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Anopheles gambiae gonotrophic cycle duration, biting and exiting behaviour unaffected by permethrin-impregnated bednets in The Gambia

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Abstract. Permethrin-impregnated bednets protect children against malaria in The Gambia, where *Anopheles gambiae* complex mosquitoes are the main vectors of malaria. However, no effect has been found on mosquito density, parous rates or sporozoite rates in *An.gambiae sensu lato* populations; only a reduction in the numbers of mosquitoes resting indoors in rooms with treated bednets. A possible explanation for this paradox is that exposure to treated bednets leads to changed vector behaviour such as a shift in biting time, a diversion to biting outdoors instead of indoors, to biting animals instead of humans, or to increased duration of the gonotrophic cycle. To investigate these possibilities, we observed the biting and exiting behaviour of *An.gambiae* in ten pairs of villages, in half of which the residents used permethrin-treated bednets. The possible influence of treated bednets on the gonotrophic cycle length was evaluated by mark–release–recapture experiment.

No significant difference was found between villages with treated and untreated bednets in the indoor/outdoor ratio of human biting, in mean biting times or in human blood indices of *An.gambiae* females found resting indoors in the mornings. The proportions of unfed, fed or gravid *An.gambiae* females collected in exit traps, and the number of females exiting showed no significant differences between rooms with treated and untreated bednets. Indications for a gonotrophic cycle length of 2 days were found. No evidence for any change in duration of the gonotrophic cycle in relation to exposure to treated bednets was found, although the number of recaptures was low in the villages with treated bednets.

Since equal numbers of infective *An.gambiae* were found in villages with treated or untreated bednets, and no changes in mosquito behaviour were detected, we cannot account for how children are protected against malaria by treated bednets. One possibility is that mosquitoes divert to bite other hosts, including adults.

Key words. *Anopheles gambiae*, impregnated bednets, malaria control, vector control, permethrin, The Gambia.

Introduction

Exposure to insecticide-impregnated bednets could have several effects on mosquito populations. Exposure might reduce mosquito survival and thus lower the malaria sporozoite rate in *Anopheles* mosquitoes (Diptera: Culicidae). Insecticide-impregnated bednets might also have important effects on mosquito behaviour. Behavioural effects on *Anopheles* mosquitoes have been described in studies carried out in the laboratory, experimental huts and at village scale. Feeding inhibition of mosquitoes exposed to treated bednets has been observed both in the laboratory and in experimental hut studies. For example, no mosquitoes fed on an arm pressed against a permethrin-impregnated bednet (Hossain & Curtis, 1989). In experimental hut studies a reduction in the proportion of mosquitoes feeding has been found in Burkina Faso (Darriet *et al.*, 1984; Carnevale *et al.*, 1992), Tanzania (Lines

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et al., 1987; Curtis *et al.*, 1992), The Gambia (Lindsay *et al.*, 1991, 1992; Pleass *et al.*, 1993), China (Li *et al.*, 1987) and Suriname (Rozendaal, 1989). Deterrency (a reduction in the number of mos-quitoes entering a room) has been found in various experimental hut studies (Darriet *et al.*, 1984; Miller *et al.*, 1991). An increase in the proportion of mosquitoes exiting from experimental huts containing permethrin-treated bednets was found in experiments in Burkina Faso (Darriet *et al.*, 1984) and Tanzania (Lines *et al.*, 1987), but not in The Gambia (Lindsay *et al.*, 1991; Pleass *et al.*, 1993).

Other behavioural changes of *Anopheles* females confronted by treated bednets were noted in a village-scale study undertaken in Papua New Guinea (Charlwood & Graves, 1987). Comparisons made before and after the introduction of permethrin-impregnated bednets in one village showed a reduction in the human blood index of mosquitoes collected resting indoors, implying a diversion to animal feeding. In addition, the post-midnight peak of biting activity shifted towards a pre-midnight peak for both local vectors: *An.farauti* Laveran and *An.koliensis* Owen. There was also a disruption in the regularity and an increase in the duration of the oviposition cycle of *An.farauti*. The interpretation given to the latter finding was that mosquitoes were diverted outside by the presence of the impregnated bednets, and thus prevented from feeding on the same night as oviposition. Diverted females had to return to feed the following night, biting in the early evening, thus increasing the length of their gonotrophic cycle. Changes in the biting pattern of *An.arabiensis* Patton were also found after the use of bednets impregnated with deltamethrin or lambda-cyhalothrin in Tanzania (Njau *et al.*, 1993). The explanation for the shift in biting time observed in Tanzania is probably different from the one proposed by Charlwood & Graves (1987) since, in this case, the contrast was seen between treated and untreated houses within the same village. In the Solomon Islands, a change from 3 to 4 days in the duration of the gonotrophic cycle length of *An.farauti* was found in a village with permethrin-treated bednets, compared with a DDT-sprayed village and an untreated village (Hii *et al.*, 1995).

