BIONOMICS OF *ANOPHELES MACULATUS* COMPLEX AND THEIR ROLE IN MALARIA TRANSMISSION IN THAILAND

E.S. UPATHAM, C. PRASITTISUK**, S. RATANATHAM, C.A. GREEN, W. ROJANASUNAN, P. SETAKANA, N. THEERASILP**, A. TREMONGKOL, V. VIYANANT, S. PANTUWATANA* and R.G. ANDRE***

Departments of Biology and *Microbiology, Faculty of Science, Mahidol University, Bangkok; **Malaria Division, Department of Communicable Diseases Control, Ministry of Public Health, Bangkok, Thailand; and ***Walter Reed Army Institute of Research, Washington, D.C., U.S.A.

INTRODUCTION

Anopheles (Cellia) maculatus Theobald occurs throughout the Oriental zoogeographic region. It is highly variable in both its morphology and ability to transmit malaria (Reid, 1968), and may represent another complex of species having little or no morphological differentiation (Reid, 1970). Studies on its biological difference were done by several authors (Christophers, 1931; Reid *et al.*, 1966). Cytogenetic evidence for a complex of species within the taxon of this species was reported by Green *et al.*, (1985).

In Thailand, *An. maculatus* is a widespread species, particularly in the upper central, northern and northeastern parts of the country, and is found in forested and foothill areas. Evidently, it is one of the main malaria vectors in Malaysia, and has been incriminated only once in Wang district of Narathiwat province, southern Thailand, along the border with Malaysia. The breeding sites of this species are very similar to those of *An. minimus* which is prevalent throughout the year (Ismail *et al.*, 1974, 1975 and 1978).

There are no recent studies on the bionomics and vectors potential of *An. maculatus* complex in Thailand prior to this investigation. Therefore, a series of investigation were conducted into the life history, bionomics and role in transmission of this mosquito which might not only settle its status vis-a-vis malaria, but also might provide biological and/or environmental indicators which could be employed to signal changes in receptivity levels. The information obtained also could be used for planning of effective control strategies by the Thai Anti-Malaria Programme.

MATERIALS AND METHODS

Study areas: Two villages, Ban Moh Mai Khaen and Ban Yang Ko, each with 130 houses and 590 inhabitants and 212 houses and 600 inhabitants, respectively, were selected as study areas for this investigation (Fig. 1).

Ban Moh Mai Khaen is located in village no. 5, Mou Si canton, Pakchong district, Nakhon Ratchasima province, central Thailand. The area is mountainous and covered with forest. The main occupation of villagers is maize farming and forest reservation park labourers. This area has a long

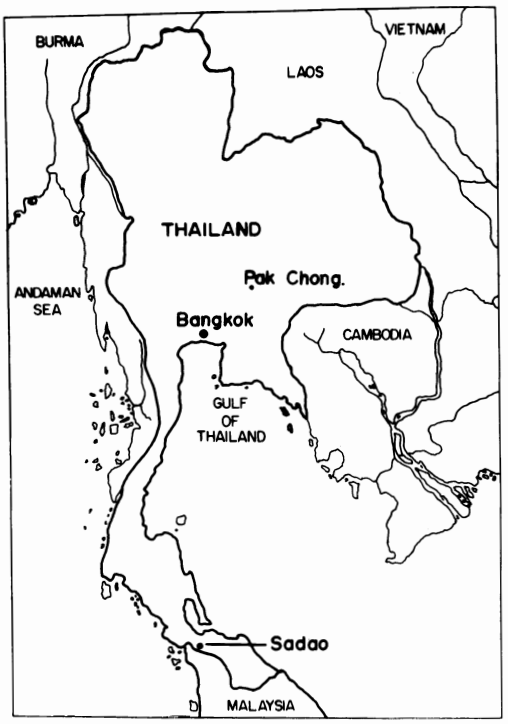

Fig. 1–Map of study areas.

history of malaria since the beginning of the malaria eradication programme. Residual indoor spraying with DDT at 2 g/m^2 has been used since 1960.

