The late biting habit of parous *Anopheles* mosquitoes and pre-bedtime exposure of humans to infective female mosquitoes

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Abstract

Using the all-night landing catch method (18:00–06:00) we showed, for *Anopheles gambiae* in Sierra Leone and *A. punctulatus* in Papua New Guinea, that parous females have a tendency to bite later than nulliparous ones. The biting habit of sporozoite-infected *A. punctulatus* was also investigated. The sporozoite rates for *Plasmodium falciparum* and *P. vivax* were 1·8 and 1·4% respectively, but only one (1·3%) of 76 females infected with *P. falciparum* was caught between 18:00 and 21:00. A significantly higher proportion (11·6%) of mosquitoes infected with *P. vivax* was caught in the same period. The late biting habit of mosquitoes infected with *P. falciparum* is discussed in relation to the differential biting habits of parous and nulliparous females. We conclude with a hypothesis that, in areas where *Anopheles* mosquitoes have a late-biting cycle and low parous rate, exposure to mosquitoes infected with *P. falciparum* during the pre-bedtime period (18:00–22:00) is very low. This hypothesis could explain why insecticide-treated bed nets protect children better in areas of seasonal transmission, where nulliparous females tend to predominate, than in areas of perennial transmission, where parous females are usually more numerous. The same hypothesis is compatible with the finding in Papua New Guinea that insecticide-impregnated bed nets are more protective against *P. falciparum* than against *P. vivax* malaria.

Keywords: malaria, *Plasmodium falciparum*, *Plasmodium vivax*, *Anopheles gambiae*, *Anopheles punctulatus*, sporozoite rates, parity, biting habits, Sierra Leone, Papua New Guinea

Introduction

Studies with the malaria vectors of the *Anopheles gambiae* complex in Burkina Faso (HAMON *et al.*, 1961) and the *A. punctulatus* complex in Papua New Guinea (CHARLWOOD *et al.*, 1986) have shown that parous females of these species tend to bite later than nulliparous ones. Since both species have late biting cycles, a late feeding habit of parous females, which are more likely to be infected with malaria sporozoites, would reduce exposure to infective mosquitoes during the early hours of the night when people are engaged in outdoor activities. In places where people use bed nets to prevent mosquito bites, pre-bedtime (18:00 to 22:00) exposure to infective mosquitoes could be an important factor determining the degree of protection from disease transmission offered by the nets. It is therefore important to determine to what extent the age composition of malaria vectors influences the outcome of insecticide-treated bed net interventions.

In The Gambia, the mean annual parous rates of *A. gambiae s.l.* were below 46% in 4 villages in an area where impregnated bed nets provided protection against clinical attacks of malaria (LINDSAY *et al.*, 1989). In Tanzania and Papua New Guinea, where less favourable results have been achieved with treated bed nets (GRAVES *et al.*, 1987; LYIMO *et al.*, 1991), the mean annual parous rates of the main vectors, *A. gambiae s. l.* and *A. punctulatus s. l.* respectively, tend to be higher than 60% (CHARLWOOD & GRAVES, 1987; MAGESA *et al.*, 1991). In this paper, we report observations of the feeding habits of nulliparous, parous, and sporozoite-positive malaria vectors in Sierra Leone and Papua New Guinea, in hourly catches between 18:00 and 06:00. We attempt to explain why insecticide-treated bed nets tend to protect children better in areas of seasonal transmission, where nulliparous females predominate, than in areas of perennial transmission, where parous females are usually more numerous.

Materials and Methods

Study area

Studies involving *A. gambiae* were carried out in Bayama village, Bo District, southern Sierra Leone, about 3 km north of Bo town in a zone of secondary forest which receives on average 2500 mm of rainfall per year, mostly (>90%) in May–November.

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Studies with *A. punctulatus* were conducted in 5 villages in East Sepik and one village, Yankok, in West Sepik Province of Papua New Guinea. The 5 villages in East Sepik Province, Albulum, Ngahmbule, Nanaha, Peneng and Yauatong, are all situated along the Maprik–Lumi highway, on the southern foothills of the Torricelli Mountains. Yankok village is also located on the Maprik–Lumi highway, about 50 km west of Yauatong. The inhabitants are subsistence farmers cultivating mainly taro, sago and vegetables. Rainfall is approximately 1600 mm per year with most rain occurring from December to June; however, rain falls throughout the year. Mean annual daily temperature is about 30°C.