In The Gambia, permethrin-impregnated bednets reduce morbidity and mortality in children due to malaria (Snow *et al.*, 1987a, 1988; Alonso *et al.*, 1993, D'Alessandro *et al.*, 1995). However, these epidemiological findings are not associated with the kind of 'mass-killing effect' described in other countries, e.g. Tanzania (Magesa *et al.*, 1991) and Zaire (Karch *et al.*, 1993), where clear reductions in density, parous rates and sporozoite rates were found after the introduction of insecticide treated bednets. In The Gambia, mosquitoes of the *Anopheles gambiae* Giles complex generally remain as abundant, as long-lived and as likely to be infected in villages with treated bednets compared to villages with untreated bednets (Lindsay *et al.*, 1993; Thomson *et al.*, 1995a; Quiñones, 1996). This is partly attributed to mixing of vector populations between villages (Thomson *et al.*, 1995b). However, changes in behaviour may enable mosquitoes to survive in villages with treated bednets.

This paper gives the results of two studies in which attempts have been made to detect changes in behaviour of *An.gambiae sensu lato* in relation to village-scale use of permethrin-impregnated bednets. One study involved a survey of biting and exiting behaviour of *An.gambiae s.l.* in twenty villages, half of which used treated bednets and the others used untreated bednets. The second study investigated effects of the impregnated bednets on duration of the gonotrophic cycle, using a mark–release recapture technique (Thomson *et al.*, 1995b) and a cross-over design. The effects of treated bednets on mosquito densities, parous rates and sporozoite rates in these villages are given elsewhere (Quiñones, 1996).

Materials and Methods

Mosquito biting and resting behaviour. This study was undertaken during a 3-week period of July 1992 in twenty villages of mid-Gambia, (see Fig. 1 in Alonso *et al.*, 1993) where people mostly (~90%) sleep under bednets at night for personal protection against anthropophilic mosquitoes (Aikins *et al.*, 1993). Villages with treated bednets were paired with their nearest control village with untreated bednets. All-night, human-landing mosquito collections were carried out on one occasion in each village, indoors and outdoors simultaneously, between 19.00 and 07.00 hours. Pyrethrum spray catches were carried out in five rooms of each village on the morning after the human-landing collections. An ELISA (Service *et al.*, 1986) was used to determine the Human Blood Index (HBI) of freshly blood-fed female *An.gambiae s.l.* mosquitoes.

Further collections were made in these villages in October 1992, when three window exit traps (WEX) were set in each village for one night in rooms with treated bednets in treated villages (30 WEX nights) and in rooms without a treated bednet in untreated villages (30 WEX nights).

Mark–release–recapture experiment to determine duration of the gonotrophic cycle. A cross-over study was carried out in two of the villages, Madina and Jakoto (see Fig. 1 in Thomson *et al.*, 1995b). Bednets impregnated with Permethrin 500 mg/m^2 were given to the people of Madina village during the first 2 weeks, whereas in Jakoto the people continued using their own untreated bednets. After 2 weeks this was reversed: new impregnated bednets were given to the people in Jakoto, the impregnated bednets were collected from Madina, and the people there were asked to use their own untreated bednets again.

Mark–release–recapture procedures were carried out twice in the two study villages, once during the 2 weeks before the cross-over of the treated bednets and again during the 2 weeks after. Resting fed females of the *An.gambiae* complex were collected daily from *c.* 600 untreated bednets sited in both the untreated study village and in four neighbouring untreated villages. During the first 6 days of each experiment, half the females collected were transferred to Jakoto for marking and release, whilst the other half were transferred to Madina. Marking, releasing and checking for recaptures took place in a designated hut (mini-laboratory) in the centre of each village. On days 7–11 the mosquitoes collected were only checked for recaptures.

