

CHANGES IN MALARIA VECTOR DENSITIES OVER A TWENTY-THREE YEAR PERIOD IN MAE HONG SON PROVINCE, NORTHERN THAILAND

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Abstract. Mae Hong Son Province in northwestern Thailand has a long history of malaria. During the last two decades the province has had one of the highest malaria incidences of all provinces in Thailand. Data were analyzed to determine whether the vector populations were stable or increasing during the last two decades and to determine the seasonal prevalence of the main vectors, and whether or not they were related to the malaria transmission peak, in the wet season. We compiled and analyzed accumulated entomological records from 1977 to 1999. The aim was to investigate long-term changes in mean densities of malaria vectors between two periods (1977-1989 and 1990-1999), and the differences in vector densities between two seasons (wet and dry). A total of 141,144 adult anophelines of 29 species were collected on indoor and outdoor human baits and animal baits during the study period. Of the main malaria vectors, the densities of *Anopheles minimus* s.l. and *Anopheles maculatus* complex increased significantly. *Anopheles dirus* s.l., however, was stable between the two periods. These vector populations were associated with consistently high malaria incidence in the province during the last two decades. *An. minimus* s.l. density was not significantly different between seasons. However, in the second period, both *An. dirus* s.l. and *An. maculatus* complex showed a tendency for higher wet season densities. This can explain the high malaria incidence in the rainy season in Mae Hong Son. Environmental and climatic factors seem to have been favorable for supporting a consistently high vector population in the province, and consequently a high malaria transmission rate during the period of study.

INTRODUCTION

Long-term studies on mosquito abundance are not common, partly because regular mosquito control and surveillance activities in many countries are quite recent innovations. If standard sampling protocols have not been used, the data may be difficult to interpret and analyze. In Thailand, however, such long-term mosquito data do exist.

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Entomological surveillance was first described and established as a regular part of malaria control programs in the early 1950s. It became more organized in the 1970s and has continued to the present time. This entomological surveillance followed standard collection protocols, reporting the number of adult mosquitoes collected on indoor and outdoor human baits, as well as on animal bait when cows or buffalos were available (Bhatia and Notananda, 1953). All collected anopheline mosquitoes were counted and morphologically identified to species.

In several studies, the effects of climatic, environmental and socioeconomic factors have been used to explain long-term changes in numbers of malaria cases (Bouma *et al*, 1996; Van

der Hoek *et al.*, 1997; Hu *et al.*, 1998; Mouchet *et al.*, 1998). However, none of these studies used detailed data on vector populations as a causal factor, although the probability of malaria transmission largely depends on the vectorial capacity of the mosquito population (Onori and Grab, 1980). Information from long-term entomological studies is valuable for analyzing the effect of temporal changes in vector densities on malaria incidence. Our intention was to describe changes in anopheline mosquito densities over 23 years in Mae Hong Son, a malaria hyper-endemic province in northwest Thailand.

The malaria history in Mae Hong Son Province shows that transmission occurs throughout the year with a major peak during the early part of the wet season (May to August) and a smaller one in the dry season (November to January), and this general pattern did not change between the last two decades (OVBD2, 2000). This transmission coincides with intense agricultural activity after the first monsoon, when farmers stay in farm huts which rice cultivating and are exposed to vectors (Somboon *et al.*, 1998). Furthermore, transmission is closely related to forest locations where there is an abundance of malaria vector breeding sites (Ketrangsee *et al.*, 1991), various occupational factors encourage population movement or the influx of refugees (Singhanetra-Renard, 1986; Butraporn *et al.*, 1995; Stern, 1998), socioeconomic factors affecting malaria (Fungladda *et al.*, 1987), and a high degree of drug resistance (Wernsdorfer *et al.*, 1994).

Mosquito collection data from northern Thailand have accumulated at the Office of Vector-Borne Disease Control No.2, Chiang Mai, without any formal analysis. We constructed a database of records from 1977 to 1999, with the objective of retrospectively studying temporal and seasonal variations in anopheline densities in Mae Hong Son Province. We investigated whether stable or increasing densities of the main malaria vectors during the study period were associated with the consistent high malaria incidence in the province. Malaria transmission in northern Thailand usually takes place during the wet season (Ismail *et al.*, 1974). Therefore, we hypothesized that malaria vector density would be higher in this season than in the dry season.

MATERIALS AND METHODS

Study area

Mae Hong Son Province is located on the Thai-Myanmar border in northwestern Thailand (Fig 1). The province is geographically homogenous with 90% of the area covered with mountains and about 70% with mainly mixed deciduous and dry dipterocarp forests. The rapid socioeconomic changes in Thailand of recent decades has mainly taken place in larger population centers and focal areas in the central valley of Mae Hong Son Province. Many villages are remotely situated, with no public transport. Approximately 40% of the province has poor accessibility, and during the rainy season accessibility is reduced even more. In a large part of the province, there is limited land for paddy fields and the average size of farms is small. The province is administratively divided into 7 districts (amphoe), 45 cantons (tambon), and 395 villages. The population consists of Thai nationals and many hill tribes of various ethnic groups. Many refugee camps are situated along the border, housing a large number of displaced people from conflict areas within Myanmar. The population in 1977 was 123,816 and in 1999 it had increased by 58% to 195,209 (Office of Vector Borne Disease Control No.2, 1978-2000).

Available records and data analysis

Accumulated mosquito collection data at the Office of Vector Borne Disease Control No. 2 in Chiang Mai (OVBD2), from 1977 to 1999, were compiled and analyzed. Data from before 1977 were few and incomplete and were therefore not used. Each record included the name of the village where mosquitos had been collected, the number of adult anopheline species collected by indoor human bait, outdoor human bait and animal bait, number of collection nights, number of collectors, village population, and number of recorded malaria cases. In human bait collections, two persons collected mosquitos indoors and outdoors, respectively. Half-night collections were undertaken, *ie* from sunset to midnight. Simultaneous animal bait collections were conducted using one cow or buffalo, if available. Each record was standardized by calculating the density of mosquitos, given as the number of mosquitos per bait per half-night.

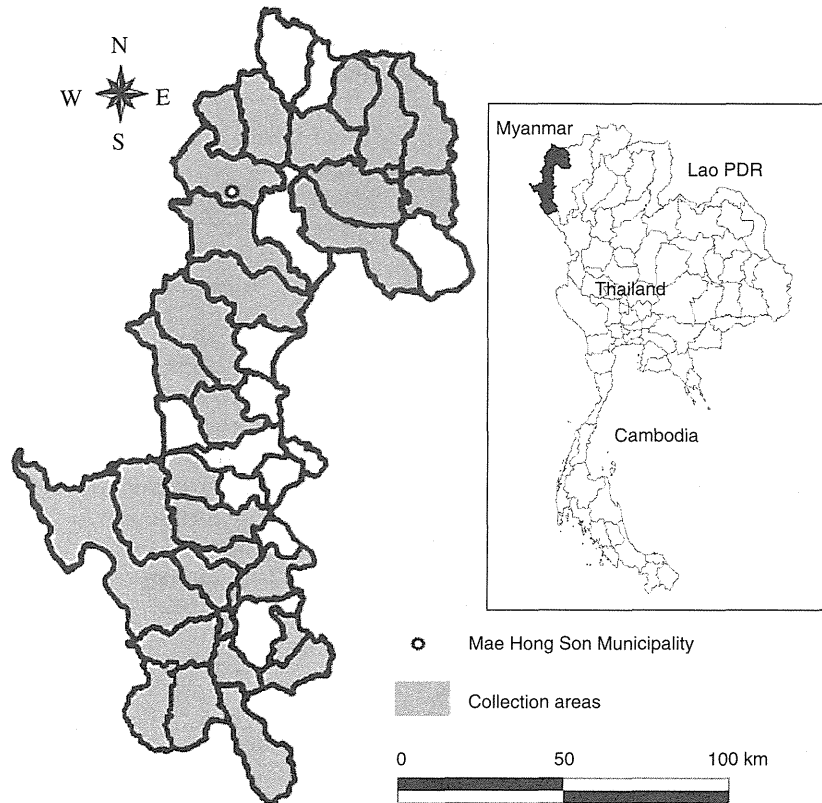


Fig 1—Location of Mae Hong Son Province and cantons (in gray) where entomological data were collected from 1977-1999.

Mosquito collections were undertaken in 97 villages (about 25% of all villages in the province) in 32 cantons (71% of all cantons) (Fig 1). We separated all records into two time periods, the first from 1977 to 1989 and the second from 1990 to 1999, and into two seasons, a wet season (May-September) and a dry season (October-April). Because of relatively few data from the early 1980s, we added available data from the end of the 1970s to the first period, so that replications were as much as possible comparable for each period. It was difficult to find long-term continuous records from single villages during the whole period. For this reason, in our analysis, villages were pooled into the next larger administrative unit, the canton. Thus, a total of 321 records of indoor human bait collections, 501 records of outdoor human bait collections, and 271 records of animal bait collections were available.

