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The effect of permethrin-impregnated bednets on a population of *Anopheles farauti* in coastal Papua New Guinea

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ABSTRACT. The effect of introducing bednets impregnated with 0.4 g/m^2 permethrin on local populations of the malaria vector mosquitoes *Anopheles farauti* Laveran and *An.koliensis* Owen was monitored in a coastal village of Papua New Guinea. Whole-night landing collections were undertaken for 25 consecutive nights before and 21 nights after the introduction of the nets. Capture–recapture experiments and resting collections were also performed before and after the introduction of the nets.

Following the introduction of treated nets, the biting population of *An.farauti*, the predominant vector, dropped from an average of 689 to 483 per man-night and the oviposition cycle became irregular, although survival rates (determined by time series analysis of the landing catches and log regression of recapture rates) were not significantly affected. The densities of *An.farauti* resting in and around houses and the human blood index of the engorged females also decreased significantly after introduction of the treated bednets.

The population of *An.koliensis* dropped prior to the introduction of the nets. However, the number of nulliparous females in the landing catches remained more or less constant which implies that, in this species, survival rates were affected by the nets but that recruitment to the population was not.

Key words. *Anopheles farauti, Anopheles koliensis,* permethrin, bednets, Papua New Guinea, mosquito control, parous rate.

Introduction

Reducing man–mosquito contact by the use of bednets (mosquito-nets) may lower the incidence of mosquito-borne diseases. In The Gambia, for example, children using bednets regularly had lower spleen rates than non-users which probably indicates that they gained some protection against malaria (Bradley *et al.*, 1986). In Papua New Guinea (PNG), many people in the Madang area use bednets but, despite reducing biting rates (Charlwood, 1986), their efficacy in reducing malaria is equivocal (Cattani *et al.*, 1986).

In many areas people use torn bednets which, although better than nothing, are less effective than undamaged ones (Port & Boreham, 1982). Apart from sewing-up the holes, one way of

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increasing the effectiveness of a bednet, even if it is damaged, is to impregnate it with an insecticide or repellent (Curtis & Lines, 1985; Schreck & Self, 1985; Lines *et al.*, 1987). Particular attention is being given to permethrin and other synthetic pyrethroids for this purpose. Although intended primarily for personal protection, if a sufficiently large number of people use such impregnated nets they may control vectors on a community basis. Despite a growing interest in such measures, however, little is known about their effect on mosquito population dynamics.

In PNG, malaria is transmitted by three species of the *Anopheles punctulatus* Dönitz complex. Behavioural differences between the three species, *Anopheles punctulatus, An. koliensis* Owen and *An.farauti* No. 1 (*sensu* Bryan, 1973), may influence the effectiveness of bednets and hence their role in reducing disease transmission. For example, *An.punctulatus* and *An.koliensis* bite later at night, spend longer in houses and are more anthropophilic than *An.farauti* No. 1, the vector on the coast (Charlwood *et al.*, 1986a). Thus, nets may be less effective against *An.farauti* No. 1 (hereafter called *An.farauti* Laveron) than the other two species. An experiment was therefore conducted in a coastal village of PNG in which the effects of permethrin-impregnated bednets on survival rates, oviposition cycle lengths and feeding behaviour of *An.farauti* and *An.koliensis* were determined.

Methods

The experiment took place on the northern coast of PNG, 7 km south of the town of Madang, 22 July to 5 September 1985, in an isolated hamlet of the village of Umuin previously described by Charlwood *et al.* (1986b). The inhabitants are relatively new arrivals to the area and there is a considerable traffic between the hamlet, called Agan by the villagers, and their original home 60 km inland. Bednet usage was determined by house-to-house surveys both before and after the introduction of the treated nets. The purpose of the experiment was explained to the villagers, all of whom were provided with permethrin-impregnated bednets 3 weeks after the start of mosquito sampling. The number of new nets provided was determined by the villagers who were given the option of buying or returning the nets at the end of the experiment.

All new, and some old, nets were impregnated with permethrin. Sufficient dilute insecticide was applied to the nets so that they were soaked through but did not drip. Before being hung up to dry they were laid on plastic sheets, with their sides folded under the top, so that, in the event of any concentration anomalies, the distribution of the insecticide would even itself out. Permethrin treatment rates on net samples were analysed by the Wellcome Laboratories at Berkhamsted.

