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SEASONAL ABUNDANCE AND PARITY RATES OF *ANOPHELES* SPECIES IN SOUTHERN THAILAND[1]

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ABSTRACT. Mosquitoes were collected with human bait, animal bait, and CO_2-baited light traps during a 13-month period in 5 villages located along the Myanmar border in southern Thailand. Altogether, 11,608 adult females consisting of 21 anopheline species were collected. Abundance of species varied according to the village where collections were made. In one village that was studied most intensely, *Anopheles minimus* was the most commonly collected species, representing 55.6, 10.6, and 56.5% of the human bait, cattle bait, and light trap collections, respectively. In addition, *An. minimus* was abundant throughout the year, whereas *Anopheles maculatus* was most commonly collected in July and August coincident with the rainy season. *Anopheles sawadwongporni,* abundant at the beginning and the end of the rainy season, was most commonly collected in June. *Anopheles dirus* was most abundant in April; however, it represented only 3.6% of all mosquitoes collected that month. Of 813 females collected in Palao-U Village and dissected, 38.9% were parous.

INTRODUCTION

Malaria in Southeast Asia is closely associated with forest or scrub ecotypes (Rosenberg et al. 1990). In peninsular Thailand these ecotypes are found along the western border with Myanmar. Because it is a zoogeographic transition zone between the Oriental and Indomalayan faunal areas, peninsular Thailand has an abundant and diverse mosquito fauna (Belkin 1962). Among the *Anopheles* (*Anopheles*), 26 species have been found in this region, which encompasses an area of only 84,000 km^2 (Harrison and Scanlon 1975). Little information exists regarding the biology of anopheline species in this region.

In preliminary surveys made in southern Thailand in May 1992, the species most frequently collected at human bait were *Anopheles maculatus* Theobald, *Anopheles minimus* Theobald, and *Anopheles sawadwongporni* Rattanarithikul and Green. More than 3% (3/82) of the *An. sawadwongporni,* a member of the Maculatus Complex (Rattanarithikul and Green 1987, Harbach 1994), tested by ELISA contained circumsporozoite antigen, indicating that they were potential vectors of *Plasmodium falciparum* (Rattanarithikul et al. 1995). In this study we report observations on the seasonal abundance and parity rates of the most common malaria vectors and potential malaria vectors in 5 villages located in Phetchaburi, Prachaub Khiri Khan, and Chumphon provinces in southern Thailand.

MATERIALS AND METHODS

Study area: Collections were made in Phetchaburi, Prachaub Khiri Khan, and Chumphon provinces in southern Thailand (centered at 12°31′N, 99°33′E; Fig. 1). These provinces cover an area of slightly more than 18,000 km^2 with approximately 50% of the land forested. Regular monthly collections were made at the village of Palao-U (350 population, 70 houses), Hua-Hin district, Prachaub Khiri Khan Province. Annual mean temperature in this province is 28°C with a relative humidity ranging from 87 to 100%, and the mean annual rainfall is 944 mm. This village is located in a valley adjacent to hills that are completely forested. Villagers maintain cats, dogs, cows, fowl, and pigs. Most villagers remain permanently on the Thai side of the border, earning their living by planting, farming, and seeking forest products. Cultivated areas consist of fruit orchards (pineapple, banana, and mango), and there is no rice agriculture in this district. Houses or cottages are made of wood and bamboo and are sprayed with DDT by government malaria-control workers once during the early rainy season in May and once after the rains in November.

Additional collections were made at various times in 4 other villages that are also located near the Myanmar border (Fig. 1). In general, the climate, living conditions, and geography of these villages are similar to those of Palao-U.

Mosquito collections: In October 1992, collection of mosquitoes was conducted on a regular basis at those study areas in which numerous females were collected during preliminary studies. These collections were continued throughout the year at Palao-U Village. Mosquitoes were col-

[1] The views of the authors do not purport to reflect the positions of the Department of the Army or the Department of Defense.