Mosquitos were morphologically identified to species using the keys of Peyton and Scanlon (1966); Sawadwongporn (1972); Rattanaarithikul and Harrison (1973); Harrison and Scanlon (1975); Peyton and Harrison (1979); Harrison (1980); Rattanaarithikul and Green (1986); Rattanaarithikul and Panthusiri (1994). Recently, *An. minimus* and *An. dirus* were genetically found to be species complexes of at least three and five sibling species, respectively (Baimai, 1988; Baimai *et al*, 1988; Sucharit *et al*, 1988; Green *et al*, 1990; 1992; Sharpe *et al*, 1999, Walton *et al*, 1999). However, in this study these two species were morphologically identified as *An. minimus* s.l. and *An. dirus* s.l., respectively. Before 1989, all species belonging to the *An. maculatus* complex were collectively identified as *An. maculatus*. Since 1990, individual members of the complex have been morphologically identified as *An.*

Table 1
List of anopheline species collected in Mae Hong Son from 1977 to 1999, their mean densities (no. of mosquitos/bait/half-night) and \pm SE.

Species	Mean densities \pm SE		
	Human bait		Animal bait
	Indoor	Outdoor	
<i>An. dirus</i> s.l.	0.08 \pm 0.02	0.15 \pm 0.05	0.32 \pm 0.01
<i>An. minimus</i> s.l.	2.11 \pm 0.29	4.1 \pm 0.27	6.9 \pm 0.75
<i>An. maculatus</i> complex	0.55 \pm 0.09	3.2 \pm 0.18	12.3 \pm 1.27
<i>An. aconitus</i>	0.19 \pm 0.06	1.2 \pm 0.18	8.4 \pm 2.58
<i>An. philippinensis</i>	0.1 \pm 0.05	0.5 \pm 0.15	0.8 \pm 0.33
<i>An. barbirostris</i>	0.1 \pm 0.03	0.3 \pm 0.06	9.3 \pm 2.87
<i>An. annularis</i>	0.5 \pm 0.14	2.6 \pm 0.67	11.8 \pm 2.32
<i>An. barbumbrosus</i>	<0.01	<0.01	<0.01
<i>An. campestris</i>	<0.01	0.01 \pm 0.005	<0.01
<i>An. culicifacies</i>	0.01 \pm 0.02	0.01 \pm 0.003	1.3 \pm 0.81
<i>An. hyrcanus</i> group	0.21 \pm 0.05	1.0 \pm 0.15	9.6 \pm 1.66
<i>An. jamesii</i>	<0.01	0.01 \pm 0.003	<0.01
<i>An. jeyporiensis</i>	<0.01	0.06 \pm 0.003	<0.1 \pm 0.08
<i>An. karwari</i>	<0.01	<0.01	<0.01
<i>An. kochi</i>	0.1 \pm 0.03	0.2 \pm 0.03	9.8 \pm 1.4
<i>An. nivipes</i>	<0.1	0.2 \pm 0.05	2.2 \pm 0.6
<i>An. pseudojamesi</i>	<0.01	<0.01	0.02 \pm 0.02
<i>An. splendidus</i>	0.1 \pm 0.03	0.3 \pm 0.06	1.0 \pm 0.47
<i>An. tessellatus</i>	<0.1 \pm 0.004	0.1 \pm 0.05	0.4 \pm 0.07
<i>An. vagus</i>	<0.01	0.1 \pm 0.01	15.4 \pm 3.8
<i>An. varuna</i>	<0.01	0.1 \pm 0.02	<0.01
<i>An. umbrosus</i>	<0.1 \pm	<0.1 \pm	<0.1 \pm
Species of <i>An. maculatus</i> complex			
<i>An. dravidicus</i>	<0.01	0.1 \pm 0.02	0.3 \pm 0.06
<i>An. maculatus</i> s.s.	0.1 \pm 0.02	1.2 \pm 0.19	6.75 \pm 1.13
<i>An. notanandai</i>	<0.01	<0.01	<0.01
<i>An. pseudowillmori</i>	0.05 \pm 0.2	0.14 \pm 0.04	0.2 \pm 0.06
<i>An. sawadwongporni</i>	0.2 \pm 0.04	0.7 \pm 0.14	2.5 \pm 0.47
<i>An. willmori</i>	0.12 \pm 0.02	0.4 \pm 0.05	2.01 \pm 0.31
Species of <i>An. hyrcanus</i> group			
<i>An. nigerrimus</i>	<0.01	<0.01	<0.01
<i>An. peditaeniatus</i>	<0.01	<0.02 \pm 0.01	0.61 \pm 0.19
<i>An. sinensis</i>	0	<0.01	<0.16 \pm 0.12

maculatus s.s., *An. dravidicus*, *An. notanandai*, *An. pseudowillmori*, *An. sawadwongporni*, and *An. willmori* (Rattanaarithikul and Green, 1986).

The three major malaria vectors, *An. dirus* s.l. (Scanlon and Sandhinand, 1965; Peyton and Harrison, 1979), *An. minimus* s.l. (Ayurakitkosol and Griffith, 1963), and the *An. maculatus* complex (Reid, 1968; Upatham *et al*, 1988; Rattanaarithikul and Green, 1986) were subjected

to statistical analysis. To determine differences in mean mosquito density, two-way analysis of variance was conducted. The variation of adult density found in indoor, outdoor, and animal bait collections was analyzed separately for each period (1st period: 1977-1989 or 2nd period: 1990-1999) and season (dry or wet). All statistical analyses were performed using SYSTAT statistical software (Wilkinson, 1996).

Table 2

Comparison of mean densities (number of mosquitos/bait/half-night) of three main malaria vectors in Mae Hong Son, between two periods (1977-1989 and 1990-1999) and between two seasons (dry and wet); pairs of means in boxes and in boxes with highlight are significantly different ($p < 0.05$).

<i>Anopheles</i> species	Seasons	Human bait indoor		Human bait outdoor		Animal bait	
		1 st period	2 nd period	1 st period	2 nd period	1 st period	2 nd period
<i>An. minimus</i> s.l.	Dry	0.825	2.002	4.044	4.502	3.143	7.097
	Wet	0.845	2.766	2.680	4.417	4.337	8.484
<i>An. dirus</i> s.l.	Dry	0.017	0.018	0.019	0.059	0.004	0.050
	Wet	0.005	0.181	0.231	0.231	0.000	0.033
<i>An. maculatus</i> complex	Dry	0.004	0.218	3.435	1.621	14.494	8.743
	Wet	0.010	1.120	2.171	4.859	11.223	14.289

1st period = 1977-1989, 2nd period = 1990-1999; dry season = October-April, rainy season = May-September.

RESULTS

During the study period, a total of 141,144 anophelines of 29 species were recorded in Mae Hong Son Province, of which 64,469 were collected on outdoor human bait, 63,416 on animal bait, and 13,259 on indoor human bait. The mosquito indices are expressed as the mean number of mosquitos per bait per half night. Table 1 gives a list of all anopheline species, and the mean densities (\pm SE) by the three different collection methods. Of all anophelines collected, *An. minimus* s.l. was predominantly biting on humans with a mean density of 2.1 and 4.1 from indoor and outdoor collection, respectively (Table 1). On animal bait, the most abundant species was *An. vagus* (15.4), followed by *An. maculatus* complex (12.3), and *An. annularis* (11.8) (Table 1).

Malaria vectors

***An. minimus* s.l.** The results of the ANOVA on the whole data set for this species showed that there were significant differences between periods (1977-1989 and 1990-1999) ($F_{1,1093}=13.40$, $p < 0.001$) and there were no significant interactions between any of the factors ($p > 0.05$). Also, for each season, there were significant differences between periods (dry: $F_{1,525}=4.69$, $p < 0.05$; and wet: $F_{1,568}=9.11$, $p < 0.01$), but when separated by collection method, only an increase in outdoor human bait in the wet season was significant (Table 2). No significant differences

were found between seasons in the whole data set ($F_{1,1093}=0.27$, $p=0.60$), nor in each period ($p > 0.2$).

***An. dirus* s.l.** In two-way analysis of all the data, there were no significant differences in density between any of the factors analyzed (period, season). However, when the periods were analyzed for this species separately, in the second period, a higher indoor density during the wet season was found (Table 2).

***An. maculatus* complex.** For this species complex, there was no difference in density between the two periods ($F_{1,1093}=2.04$, $p > 0.05$). However, there was a significant interaction between periods and seasons in the whole data set ($F_{1,1093}=5.05$, $p < 0.05$), which is reflected by a significant difference between periods in the dry season ($F_{1,525}=9.61$, $p < 0.01$), but not in the wet season ($F_{1,568}=2.70$, $p > 0.05$). Furthermore, we found contrasting results when analyzing differences between periods for each collection method and each season. For the outdoor human bait collections, there were significant decreases in the dry season and significant increases in the wet season (Table 2). The human indoor collections increased significantly between periods in both seasons. There were no differences between seasons in the first period, but in the second period there were significantly higher densities in the wet season for all collection methods.

DISCUSSION

We found that the densities of the three main malaria vectors either increased or were stable in Mae Hong Son Province during the study period. The density of *An. dirus* s.l., a more efficient vector than *An. minimus* s.l. (Gould *et al.*, 1966; Ismail *et al.*, 1974; 1975), was stable throughout the period. However, the density of *An. minimus* s.l. and *An. maculatus* complex had significantly increased from the first period (1977-1989) to the second period (1990-1999). Specifically, the density increase in the latter two species was mainly observed in the wet season. These facts support our hypothesis that large or increasing vector populations could be a contributing factor for the consistent high malaria incidence in the province.

