Hyperendemic Malaria in a Thai Village: Dependence of Year-Round Transmission on Focal and Seasonally Circumscribed Mosquito (Diptera: Culicidae) Habitats

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ABSTRACT In a longitudinal study of hyperendemic malaria in a village in eastern Thailand (from October 1985 to November 1987), man-biting anopheline mosquitoes were collected for 16 man-nights per month in 22 of 26 mo. Mosquitoes were separated according to collection sites (inner, central, more populated, outer, peripheral, more forested), biting period, and parity, and then they were tested for sporozoite antigen using an enzyme-linked immunosorbent assay (ELISA). Abundance of *Anopheles dirus* Peyton & Harrison was greater in outer than inner village sites, with bimodal peaks in the postmonsoon (October–November) and early rainy (April or May) seasons. Parity rates at both sites were high in postmonsoon seasons (ẗ about 67%), low in cool dry (December–January, <56%) and monsoon (June–September, <60%) seasons, and variable in other seasons. Of 1,861 *An. dirus* collected, 16 (0.9%) were positive for *Plasmodium falciparum* (PF) and nine (0.4%) for *P. vivax* (PV), whereas of 886 *An. minimus*, one (0.3%) and three (0.8%) were PF- and PV-positive, respectively. Entomological inoculation rates (EIR) were higher in outer (ẗ PF = 0.91, PV = 0.34) than inner village sites (ẗ = 0.01 for PF and PV). The EIR of PF appeared bimodal, high in postmonsoon (October–November) and early rainy (April or May) seasons, low in monsoon seasons, and variable in other seasons. The vectorial capacity of *An. dirus* was higher than that of *An. minimus*, indicating that the two species were primary and secondary vectors, respectively. Human malaria prevalence data indicated that transmission depended greatly on the higher year-round vector abundance in outer than in inner village sites.

KEY WORDS Insecta, *Anopheles* spp., malaria transmission, Thailand

Anopheles dirus Peyton & Harrison is the most important malaria vector in several provinces in eastern Thailand along its mountainous border with Cambodia (Wilkinson et al. 1978, Rosenberg et al. 1990), although other vector species also occur there (Prasittisuk 1985). Originally considered a species of heavily forested mountainous regions, *An. dirus* has adapted to peripheral areas where natural forests were replaced with orchards, tea, coffee, and rubber plantations (Rosenberg et al. 1990). In Chantaburi Province, eastern Thailand, Rosenberg et al. (1990) conducted a study from 1983 to 1985 in Ban Phluang, a village where malaria was hyperendemic, describing in detail the epidemiology of malaria transmission. Only *An. dirus* was incriminated; 76% of infected mosquitoes contained *Plasmodium falciparum* Welch (PF) sporozoites, the remainder being infected with *P. vivax* Grassi & Feletti (PV). Transmission was focal even in the central, most populated area of the village, and afflicted villagers were largely asymptomatic. In the dry season, *An. dirus* larvae were found once in a deep, man-made well (Rosenberg et al. 1990).

We studied the same village from October 1985 to November 1987. Our objectives were to determine possible changes in malaria transmission dynamics, with emphases on malaria vector incrimination, focality of transmission, malaria prevalence–incidence, vector infection indices, seasonal and diurnal abundance, and *An. dirus* larval breeding habitats.

Materials and Methods

Study Sites. Details of the location, demography, dwellings, terrain, vegetative composition, and economy of the village, Ban Phluang, were described previously (Rosenberg et al. 1990). Four mosquito collection sites were designated sites A and B were the original inner (central, more populated) village collection sites of Rosenberg et al. (1990), site C was about 900 m distal of site A at a house surrounded by fruit orchards and about 400 m from the base of a hill containing mostly mature rubber trees, site D was about 900 m distal

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of site C at a house surrounded by orchards and about 600 m from the base of a densely forested mountain. Peripheral (outer village) sites C and D were more forested and less populated. The area between site D and the base of the mountain was interlaced with descending streams. The 51 houses in the study area were located mostly around sites A and B, with only nine houses located near sites C and D combined. All houses were sprayed by the Malaria Control Sector Office with DDT (2 g/m²) twice per year (April and August), with an extra spraying in December 1986.