Ban Yang Ko is located in village no. 9, Prik canton, Sadao district, Songkhla province, southern Thailand. The houses are scattered in the form of small hamlets, and the area is covered with rubber plantations. Rubber tapping is the main occupation of the local people, facilitating malaria transmission in the area. As in Ban Moh Mai Khaen, residual indoor spraying with DDT was initiated in 1965, and is still continued.

Entomological study: Mosquito catchings on human and animal baits were conducted monthly, between January 1984 and June 1985, from 18 00 to 06 00 hours with 10 min rest for each hour. Four humans, 2 indoors and 2 outdoors, and one cow was used as bait to collect mosquitoes for 6 consecutive nights of each month studied.

The captured female mosquitoes were preliminary sorted into *An. maculatus* and other anophelines. Then, they were kept in mosquito cups marked with identification sites and hours of catching, and were held in a cooler at ambient temperature. All *An. maculatus* were blood fed until satiated at 24 00 and 06 00 hours each day to become half gravid in the following day. The catchers were supplied with appropriate prophylaxis against *Plasmodium* spp.

Identification: The half gravid female *An. maculatus* were knocked out individually with ether, and then were identified using a preprint of the morphological key of Rattanarithikul and Green (1986) (species. A = *sawadwongporni*, species. B forms E and F = *maculatus* s.s., species. C = *dravidicus*). The ovaries were dissected out and fixed in Carnoy's fixative for later polytene chromosome identification. The guts and salivary glands were then examined for oocysts and sporozoites. Owing to most of the specimens being used for the polytene chromosomal identification, only some specimens of *An. maculatus* were dissected to examine the tracheoles of ovaries to determine their age composition (Detinova, 1962). Based on the parous figures and field observation, the gonotrophic cycles of *An. maculatus* complex were designated 2 days for rainy and summer seasons (March–November) and 3 days for cool season (December–February). Hence, the probability of daily survival was calculated using the formula $p = \sqrt[G]{P}$ (where P = probability of daily survival, G = gonotrophic cycle, and P = proportion of parity) (WHO, 1975). Subsequently, the life expectancy was determined using the formula $1/(-\ln p)$ (Garrett–Jones and Grab, 1964).

All other anophelines caught were processed and identified the following morning, and were dissected to examine the tracheoles of the ovaries (Detinova, 1962). In parous specimens, the guts and glands were removed and examined for oocysts and sporozoites. The results are reported elsewhere.

Temperature and relative humidity were continuously recorded by a standard automatic thermohygrograph placed in a screen. Daily precipitation and hourly wind velocity were recorded during the nightly mosquito bite collections.

RESULTS AND DISCUSSION

During the 18-month study period, 21,785 (18 species) and 5,462 (13 species) anopheline mosquitoes were caught in Pakchong and Sadao, respectively (Table 1).

Morphological identification revealed 3 species of *An. maculatus* in Pakchong study area: species A, B form F and C, and one species in Sadao study area: species B form E (identified by polytene chromosome). The results of testing the morphological key against the polytene chromosomal identification showed that 2.1% of individuals were

Table 1
Total numbers of adult anopheline species collected at Pakchong and Sadao study areas, from January 1984 to July 1985 (NF = not found).

Species of *Anopheles*	Total numbers of mosquitoes (%)	
	Pakchong	Sadao
An. maculatus A	4,994 (22.9)	NF
An. maculatus B	1,067 (5.0)	1,357 (24.8)
An. maculatus C	29 (0.1)	NF
An. dirus	115 (0.5)	110 (2.0)
An. minimus	3,137 (14.4)	NF
An. aconitus	1,350 (6.2)	981 (18.0)
An. philippinensis	5,232 (24.0)	140 (2.6)
An. annularis	NF	12 (0.2)
An. barbirostris	1,094 (5.0)	1,220 (22.3)
An. campestris	281 (1.3)	575 (10.5)
An. barbumbrosus	5 (0.0)	NF
An. hyrcanus group	409 (1.9)	485 (8.9)
An. jamesii	1,775 (8.1)	NF
An. karwari	3 (0.0)	96 (1.8)
An. kochi	74 (0.3)	237 (4.3)
An. nivipes	1,050 (4.8)	NF
An. pseudojamesi	48 (0.2)	3 (0.1)
An. tessellatus	106 (0.5)	151 (2.8)
An. vagus	1,016 (4.7)	94 (1.7)
Total	21,785 (100)	5,462 (100)

misidentified using the key at the inception of the project (Table 2).