We also found significantly higher densities of *An. dirus* s.l. and *An. maculatus* complex in the wet season than in the dry season, especially in the second period. High wet season densities of *An. dirus* s.l. and *An. maculatus* complex have also been reported in other studies (Ismail *et al.*, 1974; 1975; Rosenberg, 1982; Upatham *et al.*, 1988; Rosenberg *et al.*, 1990; Suwonkerd *et al.*, 1995; Takagi *et al.*, 1995). It is believed that *An. dirus* s.l. usually retreats to dense humid forest areas during the dry season and returns to the forest fringe and populated areas during the rainy season (Rosenberg *et al.*, 1990), thus being largely responsible for wet season transmission (Ismail *et al.*, 1974). *An. maculatus* complex has generally been thought to be an important vector in southern Thailand (Upatham *et al.*, 1988), and of little or no importance elsewhere in the country (Harbach *et al.*, 1987). However, *An. maculatus* s.s. and *An. sawadwongporni*, which are members of this complex, were incriminated in Mae Hong Son Province (Somboon *et al.*, 1994). *An. pseudowillmori*, also a member of this complex, was incriminated in the neighboring province of Tak (Green *et al.*, 1991). It appears, therefore, the species of the *An. maculatus* complex may have played a larger role in malaria transmission in northern Thailand than previously assumed. The density of *An. minimus* s.l. was not significantly different between seasons. Previous studies found that *An. minimus* s.l. was prevalent throughout the year, including a major part of the dry, cool

season, as it remained at high density from November to February, and for a shorter duration in the early part of the rainy season (Ismail *et al.*, 1974; 1975; 1978). Therefore, it seems that *An. minimus* s.l. is responsible for transmitting malaria throughout the year, thus reinforcing wet season transmission by *An. dirus* s.l. and *An. maculatus* complex. These results partly support our hypothesis that the seasonal prevalence of the main malaria vectors was higher in the wet season, thus being responsible for the higher transmission rates in this season.

Rainfall has been suggested as an important climatic factor affecting both mosquito population and malaria transmission (Bouma *et al.*, 1996; Hu *et al.*, 1998; Mouchet *et al.*, 1998). In Mae Hong Son, total rainfall varied between 1,025 and 1,650 mm/year during the years 1970-1998 (Meteorological Department, 1970-1999). About 70%-90% of the rainfall in Mae Hong Son Province was recorded in the wet season. However, no significant difference in wet season rainfall was observed between the two study periods ($t=1.70$, $p=0.104$) (Fig 2). Therefore, it seems that variation in rainfall did not have much effect on malaria vector density in the study area during the study period.

Deforestation has been associated with either increases or decreases of vector transmitted diseases (Walsh *et al.*, 1993). In Africa, where 90% of worldwide malaria morbidity and mortality occurs, deforestation is considered a factor in the increase in malaria, due to the development of anopheline larval sites exposed to the sun (Mouchet *et al.*, 1988). By contrast, in Thailand, the ecological conditions in deforested areas become unfavorable for breeding of the main malaria vectors, suggesting a reduced malaria risk (Ismail *et al.*, 1978; Rosenberg *et al.*, 1990). This corresponds with a study in Yunnan, in southern China, where dense forests were associated with a high abundance of *An. minimus* s.l., and a high incidence of malaria (Hu *et al.*, 1998). In Mae Hong Son Province, forest cover decreased from approximately 87% in 1976 to about 70% in 1998 (Royal Forest Department, 1999). However, since mosquito collections were mainly undertaken in more or less remotely located transmission areas, where, in general, little land use change has taken

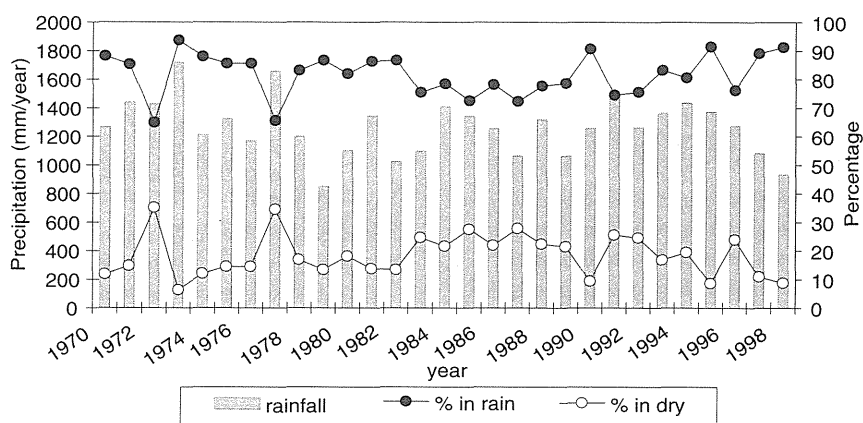


Fig 2—Yearly changes in precipitation and the proportion of rainfall recorded in wet and dry seasons from 1977-1998 in Mae Hong Son Province, northern Thailand.

place, it is reasonable to assume that there was a higher forest cover and a lower rate of deforestation in these locations. In a non-transmission area in the neighboring province of Chiang Mai, malaria vector density, especially that of *An. minimus* s.l., decreased between 1977 and 1999, and it was concluded that this might have been a result of increased landscape diversity and forest fragmentation (Suwonkerd *et al*, 2002). However, in transmission areas in Chiang Mai Province, forest cover was extensive, landscape diversity low, and malaria vector densities increased during the same period. In a comparative study of six areas in northern Thailand, of which two were situated in Mae Hong Son Province, Overgaard *et al* (in press), found that anopheline diversity and density were generally higher in forested areas with low landscape diversity than in agricultural areas with a high landscape diversity. Thus, it seems that the mosquito situation in Mae Hong Son over the last two decades resembles that of transmission areas in Chiang Mai Province.

However, apart from the density of malaria vectors, and climatic and environmental factors, there are other possible explanations for the observed high malaria rates in Mae Hong Son Province. In particular, much movement of refugees and others across the border to Myanmar, as well as a high degree of multi-drug parasite resistance, may play an important role in malaria transmission in the province.

To conclude, the increases in malaria vector densities in Mae Hong Son Province suggest that entomological factors probably play a large role in malaria transmission in the province. Furthermore, environmental and climatic factors seem to have been favorable for a consistently high vector population and relatively stable malaria transmission rate. Understanding how different factors affect malaria transmission and mosquito populations will eventually lead to better planning of malaria control.

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LABORATORY AND FIELD EVALUATION OF SPATIAL REPELLENCY WITH METOFLUTHRIN-IMPREGNATED PAPER STRIP AGAINST MOSQUITOES IN LOMBOK ISLAND, INDONESIA

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ABSTRACT. Spatial repellency of a new multilayer paper strip impregnated with metofluthrin, a newly synthesized pyrethroid, was evaluated in the laboratory and in the field at Kerandangan, Lombok Island, Indonesia, with the use of cow- and human-baited double nets. Spatial repellency was observed in both cow- and human-baited collections. Metofluthrin treatment reduced mosquito collection by >80% during the 1st 4 weeks. However, repellency seemed to reduce with the loss of metofluthrin by evaporation within 6 wk after treatment.

KEY WORDS Metofluthrin, spatial repellency, *Anopheles balabacensis*, *Culex quinquefasciatus*, Lombok Island

INTRODUCTION

Since the indoor residual spraying of insecticides lost its reliability because of the development of physiological or behaviorological resistance in mosquitoes, one of the major innovations in the field of malaria vector control during the past 2 decades has been insecticide-impregnated mosquito nets. Recent development of pyrethroids and its formulation technologies have accelerated the development of long-lasting insecticidal net (WHO 2000, N'Guessan et al. 2001). It will be the most promising measure for controlling malaria mosquitoes at low cost and high sustainability, as opposed to residual spraying. However, the exophagic behavior of mosquitoes to shift their biting preference to earlier in the evening still presents a challenge. In such cases, active people outside and inside of houses could still be exposed to the danger of malaria transmission. Mosquito coils, mats, and other formulations that prevent mosquito bites are broadly and successfully used inside houses in wealthy communities. Use of these devices is, however, limited in the poor communities because of lack of convention, electricity, and money. Moreover, their uses seem limited outside of houses because they sometimes are unable to maintain sufficient aerial concentration of active ingredient in outdoor conditions.

Metofluthrin, 2,3,5,6-tetrafluoro-4-methoxymethylbenzyl (*EZ*)-(1*RS*,3*RS*;1*RS*,3*SR*)-2,2-dimethyl-3-(prop-1-enyl) cyclopropanecarboxylate (S-1264) is a newly synthesized pyrethroid with demonstrated knockdown and lethal activity against mosquitoes (Shono et al. 2004, Sugano et al. 2004). The vapor pressure of metofluthrin (1.87×10^{-3} Pa at 25°C) is ca. >2 times and >100 times larger than *d*-allethrin and permethrin, respectively. Metofluth-

rin vaporizes at normal temperature without heating, whereas the other conventional pyrethroids need heating for evaporation. High vapor pressure and insecticidal activity of metofluthrin could lead new mosquito controlling devices that need no external energy for vaporization with low cost and long time efficacy.