Mosquito populations were monitored by all-night (18.00–06.00 hours) landing/biting collections outdoors. In previous experiments the indoor and outdoor biting populations of *An.farauti* and *An.koliensis* in Agan were found to be homogeneous (Charlwood *et al.*, 1986b). Since recruitment to a population may change, either naturally or because of an intervention, biting density or landing rate should be measured on a continuous basis (Birley & Rajagopalan, 1981; Charlwood *et al.*, 1985; Holmes & Birley, 1987). Collections were therefore made for 46 consecutive nights: 25 before and 21 after the introduction of impregnated nets into the village.

Twelve experienced mosquito collectors, organized into three teams, were recruited from the village. Each team worked in pairs for 7 consecutive nights, each pair working alternate 6 h shifts. In order to maintain enthusiasm and alertness, refreshment was routinely provided and an IMR staff member, who organized the collections, stayed in the village.

After identification, *An.farauti* and *An.koliensis* from the landing catches were released into separate cages and a random selection from each dissected for parity determination. At least fifty *An.farauti* and up to forty *An.koliensis* were dissected each day, depending on the numbers collected. On weekday mornings the two teams not involved in the landing catches sought indoor and outdoor resting mosquitoes. Each collector searched one house and its immediate surrounds. The host source of engorged specimens was determined by ELISA (Burkot *et al.*, 1981).

Two capture–recapture experiments were conducted, similar to those described in Charlwood *et al.* (1986b), one before and the other after the treated nets were introduced into the village. In the first experiment, all-night human bait collections were augmented by an additional eleven collectors, in outdoor locations throughout the village between 22.00 and 02.00 hours. In the second experiment only the all-night collection was used for the initial release and subsequent recaptures.

Results

Bednet usage

There were sixty-four people living in twelve houses at the start of the experiment. During the experiment one house fell down but three more were built. A baby was born but one person died.