Blood-fed *An.gambiae s.l.* females were marked using fluorescent powders (Fiesta Daylight Colours, SWADA, Stratford, London) according to the method described in Thomson *et al.* (1995b). The colours used were: Red (R), Blue (B), Green (G), Orange (O) and Magenta (M). In addition, powders were mixed to produce further seven colours: Purple (P) (1 B + 2 R), Turquoise (T) (1 G + 2 B), Dark Blue (DB) (1 M + 1 B), Light Blue (LB) (1 Yellow + 1 B), Dark Green (DG) (1 G + 1 R + 1 B), Yellowish

(Y) (1 O + 1 R + 1 G) and Bright Green (BG) (1 Yellow + 1 G), all of which could be distinguished easily under a dissecting microscope with ultraviolet (UV) illumination. Of these twelve colours, six were used in each village, being changed every day for six consecutive days. Thus, the colour of a recaptured mosquito indicated the day and village of its release.

To search for marked *An.gambiae s.l.* mosquitoes were collected in both villages for 11 days by means of pyrethrum knock-down spray catches in ten rooms and five window exit traps in each village. *An.gambiae s.l.* females were checked for marks, initially in the village 'mini-laboratory' using a UV lamp; those identified as probably or possibly marked were checked later under a microscope in order to confirm the colour. Date and village, method of collection, colour and gonotrophic stage were recorded for each recapture.

Palps of a sample of *An.gambiae s.l.* females were dissected and measured for species determination according to the palpal ratio (Coluzzi, 1964). Specimens with a ratio of 0.81 and above were ascribed to saltwater *An.melas* Theobald and those with a ratio less than 0.81 to freshwater *An.gambiae s.l.* (Bryan, 1980). Among the latter, a sub-sample were examined chromosomally to determine their sibling species identity (Coluzzi *et al.*, 1979, 1985).

Results

Human-landing collections indoors and outdoors

Ratios between the numbers of *An.gambiae s.l.* females collected landing on human baits indoors *v.* outdoors showed wide variation, ranging from 0.6 to 2.5 in treated villages and from 0.5 to 8.3 in untreated villages (Table 1). Overall ratios in villages with treated and untreated bednets did not differ significantly (Wilcoxon signed rank test, $P = 0.064$); the pooled ratios (1.45 and 1.39) were remarkably similar.

Table 1. Indoor:outdoor (I:O) ratio of *Anopheles gambiae s.l.* females from all night human-landing collections in ten pairs of villages. (*n* = total number collected, indoors + outdoors.) Dates in July 1992.

Date	Village pair*	Treated I:O ratio (*n*)	Untreated I:O ratio (*n*)
09	1	1.29 (470)	1.17 (737)
10	2	1.31 (150)	3.43 (31)
15	3	2.50 (210)	8.33 (48)
17	4	1.96 (77)	2.17 (38)
21	5	0.69 (44)	2.56 (57)
22	6	0.76 (44)	1.22 (140)
24	7	2.16 (112)	1.28 (155)
28	8	0.87 (28)	1.42 (29)
29	9	0.63 (13)	0.50 (15)
31	10	0.00 (5)	1.33 (7)
Overall		1.45 (1158)	1.39 (1293)

* Pairs of villages: treated–untreated: (1) Jafaye–Sotokoi, (2) Jahally–Saruja, (3) Kudang–Mbien, (4) Madina Nfally–Taifa, (5) Pacharr–Kerewan, (6) Brikamaba–Tabananeh, (7) Mbaien Maka–Mamufana, (8) Pathe Same–Batinjol, (9) Sare Futa–Fula Bantang, (10) Fas Abdou–Boweram.