Collection and processing of mosquitoes

The human-bait method was used to catch mosquitoes landing on adult males between 18:00 and 06:00. Ovaries were dissected to establish parity according to the method of DETINOVA (1962). An enzyme-linked immunosorbent assay was used for the determination of malaria sporozoite antigens (WIRTZ *et al.*, 1987). Some mosquitoes from Yauatong village, East Sepik, were preserved in 70% ethanol and later stained for filarial parasites using Mayer's acid haemalum (NELSON, 1958).

Results

At Bayama, Sierra Leone, 6493 female *A. gambiae s.s.* were collected on 30 consecutive nights in the wet season of 1991; 3191 were dissected for parity determination (Table 1). The differences between the parous rates were highly significant ($\chi^2=37{\cdot}97$, degrees of freedom [df]=2, $P<0{\cdot}001$). The catch in the first 4 h contained a higher percentage of nulliparous females than those in the remaining 8 h.

In Papua New Guinea, parous rates of A. punctulatus were determined only in Yauatong village between No-

Table 1. Parous rates of female *Anopheles gambiae* from outdoor human-bait catches at Bayama, Sierra Leone

	Number of mosquitoes		
Time	Caught	Dissected	Parous
18:00–22:00	104	91	40 (44·0%)
22:00–02:00	2016	1269	740 (58·3%)
02:00–06:00	4373	1831	1225 (66·9%)
Total 18:00–06:00	6493	3191	2005 (62·8%)

Table 2. Parous rates of combined indoor and outdoor human-bait catches of female *Anopheles punctulatus* in Yauatong, Papua New Guinea

	Number of mosquitoes		
Time	Caught	Dissected	Parous
18:00–22:00	202	171	68 (39·8%)
22:00–02:00	848	768	421 (54·8%)
02:00–06:00	1100	993	628 (63·2%)
Total 18:00–06:00	2150	1932	1117 (57·8%)

Table 3. Proportion of female *Anopheles punctulatus* infected with *Plasmodium falciparum* and *Plasmodium vivax* in hourly biting populations from 18:00 to 06:00 in six villages in East Sepik Province of Papua New Guinea

	Number of mosquitoes		
		Infected with	
Time	Processed	*P. falciparum*	*P. vivax*
18:00–19:00	82	0 (–)	2 (2·4%)
19:00–20:00	130	0 (–)	2 (1·5%)
20:00–21:00	140	1 (0·7%)	1 (0·7%)
21:00–22:00	183	3 (1·6%)	2 (1·1%)
22:00–23:00	317	9 (2·8%)	3 (0·9%)
23:00–00:00	401	10 (2·5%)	6 (1·5%)
00:00–01:00	461	9 (2·0%)	6 (1·3%)
01:00–02:00	615	2 (0·3%)	1 (0·2%)
02:00–03:00	461	8 (1·7%)	7 (1·5%)
03:00–04:00	460	10 (2·2%)	5 (1·1%)
04:00–05:00	397	10 (2·5%)	6 (1·5%)
05:00–06:00	521	14 (2·7%)	2 (0·4%)
Total 18:00–06:00	4168	76 (1·8%)	43 (1·0%)

vember 1993 and August 1994. In February, 17 consecutive nights of human-bait catches yielded 2150 female *A. punctulatus*; 1932 were dissected (Table 2). As with *A. gambiae* in Sierra Leone, the proportions of parous *A. punctulatus* caught in the 3 periods were significantly different (χ^2=37·66, df=2, P<0·0001). There was again a deficit of parous mosquitoes in the early hours of the night.