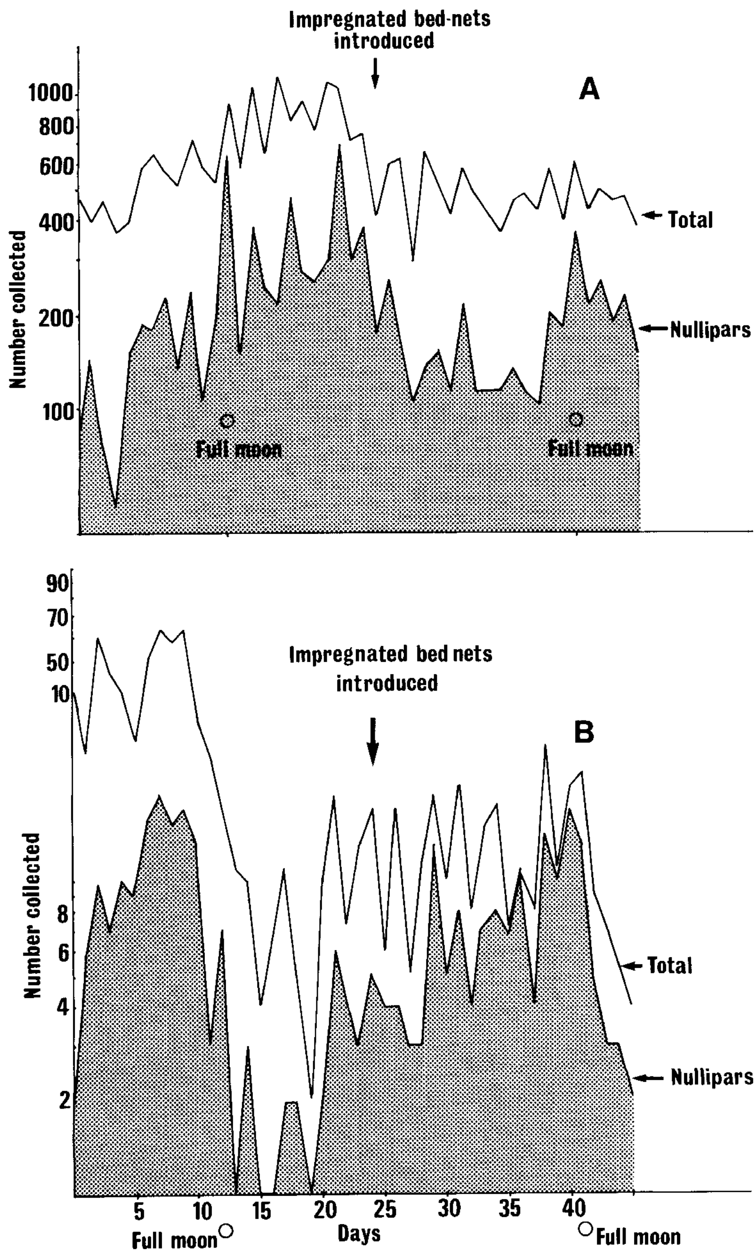

FIG. 1. Numbers of *Anopheles farauti* (A) and *An. koliensis* (B) collected in all-night landing collections in Agan village. The arrow indicates the date when the impregnated bednets were installed.

On 19 July 1985 seven people slept without bednets; nineteen people slept under their own, single-size net; two people shared a single-size net and the remaining thirty-six people slept under twelve double-size nets. Some nets were torn. On 15 August 1985, thirteen single and twelve double nets were brought to the village when they and some of the old nets were treated with permethrin. A second bednet survey a week later indicated that all but twelve people (who were sleeping under their old, untreated nets and keeping the new ones 'for best') were sleeping under treated nets. After further explanation, all of the villagers used the nets. The new permethrin-impregnated nets were all enthusiastically purchased by the villagers in the subsequent 2 weeks. Analysis of small samples indicated that the dosage of the nets was 0.4 ± 0.2 g/m^2 of permethrin.

Landing catches

A total of 27,372 *An.farauti* and 928 *An.koliensis* were caught in the 46 nights of collection, from which 2602 *An.farauti* and 656 *An.koliensis* were dissected. Numbers of each

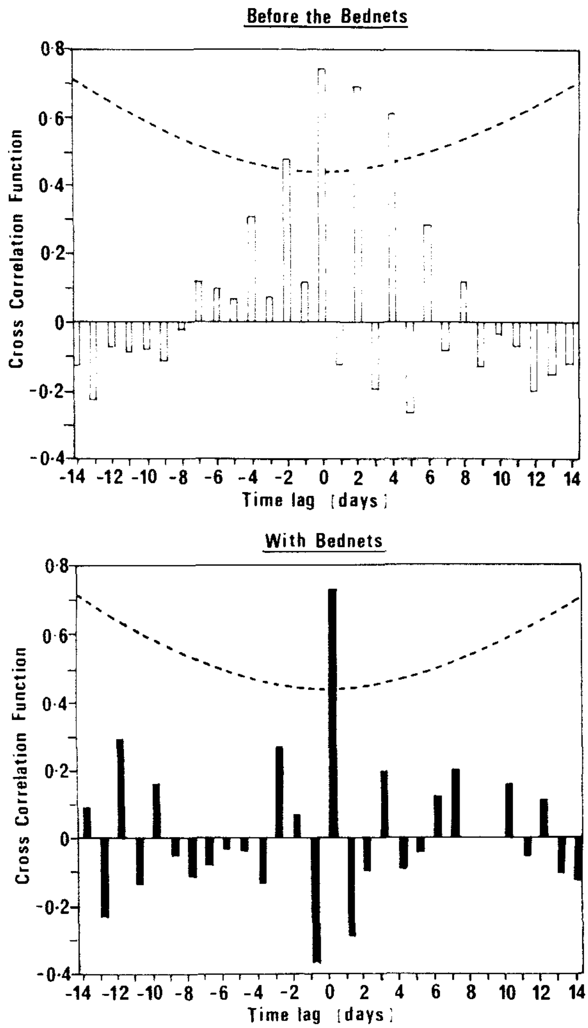

FIG. 2. Filtered cross-correlations of the total and parous *An.farauti* collected in landing catches before and after the introduction of the impregnated bednets into Agan village. The dotted line indicates the 95% significance levels.

species collected each night are shown in Fig. 1. The shaded portion of the graphs represents the estimated numbers nulliparous. The number of *An.farauti* collected was rising before the introduction of the nets but declined after their introduction.