In this paper, we report the results of laboratory and field studies that evaluate the insecticidal activity of metofluthrin-impregnated paper strip against *Anopheles balabacensis* Baisas, which is a major malaria vector in Lombok Island, Indonesia. We also report on the possibility of the use of metofluthrin-impregnated paper strip in outdoor conditions against mosquitoes, including *Anopheles* spp. and *Culex quinquefasciatus* Say.

MATERIALS AND METHODS

Formulation of metofluthrin-impregnated paper strip: Metofluthrin multilayer paper strip devices were supplied by Sumitomo Chemical Co., Ltd. (Takarazuka, Hyogo, Japan). Metofluthrin (200 mg) diluted with acetone was uniformly applied to the paper strip device, which has a multilayer and foldable structure (Fig. 1), and acetone was allowed to vaporize under ambient conditions. The folded device was compact (9 × 7 cm, ~3 mm thick), and total surface area of the unfolded paper was ~2,000 cm².

Laboratory evaluation against *An. balabacensis*: Knockdown activity of metofluthrin-impregnated paper strips was evaluated in an office of NTB Dinas Kesehatan Propinsi (Lombok, Indonesia). Room dimensions were 2.7 m wide, 3.9 m deep, and 3.2 m high. Field-collected larvae of *An. balabacensis* were reared in the laboratory and emerged adults were used in the test. Five females (3–7 days old) were released into stainless steel cages (210 × 170 × 150 mm, 11 mesh). Four pairs of cages were hung in a diagonal line at 1 m and 2.4 m (in the corner of the room) from the center of the room at a height of 130 and 30 cm, respectively, from the floor. A multilayer paper strip was hung at the center of the ceiling such that the bot-

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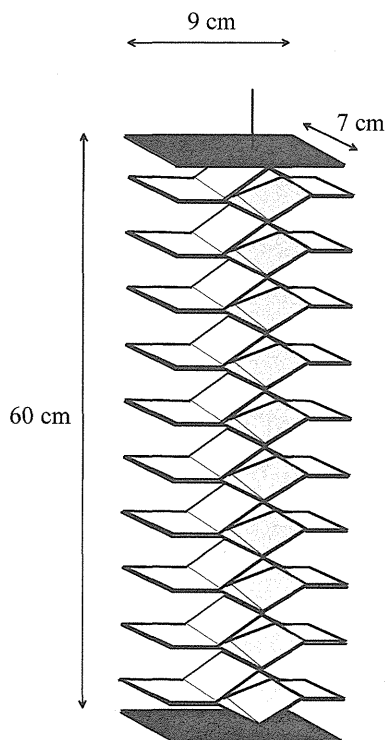


Fig. 1. Multilayer paper strip device impregnated with 200 mg of metofluthrin.

tom end of the strip was 160 cm from the floor. Knockdown of mosquitoes was observed for 1 h, and mortality was recorded at 24 h. There was no air-conditioner or ventilation system in the room, and temperature and relative humidity (RH) were recorded at 28–30°C and >70%, respectively.

Field collection of mosquitoes in Kerandangan: Weekly collection of mosquitoes was carried out at Kerandangan, a coastal area about 15 km northwest of Mataram, Lombok Island, Indonesia. A house was used for indoor human-baited collection (HBI). A bed net was hung in a room with 2 human volunteers inside, and collection of mosquitoes was made outside the bed net. Outdoor human-baited collection was carried out with the use of a double net (HBO). Collection of mosquitoes was made inside of the outer net (3 × 3 × 2 m) and outside of the inner net (1 × 1 × 2 m) in which 2 humans lay. Outdoor cow-baited collection also used a double net (CBO). Collection of mosquitoes was made inside of the outer net (6 × 6 × 2 m) and outside of the inner net (4 × 4 × 2 m) in which a cow lay. Half-night (1800–2400 h) collection of mosquitoes by aspirator was carried out at 1-h intervals. Collection time was 45 min for HBI and HBO (e.g., 1800–1845 h) and 15 min for CBO (e.g., 1845–1900 h). Field collection was carried out once a week from November 12, 2002, to February 11,

2003. Species and number of collected mosquitoes were recorded the day after collection.

Evaluation against mosquitoes in the field: Evaluation was carried out at the same place as mosquito collection in Kerandangan. Trial design is shown in Fig. 2. A room of similar size (volume 15–18 m³, floor area 5.5–8.5 m²) in 3 different houses was used for the indoor human-baited collection (the HBI-1 house was used for the 2nd, 3rd, and 4th tests and the HBI-2 house was used only for the 1st test; the HBI-3 house was used for the 1st, 2nd, 3rd, and 4th tests). The door and windows of each room were kept open during the test. Two sites were used for outdoor human-baited collection with a double net. Two sites were used for outdoor cow-baited collection with a double net. Test strips were set before the test started (1800 h). Strips were hung 10–30 cm below the ceiling of the house outside the bed nets for HBI (Fig. 3A) and in a space between the inside net and outside net of the double nets for HBO and CBO (Fig. 3B, 3C), respectively. The number of test samples hung were 1 for HBI, 2 for HBO, and 4 for CBO. The number of test samples in HBO and CBO was roughly decided according to the sizes of both nets. Half-night (1800–2400 h) collection of mosquitoes by aspirator was carried out at 1-h intervals in the same manner as in the weekly collection. In every test, 1 site was used for the treatment and the other site was left untreated. Each test was repeated 2 times on successive days at a different site. The 1st test (just after unfolding of strips) was carried out on December 22, 2002 (1800–2400 h), and December 24, 2002 (1800–2400 h); the 2nd test (after 2 wk) on January 7, 2003 (1800–2400 h), and January 8, 2003 (1800–2100 h; the test was stopped at 2100 h because of rain); the 3rd test (after 4 wk) on January 21, 2003 (1800–2400 h; replication could not be carried out because of rain); and the 4th test (after 6 wk) on February 4, 2003 (1800–2400 h), and February 6, 2003 (1800–2400 h). Test strips were preserved in unfolded condition in the room in Mataram city for the next test. Average temperature and relative humidity recorded ranged from 25 to 30°C and 70 to 90% RH, respectively. The rainy season started at the end of October 2002 and lasted throughout the test period. The number of collected mosquitoes and species identified was recorded every test day.

RESULTS

Laboratory test: evaluation of metofluthrin-impregnated paper strip against *An. balabacensis*: Knockdown activity of a metofluthrin-impregnated paper strip against female adult *An. balabacensis* is shown in Table 1. All insects were knocked down within 30 min after treatment of the strip, and mortality at 24 h was 100% irrespective of the height and position of cages. Knockdown seemed to be faster in the cages at a distance of 2.4 m (in the

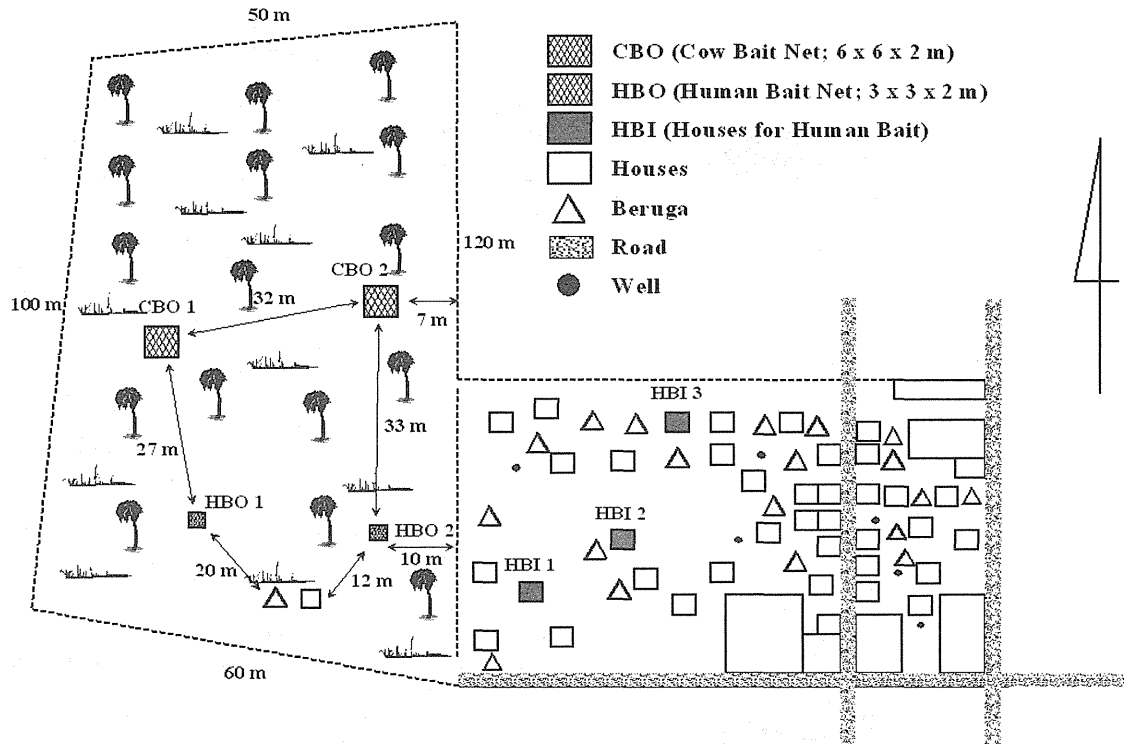


Fig. 2. Outline map of the test sites at Kerandangan. All houses are 1-story, made of wood and mud bricks, and with >2 rooms. A beruga is an arbor hut made of palm leaves; it has no walls or sometimes has simple screens in the back and on both sides.

corner of the room) than those in the cages 1 m from the strip.