Density: During the 18-month study period, the density of *An. maculatus* complex found in Pakchong was approximately 4.5 times greater than in Sadao, with their total numbers of 6,090 and 1,344, respectively. In Pakchong, the numbers of *An. maculatus* species A, B form F, and C caught were 4,994 (82.0%), 1,067 (17.5%), and 29 (0.5%), respectively. In Pakchong, *An. maculatus* species A was the most dominant species, with its mean density per night of 46.24 ± 72.04 , followed by species B form F (9.88 ± 12.62) and species C (0.27 ± 1.88). In Sadao, *An. maculatus* species B form E was the only species found, with its mean density per night of 12.44 ± 11.21 (Table 3).

Climatic influence on seasonal density: The average air temperature and relative humidity throughout the 18-month study period were, respectively, 21.72° ± 2.50°C and 78.12 ± 8.32% RH for Pakchong, and 22.65° ± 1.75°C and 91.32 ± 3.7% RH for Sadao. The fluctuation of humidity was greater in Pakchong than in Sadao. The analysis of variance revealed that humidity in both study areas differed significantly ($F = 17.607$, $p < 0.001$).

Table 2

Testing the morphological key against the polytene chromosomal identification of *An. maculatus* complex in Pakchong and Sadao study areas.

Chromosomal identification	Morphological identification		
	Correct	Wrong	
A	618	14	Pakchong
B	159	12	
C	2	1	
G	0	1	
E	159	0	Sadao

Fig. 2 illustrates monthly densities of *An. maculatus* species A, B form F, and C, together with rainfall, air temperature and relative humidity at catching sites in Pakchong study area. Both species A and B form F were abundant during the rainy season, from July to October. The densities of both species rose in July, reached their peaks in October and decreased gradually until the next rainy season. Similarly, the peak density of species C also occurred during the rainy season, although only a few specimens were caught. Table 4 shows positive correlations of the densities of both species A and B form F with monthly rainfall and relative humidity

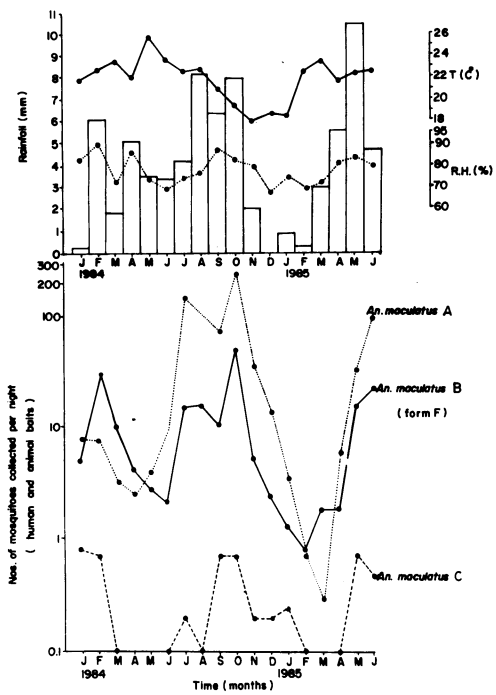

Fig. 2–Total numbers of *An. maculatus* complex caught monthly on human and animal baits, together with rainfall, air temperature and relative humidity at catching sites in Pakchong study area.

Table 3

Distribution and densities of *An. maculatus* complex (per night) at Pakchong and Sadao study areas, from January 1984 to June 1985 (* = number of specimens collected for 6 night, ** = number of specimens collected per night, NF = not found)