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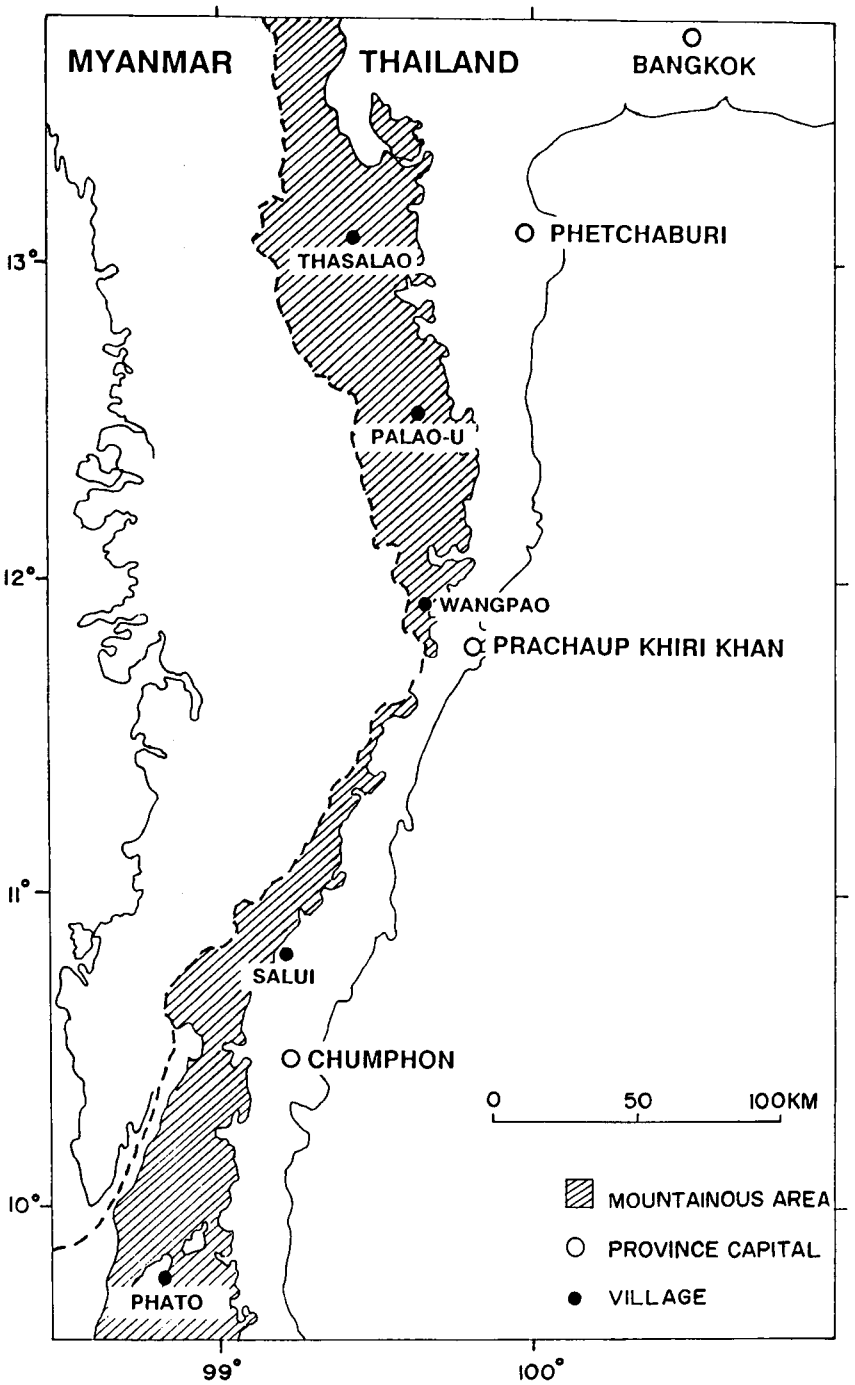

Fig. 1. Map of northern peninsular Thailand showing the 5 villages studied.