Field test: population density and species composition of mosquitoes during the test period: Species composition at each untreated test site from November 12, 2002, to February 11, 2003, are shown in Table 2. Species compositions were almost the same in HBI and HBO; >80% of the mosquitoes collected by human bait were *Culex* spp., among which, *Cx. quinquefasciatus* was dominant (>90%), and the 3-species complex of the Pyrethrophorus series (i.e., *Anopheles vagus* Doenitz, *Anopheles indefinitus* (Ludlow), and *Anopheles subpictus* Grassi) ranked next after *Culex* spp. The proportions of *An. balabacensis* and *Anopheles sundaicus* (Rodenwaldt), which are thought to be the main malaria vectors, were low (<1%) in human-baited collection during the test period. The difference in mosquito density at the different collection sites was significant by the Friedman Test ($\chi^2 = 6.42$, 1 df, $P < 0.011$). Mosquito density was >6 times higher in HBO than in HBI, which indicates the exophagy of mosquitoes. Species composition in the cow-baited collection, on the other hand, was different from those in the human-baited collections; ~70% of the collection comprised the 3-species complex of Pyrethrophorus series, and the

proportion of *Culex* spp. was lower than in the human-baited collections. The proportions of *An. balabacensis* and *An. sundaicus*, however, were low, as in the human-baited collections. Mosquito density was highest in CBO (461 per night), which was ~4 times and 20 times more than in HBO and HBI, respectively. *Anopheles* spp. and *Culex* spp. peaked in December 2002 and then gradually decreased by the end of February 2003.

Spatial repellency of metofluthrin-impregnated paper strips against mosquitoes: The number of *Anopheles* spp. and *Culex* spp. collected in metofluthrin-treated and untreated collection sites and overall changes in the total number of mosquitoes collected per hour in metofluthrin-treated and untreated sites by 3 different collections are shown in Table 3 and Fig. 4, respectively. A significant reduction in mosquito density in metofluthrin-treated sites was maintained in HBI (2-way ANOVA, $F = 7.42$, 1 df, $P = 0.034$), HBO ($F = 7.30$, 1 df, $P = 0.036$), and CBO ($F = 10.12$, 1 df, $P = 0.019$), respectively, although the density in the untreated sites of HBI and HBO were lower than those in CBO. Spatial repellency was prominent in the cow-baited collection, in which higher numbers of mosquitoes were collected than in human-baited collections. Percent reduction of mosquito density in the

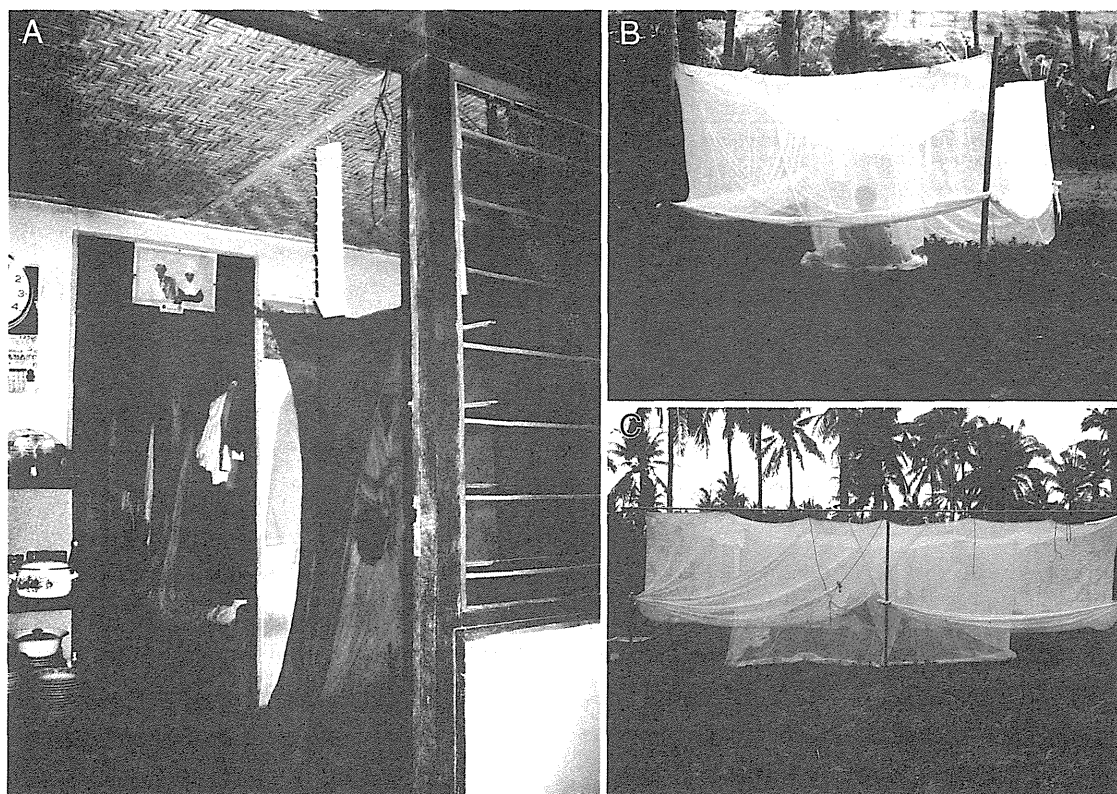


Fig. 3. Field test scene of each test site. (A) Indoor human-baited collection (HBI; a strip was hung 10–30 cm below the ceiling of the house outside the bed net). (B) Outdoor human-baited collection with a double net (HBO; 2 strips were hung in a space between the inside and outside nets). (C) Cow-baited collection with a double net (CBO; 4 strips were hung in a space between the inside and outside nets).

metofluthrin-treated CBO sites versus the untreated controls was 90.7% for *Anopheles* spp. and 92.6% for *Culex* spp. at the initial test. Percent reduction was low for *Anopheles* spp. after 2 wk (58.0%), but it recovered to 90.9% after 4 wk. For *Culex* spp., on the other hand, percent reduction was >90% un-

til 4 wk after treatment. Percent reduction decreased after 6 wk for both groups of mosquitoes. The difference between percent control in metofluthrin-treated sites at 6 wk and at 0, 2, and 4 wk were significant by the chi-squared test on the basis of the number of mosquitoes collected in CBO. For

Table 1. Knockdown activity of metofluthrin-impregnated paper strip against caged female adults of *Anopheles balabacensis* at 0, 10, 30, and 60 min.

Distance from strip (m)	Height (m)	Replicate	No. knocked down				% mortality at 24 h
			0	10	30	60	
1	1.3	1	0	3	5	5	100
		2	0	1	5	5	
		%	0	40	100	100	
	0.3	1	0	1	5	5	100
		2	0	1	5	5	
		%	0	20	100	100	
2.4	1.3	1	0	5	5	5	100
		2	0	3	5	5	
		%	0	80	100	100	
	0.3	1	0	5	5	5	100
		2	0	4	5	5	
		%	0	90	100	100	

Table 2. Species composition of mosquitoes at each collection site from November 12, 2002, to February 11, 2003.

Mosquito species	Species composition (%) ¹		
	HBI	HBO	CBO
<i>An. vagus</i> + <i>An. indefinitus</i> + <i>An. subpictus</i>	8.9	8.2	69.8
<i>An. tessellatus</i>	0.3	0.1	0.6
<i>An. annularis</i>	0	0.8	0.5
<i>An. barbirostris</i>	0	0.4	0.8
<i>An. maculatus</i>	0	0.1	0.3
<i>An. flavirostris</i>	0.3	0	0.1
<i>An. sondaicus</i>	1.0	0.2	0.3
<i>An. kochi</i>	0	0	0.0 ⁴
<i>Aedes</i> spp.	1.3	1.4	0.9
<i>Armigeres</i> spp.	0.3	2.5	1.4
<i>Culex</i> spp. ²	87.8	86.4	25.3
Total ³	100 (20.2)	100 (123)	100 (461)

¹ HBI, indoor human-baited collection; HBO, outdoor human-baited collection; CBO, cow-baited collection.

² >90% was *Culex quinquefasciatus*.

³ Number in parenthesis is average number of mosquitoes collected per night.

⁴ Calculated value was <0.05%.

Anopheles spp., $\chi^2 = 106.3$ for week 0 versus week 6 (1 df, $P < 0.0001$), $\chi^2 = 17.4$ for week 2 versus week 6 (1 df, $P = 0.00003$), and $\chi^2 = 19.6$ for week 4 versus week 6 (1 df, $P = 0.00001$). For *Culex* spp., $\chi^2 = 32.3$ for week 0 versus week 6 (1 df, $P < 0.0001$), $\chi^2 = 38.6$ for week 2 versus week 6 (1 df, $P < 0.0001$), and $\chi^2 = 12.7$ for week 4 versus week 6 (1 df, $P = 0.00037$).