Weather Data. Daily minimum and maximum temperatures and rainfall were recorded during the study by a designated village resident. Rainfall data came from the province weather station in Chantaburi, about 40 km distant.

Human Malariometry. Thin and thick smears of villagers ($n = 230$, $\bar{x} = 192$) were made bimonthly from October 1985 to June 1986 and monthly from July to September 1986 (first year). Thereafter, until November 1987, smears were made monthly (second year). Slides were Giemsa-stained, examined for malaria species, and quantified for trophozoite and gametocyte density per 500 leucocytes counted in the thick smear under an oil immersion lens (1,000×).

Treatment for malaria-positive villagers was provided by the Malaria Sector Office (Makham District) according to guidelines established by the Malaria Division, Ministry of Public Health. Adult cases of *P. falciparum* detected by the Malaria Division were given a single dose of three tablets containing 750 mg mefloquine, 1.5 mg sulfadoxine, and 75 mg pyrimethamine. Cases of *P. vivax* were given 1.5 g chloroquine base for 3 d and 15 mg primaquine for 14 d. Children received a proportionately smaller dose according to an age scale provided by the Thai Ministry of Public Health.

Prevalence was determined monthly, counting only villagers who provided ≥7 smears (first year) or ≥8 smears (second year). New cases (incidence) were estimated monthly by dividing the number of villagers with gametocytes (or parasite density >99 per 500 leukocytes) by the total who provided smears. Seasonal prevalence and incidence were obtained by dividing the total of PF (or PV) positive villagers or new PF (or PV) cases, respectively, per season by the total who provided smears in the season.

Mosquito Collections. Human-baited mosquito collections were made concurrently by two pairs of collectors at two sites per night for four consecutive nights each month (total, 4 man-nights per site) during the full moon. During the first year, collections at sites A and B were alternated nightly with those at sites C and D, whereas during the second year, collections at sites A and D were alternated with those at sites B and C. Collectors sat outdoors in chairs within 10 m of collection site houses. Occasional dry ice-baited ultraviolet light trap collections were made at the same sites when man-baited collections were not scheduled. Collections were made between 1800 and 0450 hours. Collectors worked 50 min/h until 2350 hours and were replaced at 0010 hours by a second shift of workers who collected in the same manner until 0450 hours. Details of collection and handling methods for captured mosquitoes are given elsewhere (Rosenberg et al. 1990).

Larval mosquito surveys were conducted during the same collection periods. During the first 8 mo, potential breeding sites of *An. dirus* were sampled to determine larval habitats. Deep wells were sampled with a bucket on a rope or with a plankton net. Other habitats were sampled with shallow enamel pans or long-handled dippers. Thereafter, samples were taken during each monthly survey at types of habitats (unless dry) that previously had yielded *An. dirus* larvae.

Examination of Adult Mosquitoes. The morning after a biting collection, anopheline species were identified, and the ovarian tracheoles were examined for parity (Detinova 1962). During the first 15 mo of the study, the head and thorax (HT) of each specimen were bisected from the abdomen (AB). Specimens were placed in separate 1.5-ml centrifuge tubes containing 50 or 100 μl of blocking buffer and frozen at −70°C on dry ice for future circumsporozoite antigen (CS) detection by ELISA (Wirtz et al. 1987). During the last 11 mo of the study, midguts were dissected and stained for oocysts, and salivary glands (SG) were bisected with fine dissection needles, one gland for microscopic examination of sporozoites and the other gland for testing by ELISA. Nulliparous mosquitoes were not dissected but were bisected to obtain HT and AB for ELISA as in the first 15 mo of the study. After dissections in May–July 1987, bodies of 177 mosquitoes were tested with an *An. dirus* species D-specific DNA probe to determine the proportion of *An. dirus* species D in the total *An. dirus* collections (Panyim et al. 1988).