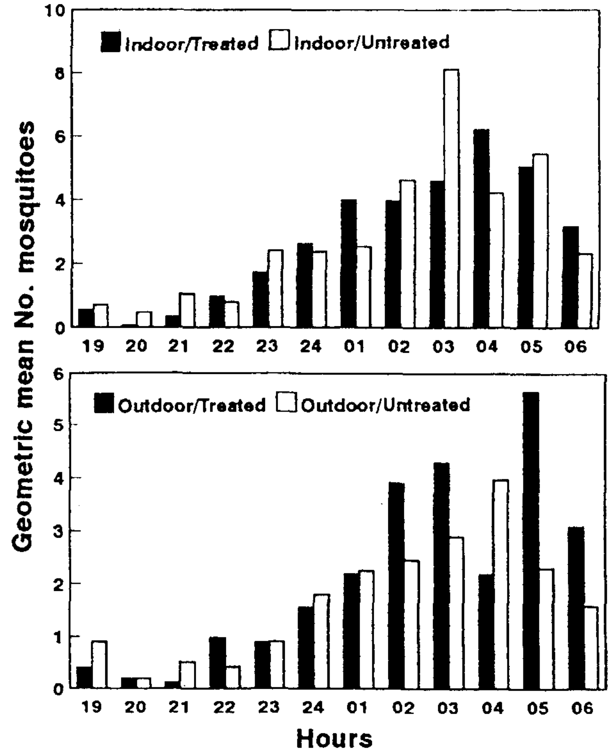

Fig. 1. Hourly geometric mean numbers of *An.gambiae s.l.* females collected landing on collectors indoors and outdoors in ten pairs of villages: see footnote, Table 1, for names and status of villages.

Biting cycle

Hourly geometric mean numbers of *An.gambiae s.l.* collected in human-landing collections indoors and outdoors are shown in Fig. 1. The mean biting time was calculated for each village (Table 2) by assuming that each mosquito was collected in the middle of the period i.e. mosquitoes collected during 02.00–03.00 hours were assumed to have been collected at 02.30 hours. Times were then weighted by the numbers biting in each hourly period. The

Table 2. Estimated mean biting time of *Anopheles gambiae s.l.* indoors and outdoors in pairs of treated and untreated villages.* (Number of mosquitoes in parentheses.) Dates in July 1992.

Date	Village pair*	Treated		Untreated	
		Indoors	Outdoors	Indoors	Outdoors
09	1	3:05 (256)	2:53 (205)	2:54 (397)	2:58 (347)
10	2	3:23 (85)	3:28 (65)	1:47 (24)	1:47 (7)
15	3	3:15 (150)	3:12 (60)	2:38 (75)	1:50 (9)
17	4	4:31 (51)	4:14 (26)	3:25 (26)	4:40 (12)
21	5	2:53 (18)	2:19 (26)	3:11 (41)	3:00 (16)
22	6	2:30 (19)	3:30 (25)	2:27 (77)	3:10 (63)
24	7	3:21 (80)	3:16 (37)	3:25 (87)	2:59 (68)
28	8	2:49 (13)	3:10 (15)	2:37 (17)	12:20 (12)
29	9	2:42 (5)	3:15 (8)	4:19 (5)	4:06 (10)
Overall		3:16 (686)	3:08 (467)	2:53 (749)	2:58 (544)

* See footnote, Table 1.

Table 3. Analysis of variance of biting times of *An.gambiae s.l.* females in relation to presence or absence of treated bednets in the villages, whether caught indoors or outdoors, and village pair.

Source of variation	DF	Sum of squares	Mean square	*F*	*P*
Treatment	1	13.47	13.47	2.0	0.16
Place	1	0.05	0.05	0.01	0.93
Pair	8	173.22	21.65	0.19	0.001
Treatment × Place	1	1.26	1.26	0.19	0.66
Treatment × Pair	8	97.76	12.22	1.81	0.07
Place × Pair	8	38.97	4.87	0.72	0.67
Error	2410	16233.9	6.74		
Total	2437	16623.5			

tenth pair of villages was excluded from this calculation since only seven and five mosquitoes were collected respectively.

No significant difference was found in the analysis of variance between the mean biting times of mosquitoes caught in villages with treated bednets or villages with untreated bednets, or between those caught indoors or outdoors (Table 3). However, there were significant differences between the mean biting times in different pairs of villages.

Host choice

Slightly fewer ($P > 0.05$) *An.gambiae s.l.* females were found resting indoors in villages with treated than in those with untreated nets (Quiñones, 1996). The human blood index (HBI) of indoor-resting females was lower in villages with treated bednets than in villages with untreated bednets (Table 4), but this difference was not statistically significant (Mantel-Haenszel Chi square = 1.47, $P = 0.225$).

Table 4. Human blood index (HBI) of *Anopheles gambiae s.l.* collected resting indoors in pairs of treated and untreated villages.* (*n* = number tested.)