Catching time (months)	*An. maculatus* A			*An. maculatus* B				*An. maculatus* C	
	Pakchong		Sadao	Pakchong (form F)		Sadao (form E)		Pakchong	Sadao
1984									
January	48*	(8.0)**	NF	34	(4.3)	30	(5.0)	5 (0.8)	NF
February	47	(7.8)	NF	78	(13.0)	56	(9.3)	4 (0.7)	NF
March	20	(3.3.)	NF	56	(9.3)	71	(11.8)	0	NF
April	15	(2.5)	NF	25	(4.2)	159	(26.5)	0	NF
May	25	(4.2)	NF	17	(2.8)	199	(33.2)	0	NF
June	59	(9.8)	NF	13	(2.2)	204	(34.0)	0	NF
July	919	(153.2)	NF	88	(14.7)	41	(6.8)	1 (0.2)	NF
August	673	(112.2)	NF	93	(15.5)	28	(4.7)	0	NF
September	470	(78.3)	NF	62	(10.3)	0		4 (0.7)	NF
October	1540	(256.7)	NF	297	(49.5)	0		4 (0.7)	NF
November	232	(38.7)	NF	31	(5.2)	3	(0.5)	1 (0.2)	NF
December	82	(13.7)	NF	16	(2.7)	2	(0.3)	1 (0.2)	NF
1985									
January	21	(3.5)	NF	8	(1.3)	12	(2.0)	2 (0.3)	NF
February	4	(0.7)	NF	5	(0.8)	140	(23.3)	0	NF
March	2	(0.3)	NF	11	(1.8)	115	(19.2)	0	NF
April	34	(5.7)	NF	11	(1.8)	97	(16.2)	0	NF
May	200	(33.3)	NF	87	(14.5)	97	(16.2)	4 (0.7)	NF
June	603	(100.5)	NF	135	(22.5)	90	(15.0)	3 (0.5)	NF
Total	4994	(46.24)		1067	(9.88)	1344	(12.44)	29 (0.27)	

(r^2 = 0.4660, $p < 0.001$ and = 0.1884, $p < 0.05$, respectively, for species A; r^2 = 0.5186 and 0.2781, $p < 0.001$, respectively, for species B form F). Wind velocity was the only factor which exhibited significant negative correlations with the densities of both species A and B form F ($r = -0.1848$, $p < 0.05$; $r = -0.2640$, $p < 0.001$, respectively).

In Pakchong, the period from mid October to mid February is considered as the cool season, as well as the dry season. During this time, the densities of adult mosquitoes were low, which corresponded to the observed low numbers of mosquito larvae, owing to the paucity of breeding places.

Fig. 3 illustrates monthly densities of *An. maculatus* species B form E, together with rainfall, air temperature and relative humidity at catching sites in Sadao study area. The densities of species B form E were high during February to June of both years 1984 and

Table 4

Pearson correlation coefficients of the densities of *An. maculatus* complex collected monthly with meteorological factors at Pakchong and Sadao study areas, from January 1984 to June 1985.

An. maculatus complex	Meteorological factors				
	Rainfall		Wind velocity	Air temperature	Relative humidity
	Daily	Monthly			
Pakchong	N=108	N=108	N=84	N=108	N=108
A	−0.1313	0.4660	−0.1848	−0.1378	0.1884
	P=0.088	P=0.000	P=0.046	P=0.078	P=0.025
B (form F)	−0.0481	0.5186	−0.2640	−0.1101	0.2781
	P=0.311	P=0.000	P=0.008	P=0.128	P=0.002
Sadao	N=108	N=108	N=87	N=107	N=107
B (form E)	−0.0294	0.3478	−0.1089	0.4723	0.2733
	P=0.381	P=0.000	P=0.158	P=0.000	P=0.002

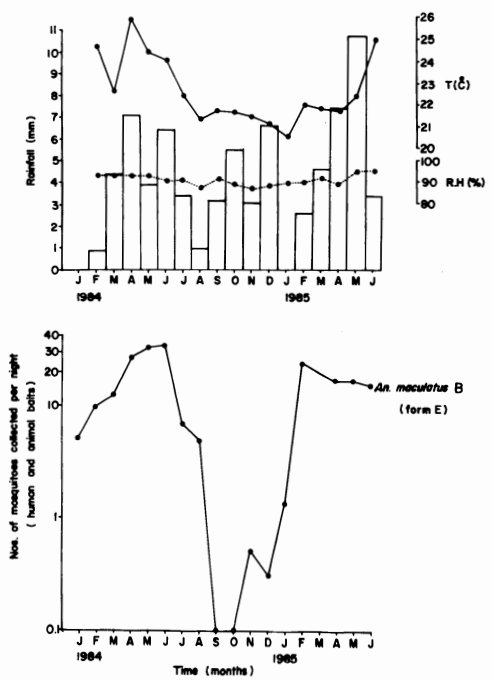

Fig. 3–Total number of *An. maculatus* species B form E caught monthly on human and animal baits, together with rainfall, air temperature and relative humidity at catching sites in Sadao-study area.