DISCUSSION

Metofluthrin and its impregnated multilayer paper strip showed promising spatial repellency against mosquitoes in the laboratory and in field conditions. Spatial repellency is thought to be caused by high knockdown activity and an intrinsic sublethal effect that also is present in other pyre-

Table 3. Number of mosquitoes collected in the metofluthrin-treated and untreated collection sites at Kerandangan.

Test site	Weeks after treatment	Replicate (day of collection)	No. collected ¹					
			<i>Anopheles</i> spp.			<i>Culex</i> spp.		
			Untreated	Treated	% control	Untreated	Treated	% control
Human bait indoor	0	1 (Dec. 22)	1	0	100	5	1	83.3
		2 (Dec. 24)	0	0	—	7	1	—
	2	1 (Jan. 7)	1	0	100	8	0	100
		2 (Jan. 8) ²	1	0	—	1	0	—
	4	1 (Jan. 21)	0	0	—	3	0	100
		— ³	—	—	—	—	—	—
6	1 (Feb. 4)	0	0	—	0	0	100	
	2 (Feb. 6)	0	0	—	7	0	—	
Human bait outdoor	0	1	0	0	—	39	4	86.0
		2	0	1	—	11	3	—
	2	1	0	0	100	10	0	100
		2 ²	1	0	—	2	0	—
	4	1	2	0	100	22	0	100
		— ³	—	—	—	—	—	—
6	1	2	1	33.3	20	14	24.1	
	2	1	1	—	9	8	—	
Cow bait	0	1	194	22	90.7 a	83	6	92.6 a
		2	96	5	—	52	4	—
	2	1	56	15	58.0 b	84	2	98.1 a
		2 ²	25	19	—	20	0	—
	4	1	22	2	90.9 a	37	2	94.6 a
		— ³	—	—	—	—	—	—
6	1	9	9	-53.6 c	48	20	48.3 b	
	2	19	34	—	41	26	—	

¹ % control = $100 \times (\text{No. untreated} - \text{No. treated}) / \text{No. untreated}$. Values in the same column followed by the same letter are not significantly different (χ^2 test, $P > 0.05$).

² Collection was terminated at 2100 h because of rain.

³ Collection was not carried out because of rain.

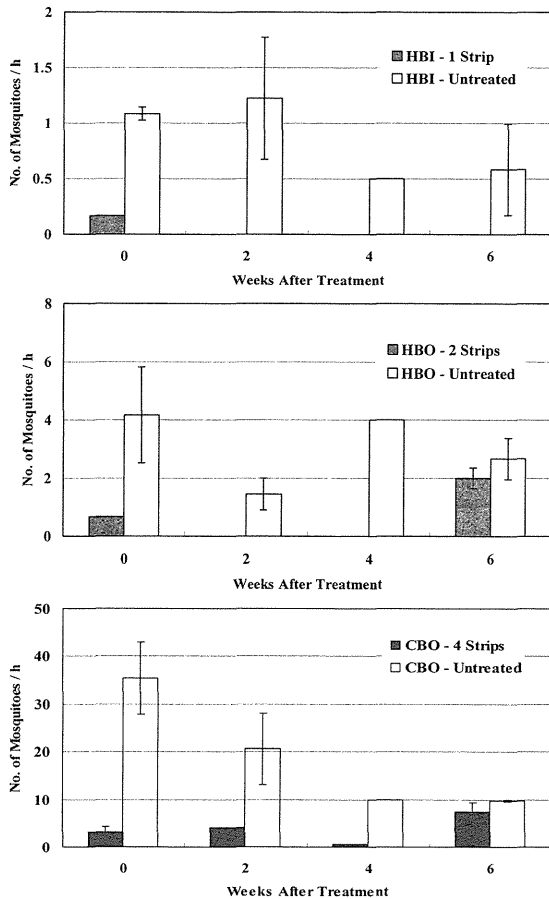


Fig. 4. Changes in total number of mosquitoes collected per hour at each test site. HBI, indoor human-baited collection; HBO, outdoor human-baited collection; CBO, cow-baited collection. Each line on the bar indicates the standard deviation.

throids. The laboratory test showed that mosquitoes were affected by airborne metofluthrin vapor and not by direct contact with the strip. The long-lasting effect of more than 4 wk in open field conditions is outstanding, even though the double net could have interfered with natural air circulation under our test conditions. Spatial repellency seemed to decrease with the loss of effective metofluthrin by evaporation within 6 wk of treatment. According to net area and number of strips treated in this study, the effective area affected by a metofluthrin-impregnated strip was estimated to be >5.5 m² for HBI, >4.5 m² for HBO, and >9 m² for CBO. A higher amount of active ingredient, improvement of slow-release devices, and additional protection for the active ingredient against degradation by external factors, such as oxidation and light irradiation, are required for the practical and successful use of metofluthrin in the field.

Our preliminary investigation on the ecology and biting habit of mosquitoes in Meninting County,

Lombok Island, including the test area of this study, has shown that mosquitoes are exophagous and their biting seems to peak in the early night (Maekawa et al., unpublished data), and it is likely that malaria transmission occurs in the early night when most people are active outside. The people of Lombok Island, like other people in tropical areas, have arbor huts made of palm leaves, called "berugas," which have no walls or have only simple screens. People use berugas to nap, pray, and converse in the evening with neighbors. Control or prevention of mosquitoes in berugas, therefore, seems to be ideal for malaria control. Use of traditional and inexpensive practices, such as mosquito coils and mats, insecticide-impregnated or untreated bed nets, and curtains have mainly been focused on the prevention of mosquito bites inside houses (Yap et al. 1990, Aikins et al. 1994, Hewitt et al. 1996). There are very few reports of the efficacy of antimosquito products in the outdoors. Jensen et al. (2000) reported that only mosquito coils and *N,N*-diethyl-3-methylbenzamide (deet) products significantly reduced mosquito landing rates relative to untreated controls in the field among the several commercial antimosquito products, including mosquito attractant, mosquito coils, ultrasonic repeller, citronella candles, and mosquito plant. Pates et al. (2002) reported unique attempts with a kerosene oil lamp to vaporize transfluthrin, a new volatile pyrethroid. They modified the lamp by mixing transfluthrin with vegetable oil and heating it to 120°C in a tin can held just above the flame to avoid decomposition of the insecticide by heat. More than 90% protection was achieved with a higher concentration of transfluthrin (0.5%) in the vegetable oil, whereas 0.1% transfluthrin gave <75% reduction in typical houses of Dar es Salaam, Tanzania. Their device will offer a cost-effective alternative to mosquito coils and mats because kerosene lamps are cheap and used widely in tropical areas. Both kerosene lamps and mosquito coils could be applicable for outdoor use. The above 2 devices, however, need heat by burning of solvents or coil materials for evaporation and diffusion of active ingredients, which have the potential danger of fire, trouble in handling, and occasional side effects for human health by smoke.

The multilayer paper strip used in this study is thought to be an ultimate device that needs no external energy for heating and no other materials for evaporation and diffusion of active ingredients with low cost and high convenience. It is noteworthy that the metofluthrin-impregnated paper strips showed significant spatial repellency against mosquitoes in open field conditions for a month at 200 mg concentration. Further evaluation in more practical conditions, such as berugas, and further studies to enhance the duration of activity will promote development of the devices for the suppression of mosquito-borne diseases.

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Photoelectric Sensing Device for Recording Mosquito Host-Seeking Behavior in the Laboratory

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ABSTRACT A new automatic device for recording the host-seeking behavior of mosquitoes was designed using photoelectric sensors. The host-seeking rhythms of several species of mosquitoes were recorded under laboratory conditions. Use of CO₂, in addition to heating and black color to activate mosquito flight, proved to be important for evaluation of the present recording device. The diel rhythms of nonblood-fed *Aedes albopictus* (Skuse), *Aedes aegypti* (L.), *Culex quinquefasciatus* Say, *Culex tritaeniorhynchus* Giles and *Anopheles stephensi* Liston females recorded by the device corresponded well with known flight and activity rhythms in field for the same mosquito species. This simple automatic recording device provided accurate information on the flight behaviors of colonized and field collected mosquitoes.

KEY WORDS *Anopheles*, *Aedes*, *Culex*, host-seeking, photoelectric sensor

ACCURATE KNOWLEDGE OF THE host-seeking flight behavior of mosquitoes is important for controlling and regulating mosquito bites. Many previous studies of mosquito host-seeking behavior have involved field tests relying on landing collections at human or animal baits, or baited traps. The field collection of mosquitoes is one of the most reliable ways of studying mosquito flight behavior. Field studies, however, are labor-intensive, expensive, time-consuming, and sometimes place human volunteers at risk of disease transmission. Certain aspects of mosquito flight behavior can be studied in the laboratory, and those results can be extrapolated to the field to help design more efficient and safe field experiments.

Extensive laboratory studies on mosquito behavior have been reported previously. These studies can be classified into several categories, including: flight behavior (Taylor and Jones 1969, Chiba et al. 1981, Jones 1981), sugar-feeding behavior (Bowen 1992, Yee and Foster 1992), and biting or host-seeking behavior (Ho et al. 1973, Klowden and Lea 1984, Yee and Foster 1992, Takken et al. 2001).