Date	Village pair*	Treated % HBI (*n*)	Untreated % HBI (*n*)
15/07/92	3	52.17 (23)	56.25 (48)
17/07/92	4	28.57 (7)	0 (1)
21/07/92	5	56.25 (16)	80.00 (20)
22/07/92	6	54.55 (11)	84.31 (51)
24/07/92	7	83.33 (36)	65.22 (46)
28/07/92	8	61.90 (21)	81.08 (37)
29/07/92	9	0 (1)	20.00 (5)
31/07/92	10	60.00 (10)	80.00 (10)
Overall		62.40 (125)	71.10 (218)

* See footnote, Table 1.

Exiting

No evidence for an effect of exposure to treated bednets was found in the mean numbers of *An.gambiae s.l.* females collected in exit traps (Wilcoxon sign rank test, $P = 0.426$), nor in the proportions by gonotrophic stage: unfeds $P = 0.426$; feds $P = 0.475$; gravids $P = 0.812$ (Table 5).

Gonotrophic cycle duration

A total of 39,199 fed *An.gambiae s.l.* females were collected from bednets in the various villages during the entire study. Of these, 11,489 were marked and released in a village with treated bednets, and 9059 were marked and released in a village with untreated bednets. PSC and WEX collections yielded 6549 and 2173 mosquitoes, respectively, in the two release villages when treated bednets were present, 28,353 and 2190 respectively when untreated bednets were present. Details are presented in Table 6.

The total number of recaptures was 148, of which 26 were collected the day following their release, as shown by their colour. These comprised 23 gravid, 1 fed and 2 unfed females. These *An.gambiae s.l.* had probably not completed their current gonotrophic cycle after being released as feds, so they were not

Table 5. *Anopheles gambiae s.l.* females collected (*n*) in exit traps in pairs of villages * with treated or untreated bednets. Percent by gonotrophic stage: unfeds (UF), blood-feds (FF) and gravids (GR) and geometric mean per trap (GM).

		Treated					Untreated				
Date	Village pair*	%UF	% FF	% GR	GM	(*n*)	% UF	%FF	%GR	GM	(*n*)
29/09/92	5	100	0	0	1.8	(61)	100	0	0	1.9	(75)
30/09/92	1	99.4	0	0.6	28.8	(165)	96.8	1.6	1.5	84.2	(856)
02/10/92	4	58.8	17.7	23.5	3.6	(13)	78.0	22.0	0	11.0	(50)
03/10/92	7	100	0	0	7.4	(24)	97.6	0	2.4	6.0	(42)
06/10/92	3	99.0	1.0	0	14.5	(102)	90.7	4.7	4.7	19.4	(43)
08/10/92	9	63.6	9.1	27.3	3.0	(6)	0	0	0	0	
10/10/92	10	100	0	0	0.4	(1)	25.0	50.0	25.0	1.3	(4)
13/10/92	9	93.6	4.7	1.8	22.4	(170)	100	0	0	11.8	(119)
15/10/92	7	96.7	3.3	0	3.2	(19)	91.7	8.3	0	0.9	(12)
16/10/92	4	84.0	10.0	5.0	4.5	(20)	89.7	8.6	1.7	12.4	(58)
Overall		95.4	2.7	2.0	6.5	(602)	95.8	2.8	1.4	5.8	(1259)

Table 6. Numbers of *Anopheles gambiae s.l.* females marked by colour (see text for colour, notation) released, collected by pyrethrum spray catches (PSC) and in window exit traps (WEX) and total number of recaptures per day (REC).

	Treated: Madina				Untreated: Jakoto				
Date	Colour	Released	PSC	WEX	Colour	Released	PSC	WEX	REC
05/10/92	BG	481			M	542			
06/10/92	R	574			G	737			
07/10/92	B	697	362	164	LB	562	160	99	1
08/10/92	O	612	563	98	Y	749	1563	82	3
09/10/92	P	838	571	109	DB	745	2461	108	6
10/10/92	T	770	439	195	DG	1027	2032	124	8
11/10/92			503	104			2890	72	8
12/10/92			793	80			2044	96	5
13/10/92			346	141			1021	126	5
14/10/92			445	51			1570	61	2
15/10/92			387	58			2379	41	1
	Treated: Jakoto				Untreated: Madina				
19/10/92	M	913			BG	641			
20/10/92	G	1077		13	R	538		134	0
21/10/92	LB	1248	320	2	B	835	1558	90	5
22/10/92	Y	1363	160	16	O	749	1801	83	13
23/10/92	DB	1372	443	20	P	1001	1649	223	9
24/10/92	DG	1544	250	147	T	933	1254	54	16
25/10/92			240	73			760	178	22
26/10/92			215	331			1644	82	23
27/10/92			281	312			1247	171	15
28/10/92			130	53			1251	231	4
29/10/92			101	206			1069	135	2
Totals	11489		6549	2173		9059	28353	2190	148