1985, but declined drastically during September to December 1984 owing to the northeastern monsoon season which washed away the mosquito larvae from their habitats. Table 4 shows significant positive correlations of the densities of species B form E with monthly rainfall, air temperature and relative humidity ($r = 0.3478$, 0.4723, and 0.2733, $p < 0.001$, respectively).

Table 5 shows monthly man-vector contact and host preference of *An. maculatus* complex in Pakchong and Sadao study areas, from January 1984 to June 1985. The results from both study areas indicated that the females of the *An. maculatus* complex preferred to feed on cattle rather than on human, that they tended to bite humans more outdoors than indoors, and thus exhibiting a zoophilic and exophagic behaviour. The ratios of mosquitoes biting human indoor to human outdoor and to animal were 0.1: 0.6: 54.9 at Pakchong, and 0.9 : 2.8 : 5.1 at Sadao (per man night or per baitnight). It is

interesting to note that *An. maculatus* species B (form E) at Sadao tended to associate with man more than *An. maculatus* complex at Pakchong (0.9 : 2.8 vs 0.1 : 0.6, respectively).

Biting cycles of *An. maculatus* species A, B (form F) and B (form E) on human and cattle interpreted in percentages of total collections in summer (February to May), wet (June to October), and cool seasons (November–January), from 18 00 to 06 00 hours, are shown in Fig. 4.

The total numbers of *An. maculatus* species A caught by human bait in summer, wet, and cool seasons were 10;99, and 8, respectively, and their biting cycles in summer and wet seasons are shown in Fig. 4a. The biting peaks of both seasons were at 19 00 h.

The total numbers of *An. maculatus* species B (form F) caught in summer, wet, and cool seasons were 15, 17 and 5, respectively, and their biting cycles in summer and wet seasons are shown in Fig. 4b. The biting peaks of both seasons were at 19 00.

The total numbers of *An. maculatus*

Fig. 4–Biting cycles of *An. maculatus* species A, B (form F) and B (form E) on human and cattle interpreted in percentages of total collections in summer (February to May), wet (June to October), and cool seasons (November to January), from 18 00 to 06 00 hours at catching sites in Pakchong and Sadao study areas.

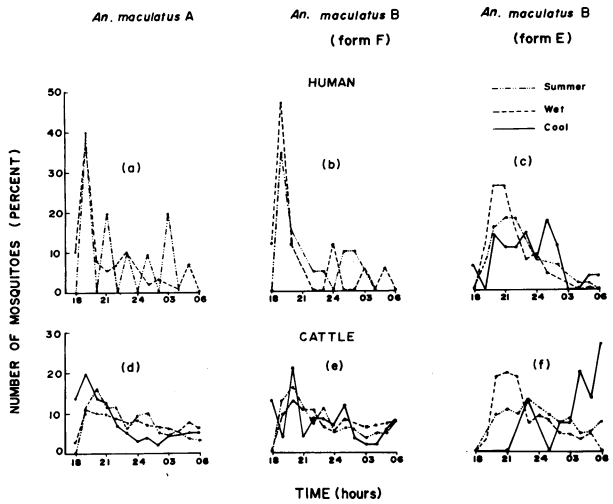

Table 5

Man– vector contact and host preference of *An. maculatus* complex at Pakchong and Sadao study areas, from January 1984 to June 1985. (* = number of specimens collected for 6 nights, ** = number of specimens collected per man night or per bait night).