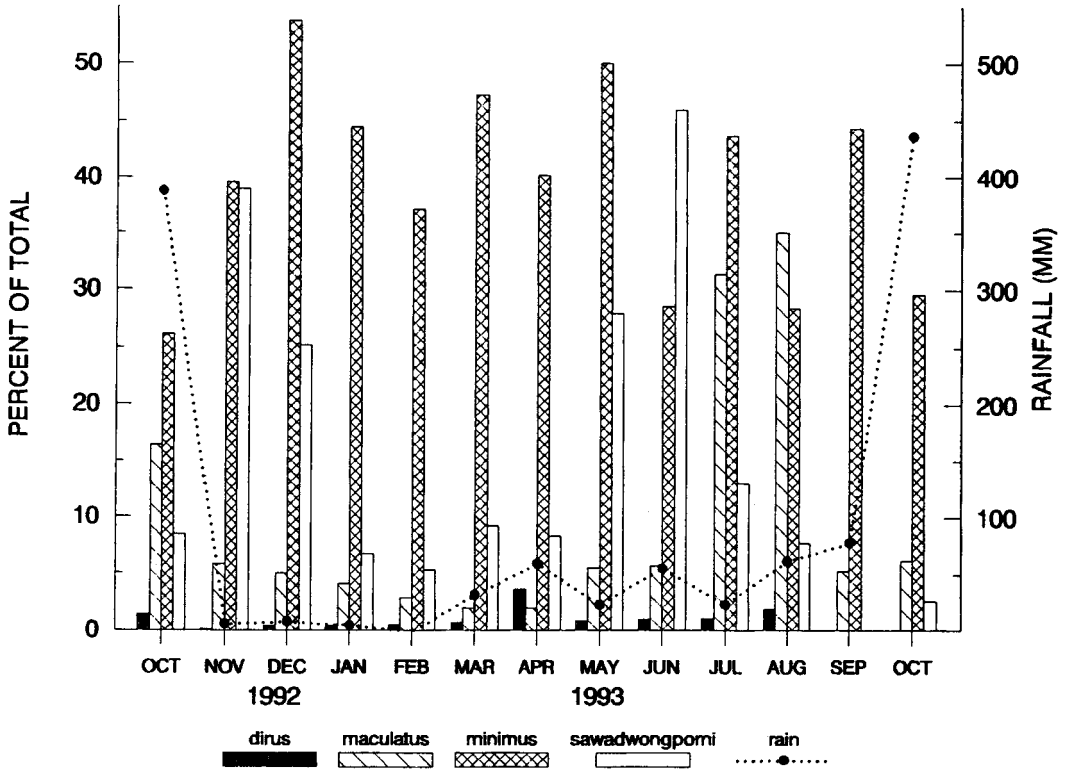

Fig. 2. Percentage of the monthly total number of *Anopheles* specimens collected by all trapping methods for 4 species and monthly rainfall in the village of Palao-U, southern Thailand.

lected with human bait, bovid bait, and light traps from October 1992 to October 1993.

Human bait (HB) collections were performed one or 2 nights per month by 2 groups of 2 people seated 2–3 m outside of a house between 1800 and 2400 h. Net traps (NT) baited with an adult cow were positioned overnight approximately 10 m from the man-biting area. Mosquitoes were collected with a battery-powered aspirator from the inside of the net at 0600 h each morning. Light trap (LT) collections were conducted with 3 CO_2-baited CDC light traps hung from trees (2 m above ground) 10–20 m from the dwelling between 1700 and 0700 h. All collections were carried out in a standardized manner to permit comparisons between collections and statistical evaluation. Monthly rainfall data were obtained from the Climatology Division, Meteorological Department, Ministry of Communications.

Anopheles mosquitoes were identified by species, and ovaries were examined to determine parity status (Detinova 1962). Mosquitoes of other genera were discarded and were not considered in this study. Field-collected specimens and progeny of some females were identified using keys to adult anopheline mosquitoes in Thailand (Harrison and Scanlon 1975, Harrison 1980, Rattanarithikul and Green 1987, Rattanarithikul and Panthusiri 1994). *Anopheles dirus* Peyton and Harrison consists of a species complex that can be distinguished only by cytogenetic or DNA probe techniques. We did not use these techniques in this study. Within the geographical range of the present study, *An. dirus* species A, C, and D have been found (Baimai 1988).