The number of *An.farauti* collected per night dropped from an average (mean±SD) of 689±241 to 483±99 after the introduction of the treated nets. The biting population of *An.koliensis* also declined from an average of 26.7±20.7 before to 12.4±6.6 after the introduction of the treated nets. The drop in *An.koliensis* biting, however, occurred before the introduction of the nets, which means that an accurate evaluation of their effect on this species cannot be made. Nevertheless, the number of nulliparous *An.koliensis* collected remained more or less constant during each part of the experiment with the result that the parous rate dropped from 0.75 to 0.44 after their introduction.

The number of *An.farauti* in landing catches can vary significantly according to moonphase (Charlwood *et al.*, 1986c) and in the present experiment the largest samples were obtained on the nights following full moon, shown as the open circles in Fig. 1. After the introduction of the impregnated nets, however, there was no rise in numbers during full moon.

Survival rate estimation

Estimates of survival rates of *An.farauti* were attempted by time series analysis as described by Charlwood *et al.* (1985). One of the constraints of the method is that the insects should have a regular oviposition cycle. Under such circumstances, significant cross-correlations between the total collected on any one day and the number of parous mosquitoes collected one or more cycle lengths later should occur (Holmes & Birley, 1987). Filtered cross-correlations for the period before and after the introduction of the nets are shown in Fig. 2. Significant cross-correlations at 2-day intervals were observed in the data before the introduction of the treated nets. This corresponds with the 2-day oviposition cycle determined by capture–recapture experiment for *An.farauti* from this village (Charlwood *et al.*, 1986b). After the introduction of the nets the cross-correlation peaks were no longer significant, although small peaks occurred at 3-day intervals (Fig. 2b). Therefore the oviposition cycle became irregular. Because of this, an assessment of survival using time-series could only be made reliably for the first part of the experiment and the only measure of survival available once the nets had been introduced was the parous rate. However, as pointed out by Holmes & Birley (1987), when large numbers of mosquitoes are dissected, and recruitment to the population is not changing dramatically, parous rates provide a reasonable estimate of survival. Daily survival rates before and after the introduction of the treated nets were similar: 0.675±0.04 and 0.63 (Table 1).

TABLE 1. Estimated survival rates of *An.farauti* before and after the introduction of impregnated bednets into Agan village; standard errors are given in parentheses.

	Time-series method	Parous rate method	Recapture method
Before	0.675 (0.04)	–	0.70 (0.2)
After	–	0.63	0.65 (0.1)

Capture–recapture experiments

1435 blood-fed mosquitoes were marked with fluorescent dust (Industrial Colors, Series 810) and released on 31 July 1985. It was estimated that 1306 of these were *An.farauti* and 129 were *An.koliensis*. 140 (10.7%) *An.farauti* and nine (7%) *An.koliensis* were recaptured. The mean oviposition interval of the *An.farauti*, derived using the formulae of Charlwood *et al.* (1986b) was 2.3±0.4 days and daily survival, determined by regression, was 0.70±0.2 (Table 1). Too few *An.koliensis* were recaptured for estimates of oviposition cycle length or survival to be obtained.

For the second capture–recapture experiment, 709 marked *An.farauti* were released on 26 August 1985 and forty-one (5.8%) were later recaptured. The mean oviposition interval of the mosquitoes returning for their first blood-meal after release was estimated to be 2.5±0.5 days and daily survival was 0.65±0.1. Table 1 gives the estimated survival rates derived from all the methods used.