ELISA Methods. The ELISA methods were those of Wirtz et al. (1987), with minor modifications. Thawed specimens were diluted to 350-μl or 400-μl volumes of blocking buffer just prior to analysis. Negative controls consisted of laboratory reared *An. dirus*, *Anopheles maculatus* Theobald, *Anopheles minimus* Theobald, and *Anopheles philippinensis* Ludlow. Three aliquots of each of the four species (total, 12 wells) were tested in each plate. Quantitative positive controls consisted of hemacytometer-counted SG sporozoites (Nonidet P-40-treated) and recombinant PF protein (Young et al. 1985) or synthetic PV peptide (Arnot et al. 1985, McCutchan et al. 1985) standardized against counted PF or PV sporozoites (from direct feeding of *An. dirus* on gametocytemic Thai patients [per Wilkinson et al. 1972]). Absorbance values (414 nm) were recorded 60 min after the addition of substrate using an ELISA microtiter plate reader (Titertek MCC, Flow Laboratories, McLean, Va.) coupled to an IBM-PC-XT computer used to store data

Mosquito triturates with absorbance values greater than the mean plus three SD of the 12 negative controls were retested. Serial 2-fold dilutions of CS antigen or counted sporozoites were used as quantitative positive controls in confirmatory tests. Mosquitoes were called CS antigen-positive if absorbance values exceeded the mean plus three SD of 12 negative control mosquitoes (Goldberg 1960) and exceeded the minimum detectable positive control sporozoite values (usually 6–12 sporozoites/50 μl). Sporozoite equivalents per positive mosquito HT or SG, based on detected CS antigen, were calculated from appropriate standard curves (per Wirtz et al. 1987) after correction for dilutions (Collins et al. 1984).

Vector Data, Calculations, and Analyses. Species, collection site, parity, biting period, and the presence–absence of CS antigen were determined for each specimen. From these data, the sporozoite antigen-positive rates, entomological inoculation rates (EIR), and vectorial capacity (VC) were calculated for each season. Entomological inoculation rates were defined as the number of sporozoite antigen-positive *An. dirus* or *An. minimus* divided by the number of man-nights of collection at the site. Vectorial capacity was $C = (ma)ap^n/-\ln p$, where ma is the average daily man-biting rate, a is the daily man-biting habit, p is the daily survival rate estimated from the parity rate, and n is the length of sporogonic cycle (Macdonald 1952). For our purposes, ma was the mean of bites per man per night, a was assumed to be 0.333, and n for PF and PV was assumed to be 12 d for PV and PF (Rosenberg et al. 1990), based on a laboratory development temperature of 26°C because no field estimates were available. Daily survival (p) was calculated from the parity rate (pr) according to $p = pr^{1/x}$ (Davidson 1954), where x is a gonotrophic duration of 3 d.

Estimates of a for *An. dirus* range from 0.25 (Ismail et al. 1975) to 0.33 (Rosenberg et al. 1990). Its feeding preference for primates is well documented (Wharton et al. 1964). Estimates of a for *An. minimus* range from 0.35 (Ismail et al. 1975) to 0.50 (Ratanatham et al. 1988). *An. minimus* prefers cattle to man, given a choice (Ratanatham et al. 1988). However, the only large mammals aside from humans in the village were dogs, not documented as preferred hosts.

Peak biting periods were determined by comparing mosquitoes collected in each of four biting intervals (1800–2100, 2101–2400, 0001–0300, and 0301–0500 hours). Vector abundance by sites (either two or four sites), seasons (either five or 11), and biting periods (four) and proportions of parous mosquitoes were analyzed by analysis of variance (ANOVA) and least significant difference (LSD, rejection level of 0.05 unless otherwise specified) after calculating mosquito bites per man per night, transformed to their square roots (SQRT). Overall site and seasonal mosquito parity differences were analyzed by Pearson's χ^2 on counts of parous and nulliparous mosquitoes, whereas proportions parous by biting period were analyzed by ANOVA after arc sine transformation. The EIR and VC were compared using the correlation coefficient (r) or Spearman correlation (Sr). Differences in malaria prevalence were determined using Pearson's χ^2 and comparisons with incidence-utilized r (Statistix, NH Analytical Software, Roseville, Minn.).