The effect of the late biting habit of parous *A. punctulatus* on pre-bedtime exposure to malaria-infective mosquitoes was investigated in all 6 Papua New Guinea study villages. A total of 85 person-nights of human-bait catches in the 5 villages in East Sepik Province yielded 4621 *A. punctulatus*. Of these, 4168 (90·2%) were processed to determine sporozoite rates for *P. falciparum* and *P. vivax* (including the variant *P. vivax*-247) (Table 3). The sporozoite rates for *P. falciparum* and *P. vivax* were 1·8 and 1·0% respectively but only one (1·3%) of the 76 females infected with *P. falciparum* was caught between 18:00 and 21:00. A significantly higher proportion (11·6%) of the 43 females infected with *P. vivax* was caught in the same period (Yates's corrected χ^2=4·14, df=1, P=0·02). *P. falciparum* sporozoite antigens were not detected in any of 202 mosquitoes caught between 18:00 and 20:00 (203 mosquitoes were caught in this period, but one was not processed). The *P. falciparum* sporozoite rate for the catch between 18:00 and 22:00 (0·7%) was lower than that between 22:00 and 02:00 (1·7%), but the difference was not statistically significant. However, the *P. falciparum* sporozoite positivity rate for the catch between 02:00 and 06:00 (2·3%) was significantly higher than that between 18:00 and 22:00 (Yates's corrected χ^2=4·37, df=6, P=0·037). *P. vivax* sporozoite antigens were detected in mosquitoes caught during each hour; the sporozoite rate for the catch between 18:00 and 22:00 (1·7%) was similar to those between 22:00 and 02:00 (0·9%) and 02:00 and 06:00 (1·1%)

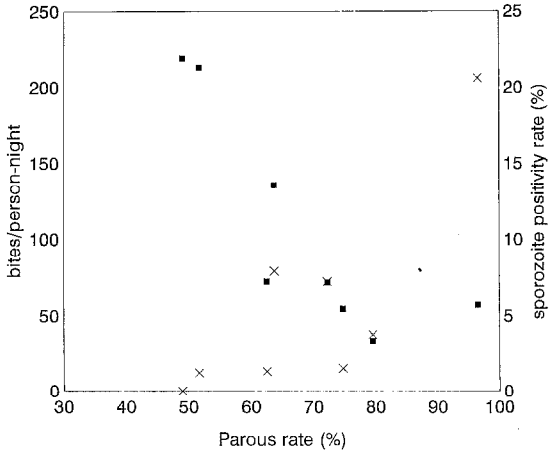

Figure. The relationships of monthly parous rates of *Anopheles punctulatus* to human biting rates (■) and sporozoite positivity rates (×) in Yauatong village, East Sepik Province, Papua New Guinea. Parous rates were determined for 8 months between November 1993 and August 1994.

In the Yauatong village catches, the monthly parous rate was significantly negatively correlated with the monthly human biting rate (r=–0·82, df=6, P<0·02) and significantly positively correlated with the monthly sporozoite rate (r=0·79, df=6, P=0·02) (Figure). In the East Sepik villages, people were exposed to many more mosquito bites during August to November (104·0 bites/person/night), the peak dry season, than during the rest of the year (19·7 bites/person/night). The mean *P. falciparum* sporozoite rates for the 2 periods were 1·2% and 3·4% respectively. However, during the peak dry season no mosquito infected with *P. falciparum* sporozoites was detected among nearly all (95·1%) of the 287 mosquitoes caught between 18:00 and 21:00.

In Yankok village, West Sepik Province, 22 person-nights of human bait collections between March and November 1994 produced 745 *A. punctulatus*; 736 were processed and the sporozoite rates were 0·8% for *P. falciparum* and 0·1% for *P. vivax*. All the 123 mosquitoes caught between 18:00 and 22:00 were tested, but none was positive for either *P. falciparum* or *P. vivax*. As in the East Sepik villages, mosquitoes infected with sporozoites tended to bite later in the night.

The hourly distribution of mosquitoes infected with different larval stages of *Wuchereria bancrofti* was investigated in the village of Yauatong; 1904 mosquitoes were dissected and the infection (L1–L3) and infective (L3) rates were 5·3% and 1·2% respectively. Infected mosquitoes were caught during each hour but only one (4·5%) of the 22 with infective (L3) larvae (>10 d old) was caught between 18:00 and 23:00. However, in the same period 14·9% of 101 mosquitoes were more than 3 d old. The difference was not statistically significant but, nevertheless, suggests that older females had a tendency to bite later than younger ones.