A simple estimate of total population size, which ignores recruitment (i.e. the nulliparous fraction of the population), can be determined

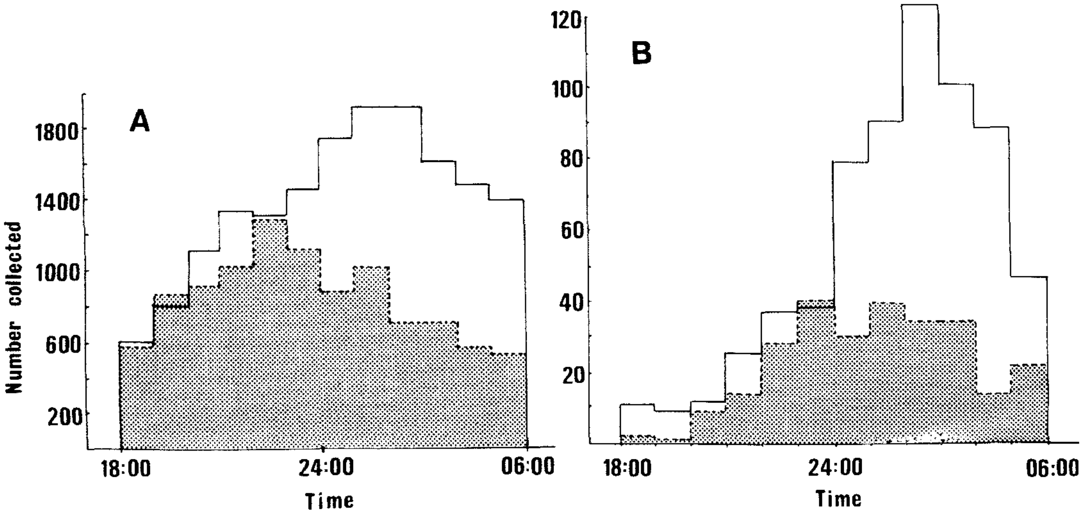

FIG. 3. Biting cycles of *An.farauti* (A) and *An.koliensis* (B) before (clear) and after (hatched) the introduction of the impregnated bednets into Agan village.

TABLE 2. The mean number of *An.farauti* per collector in resting catches, according to gonotrophic condition, before and after the introduction of impregnated bednets into Agan village.

	Unfed	Bloodfed	Semi-gravid	Gravid	Total collected
Before	0.63	5.62	0.00	0.19	807
After	0.66	0.64	0.01	0.07	127

TABLE 3. Host source of engorged *Anopheles farauti* and *An.koliensis* from resting collections before and after the introduction of impregnated bednets into Agan village.

	Host			
	Man	Pig	Dog	Mixed (man/other)
An.farauti				
Before	235	3	95	13
After	63	6	98	6
An.koliensis				
Before	29		6	
After	2			

using the formula:

$$\text{Estimated total} = \frac{\text{Total marked } \Sigma \text{ parous}}{\Sigma \text{ recaptured}}$$

The population 2 days after release was considered to provide the best estimate of total population size because, from the third day after release, the parous population would have contained post-release primiparous recruits. Before the introduction of the treated nets, the total biting population was estimated to be 22,451±4073 (mean±SD). This later dropped to 13,330±3578 after their introduction. The collectors caught an estimated 2.6% of the total population before, and 3.2% after, the introduction of the treated nets.

Biting cycles

The biting cycles of both *An.farauti* and *An.koliensis*, derived from the landing catches, changed during the course of the experiment (Fig. 3). In both species there was a shift away from a post-midnight peak towards a pre-midnight peak of biting activity.

Resting collections

The average numbers of *An.farauti* collected resting in and around houses dropped from 6.45 to 1.41 and *An.koliensis* fell from 1.4 to 0.2 per collector after the introduction of the treated nets. The ratio of engorged to unfed *An.farauti* in resting catches dropped from 9:1 to 1:1 (Table 2) and significantly fewer had fed on man (χ^2=49.8, 1 df, P<0.001) (Table 3). Too few engorged *An.koliensis* were collected for an assessment of the effect of the impregnated nets on the feeding behaviour of this species to be made.

Discussion

The effect of introducing permethrin-impregnated bednets into the village of Agan on the local *An.farauti* and *An.koliensis* populations was complex and extended beyond simply killing the mosquitoes. After the introduction of the nets there was a drop in the number of *An.farauti* collected in landing catches, an increase in the length of the oviposition cycle, a shift towards earlier biting, a drop in the human blood index and possibly a shift to outdoor feeding and resting. Thus, the insecticide apparently had an irritant/repellant effect similar to that observed in Africa against *An.funestus* Giles and *An.gambiae s.l.* (Darriet *et al.*, 1984).