Results

Weather. Seasonal temperature fluctuations in Chantaburi Province were moderate. However, combinations of rainfall and temperature patterns yielded distinct seasons defined as follows: postmonsoon, cool dry, hot dry, early rainy, and monsoon seasons (Fig. 1).

Mosquito Collections. The most abundant species caught were *An. dirus* and *An. minimus* (Table 1). Using species-specific DNA probes on 177 *An. dirus* s.l., 175 mosquitoes (99%) were *An. dirus* s.s. and two (1%) were *An. dirus* species D (S. Panyim, unpublished data). This composition corresponded closely with our morphological identification of *An. dirus* s.s. (based on Peyton & Harrison [1979]). Other anopheline species and numbers collected are listed in Table 1.

Site D yielded the largest number of adult anophelines (1,228), followed by sites C (988), A (367), and B (364). *An. dirus* constituted 85, 54, 40, and 36% of all anophelines collected at these sites, respectively. *An. minimus* were caught in inverse proportion to *An. dirus* collected at each site (i.e., 4, 14, 31, and 36% of collections at sites D, C, A, and B, respectively). The grand means of *An. dirus* parity rates ranged from 61% (site D) to 70% (site B). Similarly, the grand means of *An. minimus* parity rates ranged from 44% (site D) to 64% (site B). The grand means of parity rates for other anopheline species combined ranged from 33% (site A) to 53% (site B). The mean abundance (bites per man per night) of *An. dirus* was higher at site D (SQRT $\bar{x} = 3.2$) than at other sites in the 11 seasons of collection ($F = 6.3$, df = 3, 30, $P = 0.002$, LSD = 0.7), whereas sites A and B (SQRT $\bar{x} = 1.2$ and 1.1, respectively) were clearly similar. The mean abundance at site C (SQRT $\bar{x} = 2.3$) did not differ from site D when a rejection level of 0.01 (LSD = 0.9) was used for comparison, indicating their similarity. These findings corresponded to proximity of sites and terrain similarities. For most purposes, therefore, data were combined for sites A and B and for sites C and D to facilitate analyses (hereinafter designated sites AB and sites CD, respectively).

Seasonal and Diurnal Biting Patterns of Vector Species. Monthly mosquito collections for sites AB and CD, summed across five seasons (Fig. 1), indicated that abundance of *An. dirus* and *An. minimus* was bimodal, with a primary peak in the postmonsoon season and a secondary peak in the early rainy (*An. dirus*) and cool dry (*An. minimus*)

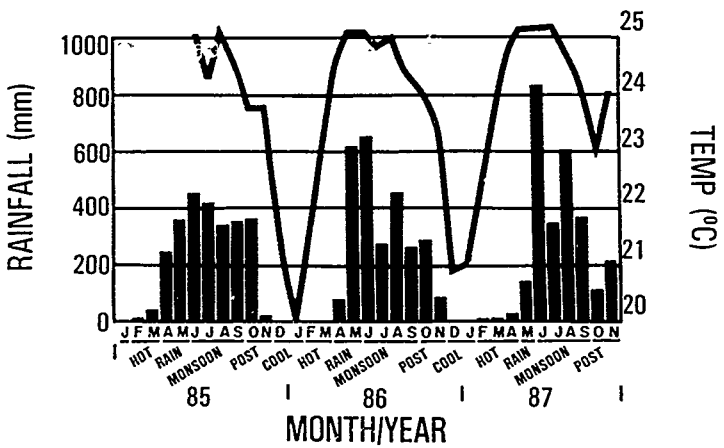

Fig. 1. Total monthly rainfall (mm) in Chantaburi Province and mean monthly minimum temperatures (°C) in Ban Phluang, 1985 to 1987. Bar graphs show rainfall, line graphs display temperatures. Postmonsoon (Post), cool dry (Cool), hot dry (Hot), early rainy (Rain), and monsoon seasons are indicated on the abscissa.