Catching time	Type of bait at Pakchong			Type of bait at Sadao		
(month)	Human (per man night)		Animal	Human (per man night)		Animal
	Indoor	Outdoor	(per bait night)	Indoor	Outdoor	(per bait night)
1984						
January	3* (0.3)**	4 (0.3)	80 (13.3)	3* (0.3)**	16 (1.3)	11 (1.8)
February	0 (0.0)	4 (0.3)	122 (10.8)	11 (0.9)	23 (1.9)	22 (3.7)
March	12 (1.0)	0 (0.0)	64 (10.7)	1 (0.1)	14 (1.2)	56 (9.3)
April	0 (0.0)	3 (3.0)	37 (6.2)	7 (0.6)	63 (5.3)	89 (14.8)
May	1 (0.1)	0 (0.0)	41 (6.8)	49 (4.1)	53 (4.4)	97 (16.2)
June	0 (0.0)	2 (0.2)	70 (11.7)	68 (5.7)	68 (5.7)	68 (11.3)
July	1 (0.1)	26 (2.2)	981 (163.5)	6 (0.5)	17 (1.4)	18 (3.0)
August	0 (0.0)	15 (1.3)	751 (125.2)	2 (0.2)	14 (1.2)	12 (2.0)
September	1 (0.1)	5 (0.4)	530 (88.3)	0 (0.0)	0 (0.0)	0 (0.0)
October	0 (0.0)	17 (1.4)	1824 (304)	0 (0.0)	0 (0.0)	0 (0.0)
November	0 (0.0)	0 (0.0)	264 (44.1)	0 (0.0)	2 (0.2)	1 (0.2)
December	0 (0.0)	6 (0.5)	93 (15.5)	0 (0.0)	2 (0.2)	0 (0.0)
1985						
January	0 (0.0)	0 (0.0)	31 (5.2)	0 (0.0)	5 (0.4)	7 (1.2)
February	0 (0.0)	0 (0.0)	9 (1.5)	9 (0.8)	60 (5.0)	71 (11.8)
March	0 (0.0)	0 (0.0)	13 (2.2)	11 (0.9)	77 (6.4)	27 (4.5)
April	0 (0.0)	1 (0.1)	44 (7.3)	2 (0.2)	53 (4.4)	42 (7.0)
May	0 (0.0)	9 (0.8)	282 (47)	12 (1.0)	72 (6.0)	13 (2.2)
June	2 (0.2)	47 (3.9)	692 (115.3)	7 (0.6)	68 (5.7)	15 (2.5)
Total	20 (0.1)	139 (0.6)	5931 (54.9)	188 (0.9)	613 (2.8)	543 (5.1)

species B (form E) caught in summer, wet and cool seasons were 523, 250, and 28, respectively. Their biting peaks in summer and wet seasons were similar, between 20 00 and 22 00, and decreased gradually until morning. However, in cool season a smaller number of mosquitoes was caught, and most of them were caught between 20 00 and 02 00 h (Fig. 4c).

The total numbers of *An. maculatus* species A caught by animal bait in summer, wet and cool seasons were 337; 4165 and 375, respectively. Their biting peaks were similar for all 3 seasons, with major peaks during the first quarter of the night. They tended to bite throughout the night until 06 00 hours (Fig. 4d).

The total number of *An. maculatus* species B (form F) caught in summer, wet and cool seasons were 275; 671, and 81, respectively,

and their biting cycles are shown in Fig. 4e. Their biting patterns for summer and wet seasons were similar to those of *An. maculatus* species A, but their biting pattern in cool season fluctuated more than that of *An. maculatus* species A. This might be owing to the smaller number of *An. maculatus* species B (form F) caught (81 vs 375).

The total numbers of *An. maculatus* species B (form E) caught in summer, wet and cool seasons were 415; 113 and 15, respectively. Their biting patterns for all 3 seasons were different (Fig. 4f). In summer season, the biting peak was at 23 00 hours, and biting occurred thoughout the night until 06 00 hours, whereas in wet season, the biting peak was between 20 00 and 22 00 hours, and biting ended between 05 00 and 06 00 hours. In cool season, however, biting peaks occurred in the early morning, between 04 00 and 06 00 hours.

Age composition: The parity ratio and life expectancy of *An. maculatus* complex varied from month to month in both study areas (Table 6).

At Pakchong, the life expectancy of *An.*

Table 6

Proportion of parity and life expectancy (age composition) of *An. maculatus* complex.