The importance of continuously monitoring populations was borne out by the results obtained with *An.koliensis*. There was a decline in the biting population of this species; however, this cannot be attributed to the bednets since it occurred before they were impregnated. It is possible that recaptures performed during the mark–recapture experiment depleted the population. Nevertheless, numbers of nulliparous *An.koliensis* collected were more or less constant throughout the experiment, which implies that, although adult survival-rates were reduced, recruitment was unaffected by the introduction of the impregnated nets.

Time series cross-correlation analysis indicated that the regularity and duration of the oviposition cycle of the *An.farauti* females was disturbed after the introduction of the treated nets. The length of the cycle was similar in engorged specimens released before and after the introduction of the nets. This indicates that the disruption to the cycle occurred before feeding.

Because of the loss in regularity of the oviposition cycle, different methods of survival analysis had to be applied to the two halves of the data. Taken in conjunction with analysis of the capture–recapture experiments, the results indicated that survival rates of *An.farauti* were not unduly influenced by the introduction of the nets. Nevertheless, the approximately 40% reduction of population density following introduction of the impregnated nets implies that they were killing the mosquitoes. In contrast to *An.koliensis*, the nulliparous fraction of the *An.farauti* population did not increase after the introduction of the nets. It is possible that nulliparous *An.farauti* are more persistent than parous individuals attempting to feed in the presence of a protected host, which would therefore make nullipars more susceptible to treated nets.

Because of a possible repellancy effect, fewer mosquitoes would have been likely to rest indoors after the introduction of the treated nets. It is assumed that indoor/outdoor sampling effort was the same throughout the experiment, although this may not have been the case. An increased outdoor-resting sample would tend to depress the human blood index because exophagic mosquitoes are more likely to be zoophagic. The highly significant differences observed (Table 3), allied to the increased proportions of unfed mosquitoes in samples from resting collections, implies that the main reason for the drop in the human blood index was due to the introduction of the impregnated nets.

In Agan there is no segregation of malaria vectors into 'indoor' and 'outdoor' biting populations and the numbers in landing collections in both situations are approximately equal (Charlwood *et al.*, 1986b). Therefore, most female *An.farauti* would be expected to be exposed to an impregnated net within two oviposition cycles. Although some insects would be killed, most would be diverted outside – possibly to feed on alternate hosts. By disrupting feeding behaviour, the impregnated nets would increase the length of the oviposition cycle of the overall population. All diverted females would have less time to feed and unsuccessful ones would have to return to feed the following night. Mosquitoes that suffer a delay after oviposition, from whatever cause, tend to bite in the early evening (J. D. Charlwood, P. M. Graves and T. Marshall, unpubl.). Thus, the observed shift to evening biting would be associated with an increase in the length of the oviposition cycle. In the Solomon Islands, after several rounds of DDT house-spraying, the biting of *An.farauti* (also species No. 1) occurred almost exclusively outdoors in the early evening (Taylor, 1975). Whether the observed changes in Agan herald such pronounced shifts as have happened in the Solomon Islands remains to be seen.

It is not known if the changes in mosquito behaviour and mortality due to the introduction of permethrin-impregnated bednets were sufficient to affect disease transmission in Agan. Paradoxically, in the absence of negative factors such as a lower survival rate or a shift to feeding on alternative hosts, an increase in the length of the oviposition cycle and a shift to early evening biting might increase the potential of the mosquito to transmit disease. However, in a parallel study, the incidence of *Plasmodium falciparum* Welch in 0–4-year-olds in four villages with impregnated nets was lower than in four control villages, while sporozoite rates in the vectors were reduced in two of the experimental villages (Graves *et al.*, 1987).

Permethrin-impregnated bednets also helped to control bed-bugs and, the villagers claimed, head lice (Charlwood & Dagaro, 1987a). The enthusiasm with which impregnated nets were adopted by the people involved in these studies indicates that the method would probably be accepted elsewhere in PNG. Impregnated bednets may well prove to be more effective against *An.punctulatus*, because of its more endophilic nature, than *An.farauti* (Charlwood & Dagaro, 1987b). In some inland areas, where *An.punctulatus* is the primary vector of both malaria and filariasis, because they are poorer, fewer people use nets than on the coast. The availability of cheap impregnated bednets may be of real benefit to people in those areas.

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