Whenever possible, specimens of the Maculatus Complex that were collected during the 2nd night of collections were bloodfed on human volunteers and the progeny were reared for taxonomic and cytogenetic studies of sibling species. Specimens of *An. maculatus* were identified by examining the ovarian nurse cell polytene chromosomes from half-gravid females (Green et al. 1985) and by examining mitotic chromosomes (Baimai et al. 1993). Consistent results were obtained by both methods. Statistical comparisons were made between parity rates for different species and seasons using multiple contingency table tests for independence (Centers for Disease Control 1994).

Table 1. Species and number of mosquitoes collected at human bait (HB), in net traps (NT), or in light traps (LT) in southern Thailand, October 1992–October 1993.

Mosquito species	No. mosquitoes collected					
	Thasalao			Palao-U		
	HB (8)[1]	NT (4)	LT (12)	HB (26)	NT (13)	LT (33)
Anopheles						
(*Cellia*)						
aconitus	3	1	2	87	38	41
annularis	—	—	—	1	—	—
culicifacies	—	—	2	—	—	—
dirus	20	1	2	31	2	5
indefinitus	—	—	—	—	—	—
jamesii	—	13	1	4	47	4
karwari	—	—	—	3	4	—
kochi	—	2	—	1	3	—
maculatus	1	35	14	144	158	6
minimus	74	38	81	1,113	137	242
nivipes	2	10	3	3	18	—
notanandai	—	—	—	—	1	—
philippinensis	—	1	—	—	3	—
pseudojamesi	—	1	—	—	—	—
sawadwongporni	5	27	40	387	352	44
subpictus	—	—	1	1	2	—
tessellatus	—	—	1	46	54	36
vagus	—	3	—	2	5	1
varuna	2	—	4	2	—	7
Anopheles						
(*Anopheles*)						
Barbirostris gp.	11	38	14	121	416	32
Hyrcanus gp.	—	11	13	56	57	10
Total/method	118	181	178	2,002	1,297	428
Total/all methods		477			3,727	

[1] Numbers in parentheses are numbers of man-nights or trap-nights.

RESULTS

We collected 11,608 adult females, representing 21 species or species groups of anopheline mosquitoes, in the 5 villages surveyed during the 13-month study period. The species and number of specimens of each species collected using different collection methods are shown in Table 1. *Anopheles minimus, An. maculatus, An. sawadwongporni, Anopheles barbirostris* Van der Wulp, and *Anopheles aconitus* Doenitz were the most commonly encountered *Anopheles* species.

Monthly variation in rainfall and the proportion of 4 species collected using all trapping methods in Palao-U are shown in Fig. 2. The dry season during this study occurred from November to February and the wet from March to October. *Anopheles minimus* was the most commonly collected species and represented more than 25% of the total anopheline collection for each month throughout the year. *Anopheles maculatus* represented more than 30% of the specimens collected in July and August. *Anopheles sawadwongporni* was abundant at the beginning and just after the end of the wet season, and in June it represented 46.1% of all specimens collected. *Anopheles dirus* represented a small proportion of the specimens collected in all months. It was most commonly collected in April, when it represented only 3.6% of the total collection.

The mean numbers of *Anopheles* collected per man or trap per night from the villages of Salui, Wangpao, Thasalao, and Phato by month for 4 species are shown in Table 2. As was observed in Palao-U, *An. minimus* was the most commonly encountered species in most months, particularly in HB and NT collections. *Anopheles maculatus* was the most commonly collected species in October 1992.

Table 1. Extended.