	An. maculatus A		*An. maculatus* B form F		*An. maculatus* B form E	
	Pakchong		Pakchong		Sadao	
Time (month)	Prop. parity	Life expectancy	Prop.parity	Life expectancy	Prop. parity	Life expectancy
1984						
January	0.00 (4)	0.0	0.0 (1)	0.0	0.47 (15)	4.0
February	0.25 (4)	2.2	0.50 (8)	4.3	0.86 (7)	19.0
March	0.00 (0)	0.0	1.00 (2)		0.57 (21)	3.6
April	0.67 (3)	5.0	0.67 (6)	5.0	0.50 (34)	2.9
May	0.50 (2)	2.9	0.33 (3)	1.8	0.52 (44)	3.1
June	0.33 (12)	1.8	0.67 (6)	5.0	0.91 (32)	21.2
July	0.10 (169)	0.9	0.15 (13)	1.1.	0.06 (17)	0.7
August	0.38 (461)	2.1	0.48 (83)	2.7	0.56 (16)	3.5
September	0.56 (399)	3.5	0.59 (56)	3.8	0.00 (0)	0.0
October	0.28 (380)	1.6	0.30 (81)	1.7	0.00 (0)	0.0
November	0.68 (113)	5.2	0.70 (20)	5.6	0.00 (0)	0.0
December	0.61 (49)	6.1	0.67 (6)	7.5	1.00 (1)	–
1985						
January	0.31 (13)	2.6	0.67 (3)	7.5	0.25 (8)	2.2
February	1.00 (1)	–	0.67 (3)	7.5	0.61 (116)	6.1
March	0.50 (2)	2.9	0.78 (9)	8.1	0.74 (82)	6.6.
April	0.46 (26)	2.6	0.72 (11)	6.1	0.51 (65)	3.0
May	0.51 (144)	3.0	0.41 (69)	2.2	0.71 (68)	5.8
June	0.74 (528)	6.6	0.71 (124)	5.8	0.83 (87)	10.7

maculatus species A ranged from 1.6 to 6.6. days, and of *An. maculatus* species B form F from 1.1 to 8.1 days. This variation of life expectancy was probably due to rainfall. At Pakchong, during the rainy season, from August to October 1984, breeding places covered wide areas, and thus giving rise to high emergence of mosquitoes. This resulted in the low proportion of parity (or high proportion of nulliparity). At Sadao, the life expectancy of *An. maculatus* species B form E was long during the first half of both 1984 and 1985 years, especially in June (21.2 and 10.7 days). This finding also was probably due to rainfall.

Very few specimens of *An. maculatus* species C were caught at Pakchong, and most of them were not dissected because their ovaries were used for polytene chromosomal identification. Therefore, the data on the proportion of parity and life expectancy of species C are not available.

Parasitological data: In Sadao, out of 1,002 guts of *An. maculatus* complex dissected, only 0.6 oocyst rate, 6%, was recorded, and out of 1,024 salivary glands examined, there was no positive sporozoite detected.

In both Pakchong and Sadao areas, 49 and 62 salivary glands of *An. dirus* were dissected respectively, and 2.0% and 1.6% were positive for *Plasmodium falciparum* respectively.

In Pakchong, out of 3,428 guts of *An. maculatus* complex dissected, only 0.1 oocyst rate, 4%, was recorded, and out of 3, 448 salivary glands examined, there was no positive sporozoite detected.

The present investigations indicate that the *An. maculatus* group do not serve as malaria vectors under field conditions. Nevertheless, there is a possibility that they may act as potential malaria vectors.

SUMMARY

The bionomics of *Anopheles maculatus* complex and its role in malaria transmission were conducted in Pakchong and Sadao districts, Nakhon Ratchasima and Songkhla provinces, respectively, from January 1984 to July 1985.

In Pakchong, *An. maculatus* species A was the most dominant species, followed by species B form F and species C which was rare. The densities of species A and species B form F were high between July and November, with their peaks in October. Biting activities of both species occurred through out the night, with a major peak during the first quarter of the night on all seasons. In Sadao, only *An. maculatus* species B form E was detected with peak densities between February and June. Biting activities of this species varied according to seasons. The prevalence of mosquitoes was influenced by monthly rainfall, relative humidity and air-temperature.

