambiae* species while *An. arabiensis* and *An. moucheti* account for most dry season infective bites. The MBR can be an important factor in the epidemiology of the disease and in estimating the vector-human contact for determining malaria transmission and for planning control programme.

The sporozoite rates for *An. gambiae* and *An. funestus* were highest during the wet season while that for *An. arabiensis* was highest during early dry season which corresponds with the period of the year when vector density was at its peak. The sporozoite rates recorded for *An. arabiensis* was relatively high compared to the previous records from the northern Nigeria[3], but was close to that reported in other parts of West Africa[34–36]. This may likely due to the attraction of this species to the human habitat where domestic animals are also kept. Moreso, *An. arabiensis* is an opportunistic feeder and will readily feed on both humans and animals[4,5]. Meanwhile, the overall sporozoite rates recorded in the wet season (3%) in this study are also close to reports obtained from the similar rain forest zones elsewhere in Africa[33,37].

The year-round transmission of malaria in the study communities which often reach the peak during the rains with the active participation of the major anopheline fauna indicates the endemicity of the disease and the need for malaria control programme in the region. Meanwhile, the information provided in the present study brings to light some aspects of the behaviour of anopheline species and the epidemiological importance of the results obtained thereof to malaria transmission in southern Nigeria.

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