No. mosquitoes collected									
Wangpao			Salui			Phato			
HB (14)	NT (7)	LT (21)	HB (22)	NT (11)	LT (33)	HB (18)	NT (8)	LT (24)	Total (254)
2	9	2	93	183	293	23	2	5	784
—	—	—	—	10	3	0	1	0	15
—	13	5	—	—	—	—	—	—	20
—	—	—	5	1	4	28	2	6	107
—	1	—	—	—	—	—	—	—	1
—	1	—	—	40	11	—	38	21	180
—	1	—	—	—	—	16	1	26	51
—	—	—	—	21	20	—	4	6	57
50	180	3	35	65	22	106	33	77	929
67	176	286	534	83	252	1,977	3	440	5,503
1	1	—	2	50	6	2	237	1	336
—	—	—	—	—	—	—	—	—	1
—	1	—	—	1	1	—	17	—	24
—	—	—	4	50	—	—	—	—	55
12	169	90	1	0	1	2	—	3	1,133
—	7	1	—	3	—	—	—	—	15
—	1	—	—	8	39	—	—	2	187
—	—	—	2	31	3	—	—	—	47
—	—	—	4	20	6	—	—	—	45
9	99	15	17	683	155	9	222	17	1,858
—	32	—	—	10	11	6	48	6	260
141	691	402	697	1,259	827	2,169	564	654	
	1,234			2,783				3,387	11,608

The mean numbers of *Anopheles* collected per man or trap per night in Palao-U in each month for 4 species using HB, NT, and LT methods are shown in Table 3. *Anopheles minimus* was the predominant species, comprising 55.6% of HB, 10.6% of NT, and 56.5% of LT collections. *Anopheles sawadwongporni* and *An. maculatus* together comprised 26.5% of the HB, 39.3% of the NT, and 11.7% of the LT collections. *Anopheles dirus* was much less abundant, representing only 1.6% of the HB, 0.2% of the NT, and 1.2% of the LT collections.

We dissected 932 mosquitoes to determine their parity status. Almost 40% of all specimens examined were parous. The percentages of *An. dirus, An. maculatus, An. minimus,* and *An. sawadwongporni* collected in Palao-U that were parous for each month are shown in Table 4. There were no significant differences in parity rates between the dry season and the wet season for *An. minimus, An. maculatus,* and *An. sawadwongporni.* In the dry season, parity rates were significantly higher for both *An. minimus* (46%) and *An. maculatus* (57%) than for *An. sawadwongporni* (21%) (chi-square, $P < 0.05$). In the wet season, parity rates were significantly higher for *An. maculatus* (51%) than for *An. minimus* (41%) (chi-square, $P < 0.05$), and both species had rates somewhat higher than those of *An. sawadwongporni* (38%). Parity rates in the wet season were significantly higher for *An. dirus* (65%) than for *An. minimus* and *An. sawadwongporni* (chi-square, $P < 0.05$).

DISCUSSION

During a period of approximately average rainfall, substantial numbers of 21 species of *Anopheles* mosquitoes were collected in 254 human bait or trap-nights in villages in the 3 northernmost

Table 2. Mean number of *Anopheles* mosquitoes collected per man per night at human bait (HB) and per trap per night for net traps (NT) and light traps (LT) in the villages of Thasalao, Wangpao, Salui, and Phato, October 1992–October 1993.

	Number of man-or trap-nights			No. mosquitoes collected *An. dirus*		
Month	HB	NT	LT	HB	NT	LT
1992						
Oct.	2	1	3	0	0	0
Nov.	6	3	9	1.0	0	0.1
Dec.	8	3	9	0	0	0
1993						
Jan.	6	3	9	0.1	0.3	0.1
Feb.	6	3	9	0.1	0	0
Mar.	6	3	9	0.1	0	0
Apr.	4	2	6	1.5	0	0.2
May	4	2	6	2.0	0.5	0.3
Jun.	4	2	6	1.5	0	0
Jul.	4	2	6	0	0	0.2
Aug.	4	2	6	3.0	0.5	0.5
Sep.	4	2	6	2.3	0.5	0
Oct.	4	2	6	0.8	0	0.5

provinces of peninsular Thailand. This large species diversity emphasizes the importance of this region of Thailand in maintaining biodiversity of the anopheline fauna of Asia and its potential for malaria transmission. Comparable studies in other parts of Thailand and Asia reported finding fewer species (Wilkinson et al. 1970, Ismail et al. 1978, Amerasinghe and Munasingha 1988, Upatham et al. 1988). Of the 21 species collected, 15 are considered to be proven or potential vectors within Asia. In Thailand, *An. dirus* and *An. minimus* are considered the most important vectors (Scanlon

Table 3. Mean number of *Anopheles* mosquitoes collected per man per night at human bait (HB) and per trap per night for net traps (NT) and light traps (LT) in the village of Palao-U, October 1992–October 1993.