All species of female *An. maculatus* complex studied prefered to feed on animal rather than on human, and tended to bite human more outdoors than indoors, and thus exhibiting a zoophilic and exophagic behaviour.

Life expectancies of *An. maculatus* species A ranged from 1.6 to 6.6 days, species B form F from 1.1 to 8.1 days, and species B form E from 0.7 to 21.2 days. The natural malaria infection rate was very low. Out of 4,430 guts dissected, only 0.23% were found infected with oocysts. There were no sporozoites detected in the 4,472 dissected salivary glands.

ACKNOWLEDGEMENTS

The authors wish to thank Dr. S. Phinichpongse, former Director of Malaria Division, Department of Communicable Diseases Control, Ministry of Public Health, Bangkok, Thailand for his support and the entomological staff to carry out this investigation. Special thanks to Mr. Weerapol Photichiti (Regional Entomologist), Mr. Sarayut Chaiaei and Mr. Chum Upathamnarakorn, team leaders of the Entomological Sections in Pakchong and Songkhla and their respective staff, Mr. Wanchad Sumanochitraporn, Mr. Sompob Boontim and Ms. Suchittra Jariyasesth. This investigation was supported by the United States National Academy of Sciences, Board on Science and Technology for International Development Research Grants Program, Number MVR-TH-583-29.

REFERENCES

CHRISTOPHERS, S.R., (1931). Studies on the anopheline fauna of India (Parts I–IV). *Rec. Mal. Sur. India, 2* : 305.

DETINOVA, T.S., (1962). Age grouping methods in Diptera of medical importance: with special reference to some vectors of malaria. *WHO. Monogr. Ser., No. 47* : 216 pp.

GARRETT–JONES, C. and GRAB, B., (1964). The assessment of insecticidal impact on the malaria mosquito's vectorial capacity, from data on the proportion of parous females. *Bull. WHO., 31* : 71.

GREEN, C.A., BAIMAI, V., HARRISON, B.A. and ANDRE, R.G., (1985). Cytogenetic evidence for a complex of species within the taxon *Anopheles maculatus* (Diptera:Culicidae). *Biol. J. Linnean Soc., 24* : 321.

ISMAIL, I.A.H., NOTANANDA, V. and SCHEPENS, J., (1974). Studies on malaria and responses of *Anopheles balabacensis balabacensis* and *Anopheles minimus* to DDT residual spraying in Thailand. I. Pre-spraying observations. *Acta Trop., 31* : 129.

ISMAIL, I.A.H., NOTANANDA, V. and SCHEPENS, J., (1975). Studies on malaria and responses of *Anopheles balabacensis balabacensis* and *Anopheles minimus* to DDT residual spraying in Thailand. II. Post-spraying observations. *Acta Trop. 32* : 206.

ISMAIL, I.A.H., PHINICHPONGSE, S. and BOONRASRI, P., (1978). Responses of *Anopheles minimus* to DDT residual spraying in a cleared forested foothill area in central Thailand. *Acta Trop., 35:* 69.

RATTANARITHIKUL, R. and GREEN, C.A., (1986). Formal recognition of the species of the *Anopheles maculatus* group (Diptera : Culicidae) occurring in Thailand, including the descriptions of two new species and a preliminary key to females. *Mosq. System., 18* : 246.

REID, J.A., (1968). Anopheline mosquitoes of Malaya and Borneo. *Stud. Inst. Med. Res.,* Kuala Lumpur, Malaysia.

REID, J.A., (1970). Systematics of malaria vectors. Anopheline systematics and malaria control, with special reference to Southeast Asia. *Misc. Pub. Entom. Soc. Amer., 7* : 56.

REID, J.A., WATTAL, B.L. and PETERS, W., (1966). Notes on *Anopheles maculatus* and some related species. *Bull. Indian Soc. Mal. Commun. Dis., 3* : 185.

WHO., (1975). Manual on practical entomology in malaria. II. Methods and techniques. *WHO Offset Pub. No. 13* : 121.