Many laboratory and field studies on mosquito attractants such as heat, color, light, and chemical substances have been reported. Several key factors, including carbon dioxide, 1-octen-3-ol, L-lactic acid, heat ($\approx 35^{\circ}\text{C}$), and black or dark color have been found to be essential for attracting mosquitoes (Takken and Kline 1989, Kline et al. 1991). These observations resulted in the development of a variety of laboratory olfactometers designed to evaluate mosquito flight behavior (Gouck and Schreck 1965, Posey

et al. 1998, Takken et al. 2001) and a variety of mosquito traps (Parker et al. 1986, Burkett et al. 1998, Kline 2002) to evaluate mosquito flight behavior in the field. In addition, recording devices were designed to automatically record the flight activity and sugar-feeding behavior of mosquitoes in the laboratory (Chiba et al. 1981, Yee and Foster 1992). Many laboratory studies of mosquito host-seeking behavior, however, have relied on visual or videorecording observations of mosquito flight behavior. The host-seeking and blood-feeding behaviors of mosquitoes in nature are known to be extremely complex. Host-seeking behavior is affected by environmental conditions, mosquito physiological conditions, and host-produced cues. Blood feeding is affected by host physiology and environmental conditions. Simple recording of mosquito flight behavior, therefore, underrepresents the true complexity of mosquito behavior. The introduction of variables such as host cues into flight experiments are needed for a more comprehensive study of mosquito host-seeking behavior. In the current study, we designed an automatic recording device by using photoelectric sensors and report the results of mosquito flight experiments in the laboratory.

Materials and Methods

A General Description of the Automatic Recording Device. The recording device was composed of four photoelectric sensors, an amplifier, a programmable controller unit, a power supply, and monitoring soft-

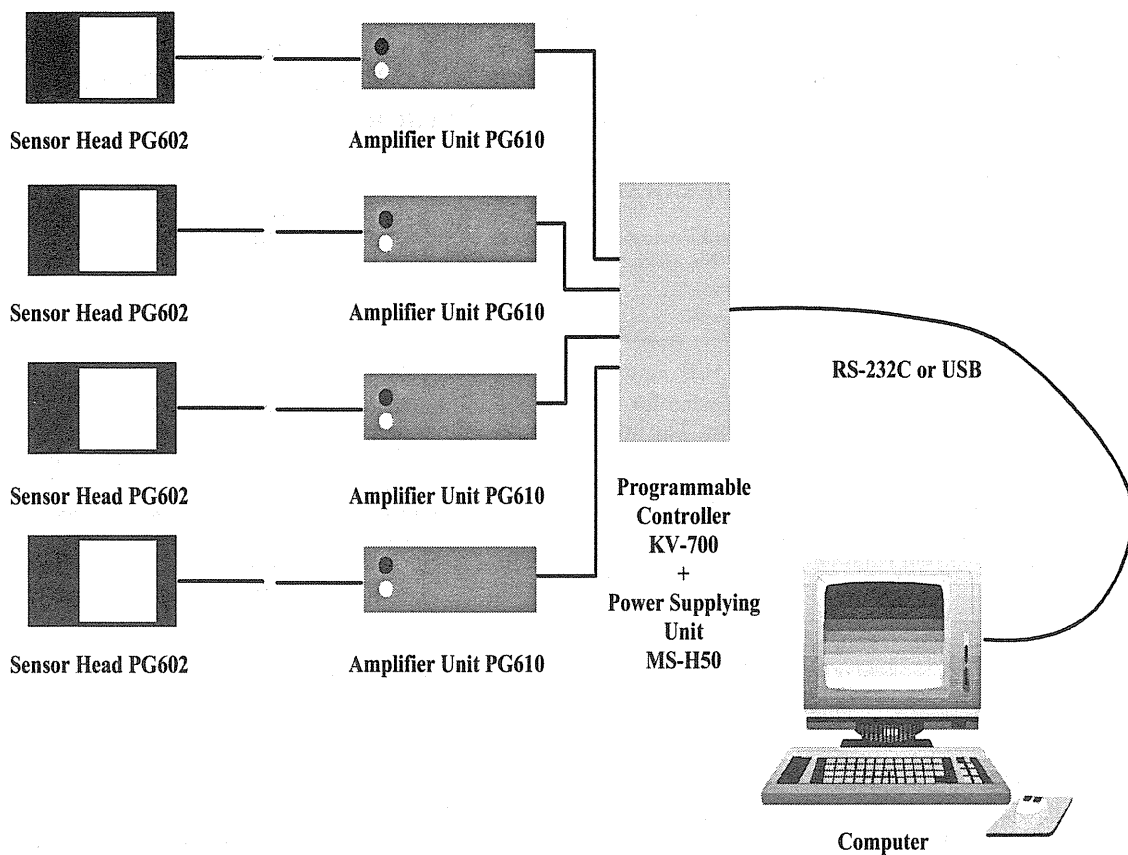


Fig. 1. Schematic view of an automatic recording device to quantify mosquito activity patterns. The device is composed of four photoelectric sensors and amplifiers, a programmable controller unit, a power supplying unit, and monitoring software installed in a computer system. Mosquitoes passing through the sensor windows, composed of an infrared LED with a light-receiving element on both sides, are detected and a signal is transmitted to the computer.

ware (Keyence Corporation, Osaka, Japan) (Fig. 1). The photoelectric sensors had a small sensor head (55 by 30 by 10 mm) with a 21 by 21-mm window. The window was composed of a pair of infrared light emission diode (LED) sensors with light receiving elements on both sides. Small objects (minimum detectable size is 0.5 mm in diameter) passing through the window were detected by the sensors, and a signal was transmitted to the computer through the controller and monitoring software. Nocturnal activity was detected by the same system by using infrared LEDs. The total cost for the recording device was \approx \$4,000 (\approx \$500 for a sensor and \approx \$2,000 for a programmable controller and monitoring software).

A water bath unit for the recording device was made from a white Styrofoam box (23 by 30 by 20 cm, 5 cm in thickness) that contained \approx 3.5 liter of water (Fig. 2). The temperature of the water was maintained at \approx 35°C by a heater with thermo-regulator (Powerthermo ET-30, Kotobuki Kogei Co., Ltd., Osaka, Japan). The box was covered with a white plastic panel that contained the four photoelectric sensors, the bottom sides of which were sealed with black plastic

plates. The top surface of the box, except for the sensors, was covered with a white plastic plate that helped maintain the temperature in the box.

The recording device was placed in a cage (80 by 80 by 80 cm) with white plastic panels that buffered the mosquitoes inside the cage from the external stimulations, such as airstreams, light, human movement, heat, and carbon dioxide (CO₂) (Fig. 3). Carbon dioxide at a flow rate of 500 ml/min was generated by a gas bomb and was released into the cage through a silicone tube, the opening of which was placed \approx 10 cm above the sensors. The CO₂ release was regulated (2 min on/13 min off) by a solenoid valve (FSD-0408C, Flon Industry Co., Ltd., Tokyo, Japan) controlled by timer. The air inside the cage was ventilated by an electric fan located in the bottom corner of the cage. The concentration of CO₂ inside the cage was measured \approx 1 cm above the sensors by a CO₂ detector (TECH-JAM Co., Ltd., Osaka, Japan). The light intensity in the laboratory was measured with a photo recorder (T&D Corp., Nagano, Japan) before starting the tests.