	Number of man-or trap-nights			No. mosquitoes collected *An. dirus*			*An. maculatus*		
Month	HB	NT	LT	HB	NT	LT	HB	NT	LT
1992									
Oct.	2	1	ND[1]	1.5	0	ND	17.0	1.0	ND
Nov.	2	1	3	0.5	0	0	17.0	3.0	1.0
Dec.	2	1	3	0.5	0	0	6.0	1.0	0
1993									
Jan.	2	1	3	0.5	0	0	5.5	0	0
Feb.	2	1	3	0.5	0	0	3.0	0	0
Mar.	2	1	3	0.5	0	0	0.5	2.0	0
Apr.	2	1	3	6.5	1.0	0.7	1.0	7.0	0
May	2	1	3	2.0	0	0	1.0	21.0	0.3
Jun.	2	1	ND	2.0	0	ND	4.5	13.0	ND
Jul.	2	1	3	0.5	1.0	0	1.5	54.0	0.3
Aug.	2	1	3	0.5	0	1.0	12.5	47.0	0.3
Sep.	2	1	3	0	0	0	0.5	6.0	0
Oct.	2	1	3	0	0	0	2.0	3.0	0

[1] ND = no data.

Table 2. Extended.

No. mosquitoes collected								
An. maculatus			*An. minimus*			*An. sawadwongporni*		
HB	NT	LT	HB	NT	LT	HB	NT	LT
23.0	133.0	0	2.5	17.0	0.3	1.5	28.0	1.0
0.2	12.3	1.9	5.5	12.7	4.3	0.7	14.7	1.7
1.8	5.3	0.7	1.8	12.0	15.1	0.4	10.3	2.3
4.2	9.0	0.1	2.8	9.7	12.1	0	10.0	0
5.7	1.7	0.2	81.0	13.3	4.4	0.2	4.7	0
2.2	1.7	6.0	60.8	1.7	26.8	0.3	0.3	0.4
0.3	11.0	0.5	14.3	22.5	40.8	1.3	19.0	15.0
0.5	0.5	1.2	10.8	27.5	5.2	0.3	5.0	0.2
2.0	3.0	0.3	332.5	3.0	25.3	0.3	0	0
0.5	9.0	0.3	49.0	2.0	5.8	0	0	0
5.3	5.0	1.7	14.0	7.5	3.7	0	0	0
3.3	12.0	0	9.5	5.0	0.5	0	0	0
3.0	4.5	2.0	3.0	0	0.8	0	0	0

and Sandhinand 1965, Prasittisuk 1985). *Anopheles sawadwongporni, An. barbirostris, An. aconitus, Anopheles hyrcanus* group and *Anopheles nivipes* Theobald, which were found to be abundant, are all considered potential vectors in Thailand (Rattanarithikul et al. 1995).

Table 3. Extended.

No. mosquitoes collected					
An. minimus			*An. sawadwongporni*		
HB	NT	LT	HB	NT	LT
19.0	18.0	ND	6.5	5.0	ND
101.0	3.0	24.3	107.0	48.0	4.0
68.0	0	1.0	29.0	6.0	0.3
43.5	4.0	9.7	6.0	2.0	1.3
21.5	0	11.3	5.0	0	0.3
26.5	15.0	1.3	3.5	5.0	0.7
50.5	3.0	25.3	2.0	18.0	5.0
102.0	16.0	2.0	4.0	114.0	1.3
33.0	44.0	ND	18.0	141.0	ND
34.5	6.0	2.0	8.5	3.0	1.3
22.5	12.0	0.7	3.0	9.0	0.3
21.0	14.0	1.3	0	0	0
13.5	2.0	1.7	1.0	1.0	0

The provinces of Chumphon, Phetchaburi, and Prachuap Khiri Khan each had malaria incidence rates more than 200/100,000 population in 1992 (Division of Epidemiology 1993). Villages in our study experienced much greater malaria incidence because they are located in close proximity to intact forests and clearings within forests that are suitable habitats for the known malaria vectors *An. minimus, An. maculatus,* and *An. dirus* to coexist. Malaria incidence at Palao-U during the first 12 months of the study was 60/350 population, based on passively detected cases (Director, Malaria Control Section V, unpublished data).