taken into account for estimating duration of the gonotrophic cycle. Of the remaining 122, a total of 93 females were recaptured either unfed or fed, and 29 were gravids. Table 7 shows the number of recaptures by day after release, by gonotrophic stage, and according to whether or not bednets in the village of recapture were treated.

Since the releases were all fed females, the fed recaptures provide the most direct information on the gonotrophic cycle duration. Recaptures of fed females showed a clear two day periodicity, at least in untreated villages. The majority of fed recaptures were found on day 2, and another peak occurred on day 4 after release. The situation is less clear for unfed females, perhaps because of the small numbers of recaptures, but the peaks of recapture of gravid females were on days 1 and 3 after release. Together, these results imply a 2-day gonotrophic cycle of *An.gambiae s.l.* in villages with untreated bednets.

Table 7. Recaptures of marked females of *An.gambiae s.l.* by day after release in villages during periods of use of treated or untreated bednets, according to their gonotrophic stage: unfed (UF), fed (FF) or gravid (GR).

	Recaptured in treated villages			Recaptured in untreated villages		
Days after release	UF	FF	GR	UF	FF	GR
1	0	1	6	2	0	17
2	0	5	0	2	36	3
3	1	2	1	1	6	15
4	1	1	0	1	16	4
5	1	0	1	2	5	4
6	0	0	0	0	5	1
7	0	1	0	0	4	0
8	0	1	0	0	1	0
9–11	0	0	0	0	0	0
12	0	0	0	0	1	0

Unfortunately, there were few recaptures when treated bednets were used, since PSCs yielded relatively less mosquitoes resting indoors in the presence of treated bednets. However, the highest number of recaptures of fed females in the villages in the presence of treated bednets was again on day 2 after release.

The mean day of recapture was calculated to be 3.47 for untreated villages and 3.27 for treated villages.

Species identification

In both studies the main species identified was *An.gambiae* Giles *sensu stricto*. From the ten pairs of villages, 96.1% (n = 154) of those identified chromosomally were *An.gambiae s.s.* savanna type of Coluzzi *et al.* (1985); the remaining 3.9% were *An.arabiensis* Patton. In the cross-over study, according to the palpal ratio, 85% (n = 321) of the *An.gambiae* complex females in the two study villages were freshwater *An.gambiae sensu lato* (either *An.gambiae s.s.* or *An.arabiensis*), whereas 15% were identified as *An.melas* Theobald, the saltwater sibling species.

For the recaptures, 93.5% of 109 were freshwater *An.gambiae s.l.*, a significantly higher proportion than among samples from the villages ($\chi^2 = 4.57$, $P = 0.032$). Therefore the results of the gonotrophic cycle length are relevant only to the freshwater sibling species, i.e. *An.arabiensis* and *An.gambiae s.s.*

Discussion

We found no evidence of changed biting behaviour of *An.gambiae s.l.* in terms of place, time and host choice or in duration of the gonotrophic cycle in villages with permethrin-treated bednets in The Gambia. As the collections were made soon after the impregnation of bednets, our measures would have detected any *immediate* phenotypic effect of the insecticide on the biting behaviour of the mosquitoes and not changes selected genetically.

Deterrence has been found in the presence of impregnated bednets in previous experimental hut studies (Darriet *et al.*, 1984; Lines *et al.*, 1987; Lindsay *et al.*, 1991; Miller *et al.*, 1991), but no evidence for this was found from human-landing collections at night in the present study. This contrasts with the deterrence observed in experimental hut studies, including those in The Gambia (Lindsay *et al.*, 1991; Miller *et al.*, 1991).

Indoor/outdoor biting ratios were similar in rooms with treated bednets to those in rooms with untreated bednets, which indicates that mosquitoes were entering rooms with treated bednets, and those without, at a similar rate. Similar results were obtained in Burkina Faso (Robert & Carnevale, 1991). A change in the ratio of biting outdoors:indoors of *An.farauti* from 1:1.17 to 1:0.47 was found in the Solomon Islands after house spraying with DDT (Taylor, 1975). The change in that case was considered not to be due to the temporary repellent effect of the insecticide, but due to selection in favour of an outside and early biting population.