Discussion

Our results clearly showed that there was a lower proportion of parous *A. gambiae* and *A. punctulatus* in the biting population before 22:00. GILLIES & WILKES (1963), working in Tanzania, ignored *A. funestus* biting before 22:00 but showed that the proportions of parous females in the 22:00–02:00 and 02:00–06:00 catches were identical. In the present study, the proportions of parous *A. gambiae* biting in the 22:00–02:00 and 02:00–06:00

catches were significantly different. The same was true for *A. punctulatus*. CHARLWOOD & WILKES (1979) also found very pronounced differences in the biting times of parous and nulliparous *A. darlingi* in Brazil.

The dearth of mosquitoes containing *P. falciparum* sporozoites between 18:00 and 22:00 may be associated with the late biting habit of older mosquitoes. It appears, from some studies in Africa, that children sleeping under insecticide-treated bed nets are better protected from infective mosquitoes during periods when the vector parous rate is low, when the early biting nulliparous females predominate. In a village (Mlingano) in a holoendemic area of Tanzania, where the parous rate of *A. gambiae* was only 40%, introduction of insecticide-treated bed nets resulted in a prolonged downward trend in the blood slide positivity rate for *P. falciparum* from 40–65% to about 20%. In another village (Umba), in the same area, where the parous rate of *A. gambiae* was over 60% when impregnated bed nets were introduced, there was only a slight decline in the slide positivity rate from about 60–80% to 50% (LYIMO *et al.*, 1991; MAGESA *et al.*, 1991). In The Gambia, treated bed nets provided protection against clinical attacks of malaria in an area where the mean annual parous rate of *A. gambiae* was less than 46% (SNOW *et al.*, 1988; LINDSAY *et al.*, 1989). However, recent studies in the same country, in an area where high sporozoite rates in *A. gambiae* were combined with low biting rates, showed that treated bed nets were ineffective in reducing childhood death rates (D'ALESSANDRO *et al.*, 1995). In the present study, a high sporozoite rate in *A. punctulatus* combined with a low biting rate was indicative of a high parous rate (Figure).

Our results also showed that mosquitoes infected with *P. vivax* have a tendency to bite earlier than those infected with *P. falciparum*, which cannot be explained solely by the difference in biting activity between parous and nulliparous mosquitoes, assuming all malaria-infective mosquitoes were parous. However, mosquitoes containing *P. vivax* sporozoites would be expected, on average, to be younger than those infected with *P. falciparum* sporozoites because the duration of *P. vivax* sporogony (7 d) is shorter than that of *P. falciparum* (9 d) at 30°C (GARNHAM, 1966)—the mean daily temperature in the Papua New Guinea study villages. A tendency for younger parous females to bite earlier than older ones may explain the early biting habit of mosquitoes infected with *P. vivax* in comparison to mosquitoes with *P. falciparum*. We showed indirectly, by looking at the biting habit of mosquitoes infected with *W. bancrofti*, that younger parous females have a tendency to bite earlier than older ones. DETINOVA's finding (1962) that samples of *A. maculipennis* with a deficit of nulliparous females sometimes also had a deficit of uniparous and biparous females suggests that young parous females tend to behave like nulliparous ones.

Our observation that there was a higher proportion of mosquitoes infected with *P. vivax* in the early part of the evening than those with *P. falciparum* is consistent with the results of 2 trials of impregnated bed nets in Papua New Guinea. MILLEN (1986), in Madang Province, found a significant reduction in prevalence of *P. falciparum* among children 1–4 years old who used nets, but there was no effect on *P. vivax* prevalence. This could have resulted from the children's having been exposed more to *P. vivax* than to *P. falciparum* before going to sleep under impregnated bed nets. Also in Madang Province, GRAVES *et al.* (1987) reported that the incidence of *P. falciparum* was significantly lower in children 0–4 years old 4–10 weeks after impregnated nets were introduced. However, they found no effect on the incidence of *P. vivax* or *P. falciparum* in 5–9 years old children, who usually go to bed later than the younger ones. Pre-bedtime exposure to sporozoite-infected mosquitoes may be another important factor in determining the outcome of impregnated bed net intervention for malaria control.

Acknowledgements

We are grateful to the residents of the study villages for their co-operation. We thank the field and laboratory staff of the British Medical Research Council Laboratory in Bo, Sierra Leone and of the Papua New Guinea Institute of Medical Research in Madang, Maprik and Dreikikir. We also thank Professor M. W. Service for comments on the manuscript.

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Received 2 May 1995; revised 31 July 1995; accepted for publication 1 August 1995