The collection of large numbers of *An. minimus* throughout the year, particularly at human bait, suggests that this species could be a potentially important vector at any time of the year. Ismail et al. (1974) reported that in northern Thailand *An. minimus* populations were lowest in August and September, but this pattern was not observed in the present study. In October 1993 in Thasalao and Wangpao, and in December and January in Salui and Phato, *An. maculatus* was more common than *An. minimus.* In Palao-U in June, *An. sawadwongporni* was the most abundant species. Parity rates for *An. minimus* were similar to those reported by Ismail et al. (1978) from elsewhere in Thailand.

During this study, we found *An. maculatus* forms B and E (Green et al. 1992) to be sympatric in Thasalao, Palao-U, and Wangpao,

Table 4. Parity rates for *Anopheles* species collected in Palao-U Village, October 1992–October 1993.

Month	Parity rate[1]				
	An. dirus	*An. maculatus*	*An. minimus*	*An. sawadwongporni*	Total
1992					
Oct.	ND[2]	50.0 (34)	55.3 (47)	20.0 (15)	47.9 (96)
Nov.	ND	0 (1)	60.4 (96)	14.6 (130)	33.9 (227)
Dec.	ND	75.0 (4)	0 (30)	44.4 (18)	21.2 (52)
1993					
Jan.	ND	66.7 (3)	ND	28.6 (7)	40.0 (10)
Feb.	0 (1)	50.0 (6)	ND	55.6 (9)	50.0 (16)
Mar.	100.0 (1)	100.0 (1)	75.0 (4)	16.7 (6)	50.0 (12)
Apr.	54.0 (13)	ND	38.6 (57)	ND	41.4 (70)
May	100.0 (1)	ND	38.5 (52)	ND	39.6 (53)
Jun.	75.0 (4)	33.3 (9)	43.9 (66)	50.0 (36)	46.1 (115)
Jul.	100.0 (1)	100.0 (3)	42.6 (68)	35.3 (17)	43.8 (89)
Aug.	ND	ND	ND	ND	ND
Sep.	ND	ND	40.5 (42)	ND	40.5 (42)
Oct.	ND	50.0 (4)	11.1 (27)	ND	16.1 (31)

[1] Percent parous (number examined).
[2] ND = no data.

whereas only form E was found in Salui and Phato (Rattanarithikul et al., unpublished data). *Anopheles maculatus* and *An. sawadwongporni* were collected most frequently host-seeking at human bait and bovid bait. Although based on small sample sizes, the high parity rates observed in January and February suggest that these species may have a potential role in malaria transmission during the dry season. Upatham et al. (1988) reported that parity rates were lower during the rainy season when there was a high emergence of mosquitoes. *Anopheles sawadwongporni* was very common during some months; however, few were collected in Salui and Phato (Table 1). Its range is now reported to extend to southern Thailand for the first time.

Anopheles dirus, the least frequently encountered malaria vector in our study, was collected primarily at human bait. As has been reported previously, population numbers peaked immediately after the end of the wet season and early in the wet season (Rosenberg et al. 1990) and were lowest during the dry season (Ismail et al. 1974).

Climate, geography, and other factors have an influence on mosquito populations and their consequential role in malaria transmission. More detailed analysis of the relationship between collection sites, larval habitats, and the population dynamics of mosquito species is required in future studies on the biology of malaria vectors in southern Thailand. The role of potential malaria vectors must be evaluated in light of many factors, such as survival and man-biting behavior, and may not be correlated with overall abundance.

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