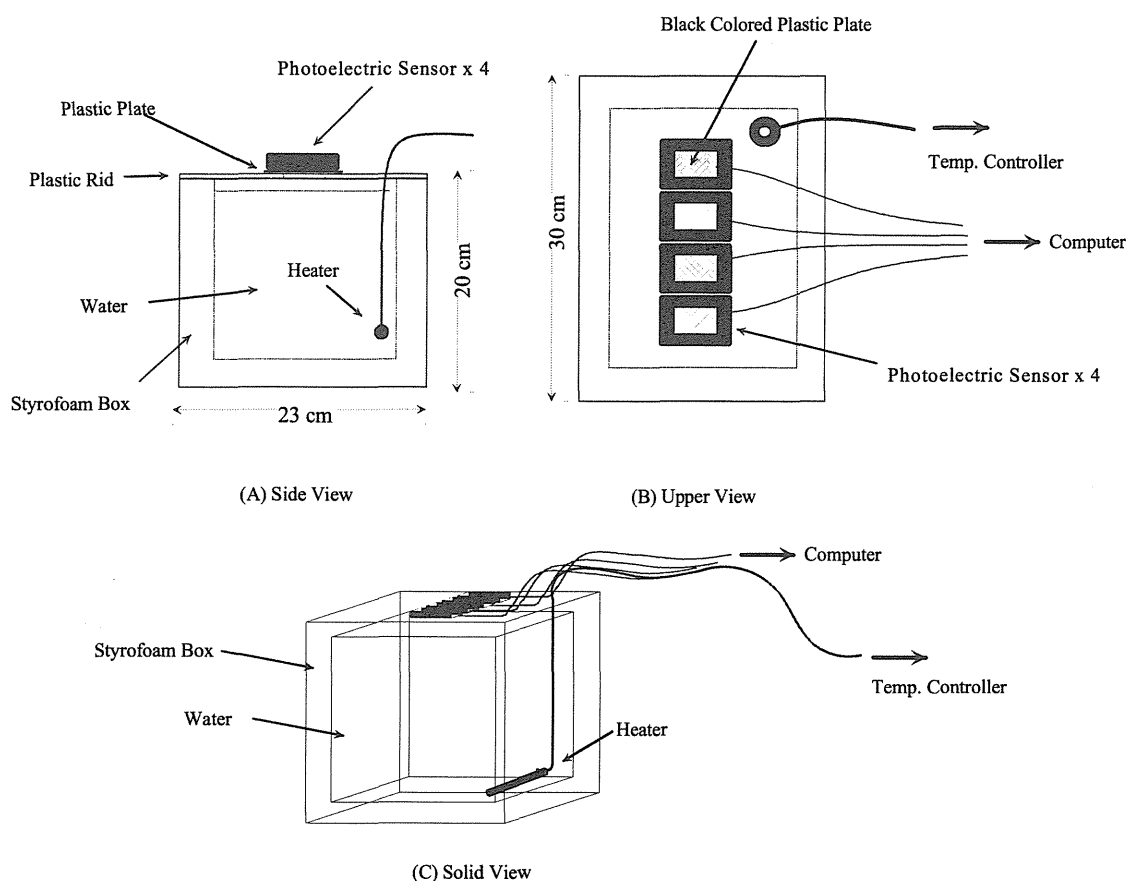


Fig. 2. Schematic view of the water bath unit of the recording device. A Styrofoam box with 3.5 liter of water kept at 35°C by a heater is covered with a white plastic panel containing four photoelectric sensors, the bottoms of which are covered with black plastic plates. The top surface of the Styrofoam box, except for the sensor plates, is covered with another white plastic plate to concentrate the heat onto the black plates.

Recording Mosquito Flight Patterns in the Presence and Absence of CO₂. Colonized *Aedes albopictus* (Skuse), *Aedes aegypti* (L.), *Culex quinquefasciatus* Say, and *Anopheles stephensi* Liston were used for this part of the study. Both *Aedes* species were collected in Singapore during 2000. *Cx. quinquefasciatus* was collected in Vietnam during 2003. *An. stephensi* was collected in India during the 1950s. Mosquitoes were maintained in the laboratory after collection at 27°C, 70% RH, and a photoperiod of 16:8 (L:D) h. Sixty, ≈10-d-old nonblood-fed females were released into the test cage in the morning between 0700 and 1000 hours. The number of mosquitoes landing on the black plastic plate containing the photoelectric sensors was counted and recorded every 60 min for 72 h. Carbon dioxide was not released during the first 24 h of the experiment and was then released for 2 min every 15 min during the last 48 h of the experiment. Mosquitoes were provided a 1% sugar solution throughout the test. The test was carried out in a room maintained under a photoperiod of 16:8 (L:D) h where the first

and last 1 h of the scotophase were in twilight, 25–27°C, and >60% RH regime.

Recording the Flight Behavior of Field-Collected and Colonized *Culex tritaeniorhynchus* Giles. Third and fourth instars of *Cx. tritaeniorhynchus* were collected from a rice paddy field in Isahaya, Nagasaki, Japan, on 14 July 2003. Larvae were reared to adults under the laboratory conditions described above. Sixty, ≈7-d-old nonblood-fed female mosquitoes were released into the test cage at 1000 hours. The number of mosquitoes landing on the black plastic plate containing the photoelectric sensors was recorded as described above. The flight behavior of colonized *Cx. tritaeniorhynchus* (collected from Nishiarita, Japan, in 1998) was recorded in the same manner and compared with the flight behavior of the wild-caught females. Sixty, ≈7-d-old nulliparous females and sixty, ≈14-d-old, parous (in their second gonotrophic cycle) colonized females were used for this test. Three replicates were carried out for each recording for both wild and laboratory colonies.

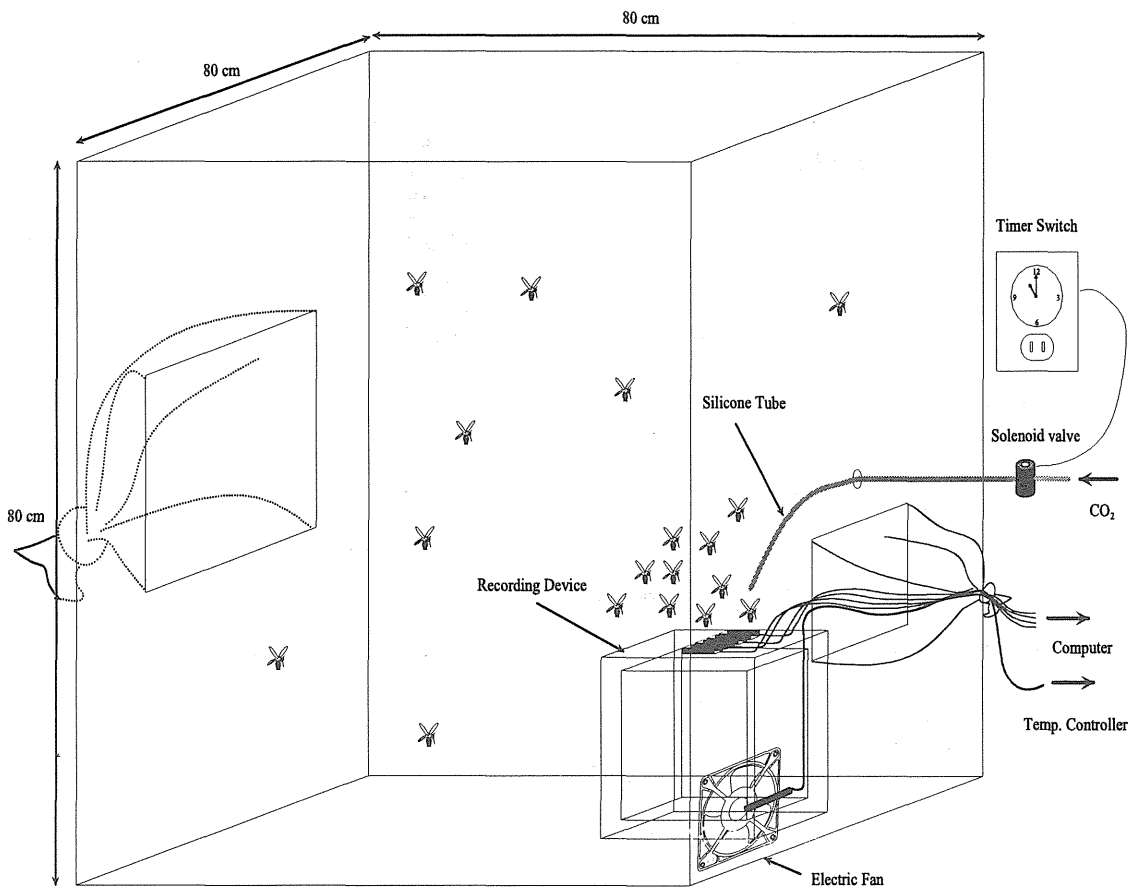


Fig. 3. Schematic view of the mosquito holding cage and recording device. Carbon dioxide was released at the rate of 500 ml/min into the cage through the silicone tube. The release of CO₂ was regulated by solenoid valve controlled by timer. The air inside the cage was ventilated by the electric fan located at the bottom corner of the cage. Mosquitoes inside the cage were provided a 1% sugar solution during the test.

Results and Discussion

The changes in CO₂ concentration in the air \approx 1 cm above the sensors inside the cage are shown in Fig. 4. The CO₂ concentration rapidly increased to $>5,000$ ppm (detective limit by the detector) immediately after the release, maintained a high value ($>5,000$ ppm) for 2–3 min, and recovered to the baseline concentration of $\approx 2,000$ ppm rapidly after closing the solenoid valve. The CO₂ baseline concentration inside the cage was the balance of the release rate of CO₂ and the ventilation capacity with a fan and was slightly higher than that in the laboratory where the CO₂ baseline was 1,600–1,800 ppm.

Figure 5 shows the changes in the intensity of illumination in the laboratory. Intensity of illumination during the photophase, twilight phase, and the scotophase was ≈ 490 , 7, and 0 lux, respectively.

Diel changes in mosquito numbers recorded by photoelectric sensors for several laboratory colonies are shown in Fig. 6. There were significant increases in mosquito activity when CO₂ was intermittently

released for all species examined in the study (two-way analysis of variance [ANOVA], $P < 0.05$). This indicates that CO₂ provided a strong stimulatory effect for all mosquito species tested. Slight amounts of flight activity were recorded for both *Aedes* species (Fig. 6A and B), even when CO₂ was not released, indicating that the presence of heat and color (the black plastic recording disk) may have stimulated flight activity in these normally diurnal species. The diel activity peaks in *Aedes* mosquitoes, however, were not clearly shown in the absence of CO₂. The stimulating effect of CO₂ was more prominently shown for *Cx. quinquefasciatus* (Fig. 6C) and *An. stephensi* (Fig. 6D) than for the *Aedes* mosquitoes. The difference in the reaction of mosquitoes to CO₂ might be attributable to the difference in the importance of attractant cues, such as heat, color, and CO₂, to each mosquito species, that is to say, *Aedes* mosquitoes might use heat and visual information as attractant cues more than *Culex* and *Anopheles*. Two peaks of *Ae. albopictus* diel activity were observed, one from 1000 to 1200, and the second