seasons (Table 2). The mean abundance of *An. dirus* was greater at sites CD and AB ($\bar{x} \pm 2$ SE = 9.0 ± 6.2 versus 1.5 ± 0.8/man per night, $P < 0.05$). Comparing abundance of *An. dirus* among sites, seasons, and biting periods indicated greatest abundance in postmonsoon and early rainy seasons (SQRT $\bar{x}$ = 3.4 and 2.2/man per period, respectively), other seasons were significantly lower, especially the hot dry seasons (SQRT $\bar{x}$ = 1.1/man per period, $F = 22.4$; df = 4, 31, $P < 0.001$, LSD = 0.6). Similarly, abundance of *An. dirus* was greatest from 2100 to 2400 hours (SQRT $\bar{x}$ = 2.4/man per period) and least from 0300 to 0500 hours (SQRT $\bar{x}$ = 1.4/man per period), with other periods similar to one or the other of the extreme periods ($F = 6.4$; df = 3, 31; $P = 0.002$; LSD = 0.5). Biting activity occurred earlier in cool dry seasons, with 35–40% of *An. dirus* caught before 2100 hours, about double the percentage biting in this period in other seasons (14–19%) (Fig. 2).

Overall proportions of parous *An. dirus* at sites AB and CD did not differ (Pearson's χ^2, $P = 0.15$). However, proportions of parous mosquitoes in postmonsoon seasons were high and similar among sites, but were low in cool dry and monsoon seasons (Table 2). Similarly, although overall proportions of parous *An. dirus* collected did not differ across five seasons, they were higher at sites CD than at AB in early rainy seasons ($\chi^2 = 14.3$; $P < 0.001$), whereas the reverse was true in hot dry seasons ($\chi^2 = 6.6$, $P < 0.01$) According to biting period, proportions of parous mosquitoes caught (arcsine-transformed and normalized) were greatest from 2100 to 2100 hours ($\bar{x} = 0.39$) and least from 0300 to 0500 hours ($\bar{x} = 0.07$), whereas periods 1800–2100 hours ($\bar{x} = 0.26$) and 2100–0300 hours ($\bar{x} = 0.27$) were similar to the peak period ($F = 7.3$, df = 3, 31, $P < 0.001$, LSD = 0.15) (Fig. 2). Parity rates of *An dirus* generally increased in consecutive biting periods up to 0300 hours in most seasons (not significant) but varied after 0300 hours However, in periods of low abundance (i.e., hot dry seasons at sites AB), parity rates in consecutive periods fluctuated.

Table 1. *Anopheles* species, number collected, and percentage of total collected from October 1985 to November 1987

Anopheles Species[a]	No.	%
aconitus Donitz	41	1.3
barbirostris Reid group	102	3.3
campestris Reid	88	2.8
dirus Peyton & Harrison	1,861	60.0
hyrcanus Reid group	87	2.8
karwari (James)	96	3.1
maculatus Theobald	52	1.7
minimus Theobald	386	12.5
nigerrimus Giles	49	1.6
nivipes (Theobald)	29	0.9
nitidus Harrison, Scanlon & Reid	73	2.3
peditaeniatus (Leicester)	97	3.1
sawadwongporni Rattanarithikul & Green	22	0.7
tesselatus Theobald	80	2.6

[a] Species with <11 mosquitoes each *Anopheles annularis* Van der Wulp, *An argyropus* (Swellengrebel), *An crawfordi* Reid, *An. kochi* Donitz, *An philippinensis* Ludlow, *An pursati* Laveran, *An sinensis* Wiedemann, *An varuna* Iyengar, *An. umbrosus* (Theobald), and *An. wharton* Reid.