The fact that in rooms with treated bednets there was a reduction in the density of indoor-resting but not of human-biting mosquitoes confirms the excito-repellent or lethal effects of permethrin (Darriet *et al.*, 1984; Lines *et al.*, 1987; Rozendaal, 1989). As the presence or absence of a treated bednet had no significant effect on the indoor/outdoor biting ratio, of *An.gambiae s.l.*, the reduction of density resting indoors during the following day must occur after mosquitoes have entered rooms with a treated bednets, probably after they have tried to bite through the impregnated net. This implies that any unprotected person sitting in a room with a treated bednet would receive the same number of bites as if they were in a room with an untreated bednet. This result contrasts with observations in experimental huts in Tanzania (Lines *et al.*, 1987), where it was shown that the presence of a permethrin-impregnated bednet reduced biting on an unprotected person in the same room, perhaps due to the relatively small size of rooms in experimental huts compared to the larger rooms of houses in villages.

Increased rates of mosquitoes exiting from rooms with a treated bednet have been described in several studies using experimental huts (Darriet *et al.*, 1984; Lines *et al.*, 1987) and in field evaluations (Snow *et al.*, 1987b; Charlwood & Dagoro, 1987; Lindsay *et al.*, 1989). If mosquitoes enter rooms with treated bednets at equivalent rates to those with untreated bednets, as shown by our human-landing collections – yet fewer were found resting in treated rooms in the morning (Lindsay *et al.*, 1993; Thomson *et al.*, 1995a; Quiñones, 1996), then one would expect to find higher densities in the exit trap collections from rooms with treated bednets than rooms with untreated bednets. However, no significant differences were found in the density of exiting mosquitoes or in the proportions of unfed, fed or gravid mosquitoes exiting from rooms with treated or untreated bednets. The majority of mosquitoes exiting were unfed females. Perhaps mosquitoes left rooms with treated bednets via the open eaves or other apertures or were killed by the insecticide, so were not collected in the exit traps.

The biting pattern and mean biting time of *An.gambiae s.l.* in the ten pairs of villages were similar to those described previously in West Africa (Gillies & De Meillon, 1968; Lindsay *et al.*, 1989, 1995). Differences between villages with treated and untreated bednets were not significant. Similarly, in the Congo deltamethrin-impregnated bednets had no effect on the biting cycle (Zoulani *et al.*, 1994).

With respect to the human blood index, we found no significant difference between villages with treated or untreated bednets; this agrees with previous experiences in The Gambia by Lindsay *et al.* (1991, 1993) and Thomson *et al.* (1995a). The possibility remains that mosquitoes were diverted to bite animals and rest outdoors due to the excito-repellent effect of the insecticide, but we did not investigate this. In Tanzania, no differences were found in the bloodmeal sources of *An.gambiae s.l.* females collected resting outdoors in pit traps before and after introduction of treated bednets (Magesa *et al.*, 1991); the majority were human fed.

Higher frequencies of recaptures on days 2 and 4 in untreated villages indicate that the gonotrophic cycle of *An.gambiae* in The Gambia is 2 days. A similar cycle duration has been reported for other countries in Africa (e.g. Muirhead Thomson, 1947), although a 3-day period between successive feeds has been reported equally frequently (Gillies & Coetzee, 1987). No evidence of any change in duration of the gonotrophic cycle in the presence of treated bednets was found in this study, although the rate of recaptures was lower when the villages had treated bednets.

Several studies on permethrin-treated bednets in The Gambia have demonstrated that the densities and sporozoite rates of *An.gambiae s.l.* are similar in villages with treated and untreated bednets, indicating that survival rates are unaffected (Lindsay *et al.*, 1991, 1993; Thomson *et al.*, 1995a; Quinones, 1996). Nevertheless, there is ample evidence that children in villages with permethrin-treated bednets are protected against malaria (e.g. Alonso *et al.*, 1993; D'Alessandro *et al.*, 1995). If there are equal numbers of infective mosquitoes in villages with treated and untreated bednets, and these are biting humans in a similar proportion, how then are children protected? One possibility is that mosquitoes are diverted to bite other hosts including adults instead of children, adults being more exposed on account of their sleeping habits.

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