Table 2. Comparison of parity rates, infection rates, number of ELISA-positive mosquitoes by season, collection site, and species

Season	*Anopheles* species	SITES AB[a]							SITES CD[a]						
		n (% P)[b]	PFINF *n* (%)[c]	PVINF *n* (%)[d]	EIRF[e]	EIRV[f]	MA[g]	VC[h]	*n* (% P)	PFINF *n*(%)	PVINF *n*(%)	EIRF	EIRV	MA	VC
Postmonsoon	*dirus*	160 (69)	0	1 (0 6)	0	0 03	4 00	0 75	971 (64)	9 (0 9)	3 (0 3)	0 23	0 08	24 28	4 55
	minimus	104 (64)	0	0	0	0	2.60	0 31	67 (74)	0	0	0	0	1.85	0 40
Cool	*dirus*	21 (55)	0	0	0	0	0 82	0 03	107 (45)	3 (2 8)	2 (1 9) (1 mixed)[i]	0 13	0 08	4 46	0 08
Dry	*minimus*	53 (55)	1 (1 9)	0	0 04	0	2.21	0 11	32 (38)	0	0	0 01	0	1 33	0 04
Hot	*dirus*	12 (42)	1 (8 3)	0	0 04	0	0 33	<0 00	93 (85)	0	0	0	0	2 07	3 49
Dry	*minimus*	16 (63)	0	1 (6.3)	0	0 03	0 86	0 09	35 (84)	0	1 (2.9)	0	0 03	1.09	0.81
Early	*dirus*	30 (83)	0	1 (3.3)	0	0 06	1 88	0 07	172 (48)	4 (2 3)	0	0 25	0	10.75	0.24
Rainy	*minimus*	9 (67)	0	0	0	0	0 56	0 09	16 (50)	0	1 (6.3)	0	0 06	1.00	0 03
Monsoon	*dirus*	51 (59)	0	0	0	0	0 56	0 07	232 (50)	0	1 (0 4)	0	0 02	3 63	0 10
	minimus	12 (50)	0	0	0	0	0 19	0 01	24 (50)	0	0	0	0	0 33	0 01
Total	*dirus*	274 (66)	1 (0 4)	2 (0 7)	0 01	0 01	1 56	0 33	1,577 (63)	16 (1 0)	6 (0 4) (1 mixed)[i]	0 91	0 34	8 96	1 09
	minimus	205 (60)	1 (0.5)	1 (0 5)	0 01	0 01	1 16	0 01	181 (60)	0	2 (1.1)	0	0 01	1.03	0 09

[a] Sites AB, outer village; sites CD, inner village.
[b] *n* (%), number and percentage parity rates.
[c] PFINF, *P. falciparum* infection rates, *n*, number of ELISA-positive mosquitoes.
[d] FVINF, *P. vivax* infection rates; *n*, number of ELISA-positive mosquitoes.
[e] EIRF, entomological inoculation rate, *P. falciparum*.
[f] EIRV, entomological inoculation rate, *P. vivax*.
[g] MA, mosquitoes per man-night
[h] VC, vectorial capacity
[i] mixed, mixed infection of *P. falciparum* and *P. vivax*

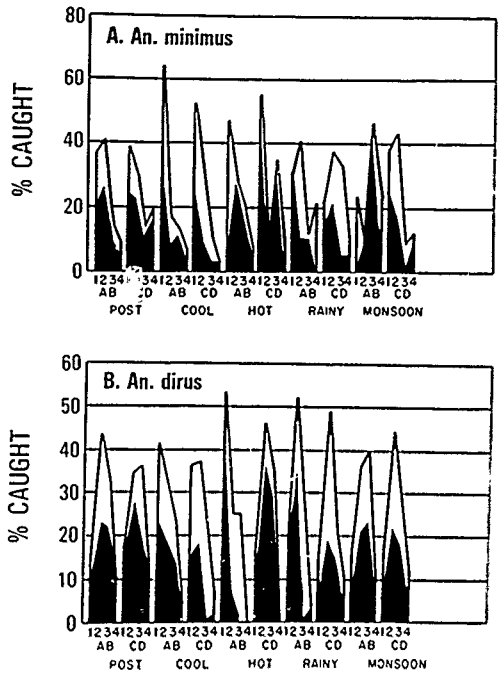

Fig 2. Biting activity and proportion of parous mosquitoes at inner village sites (AB) and outer village sites (CD) summed by season (see Fig 1). Biting activity is the percentage of total mosquitoes caught (unshaded areas) in periods 1 (1800–2100 hours), 2 (2101–2400 hours), 3 (0001–0300 hours), and 4 (0301–0500 hours) Proportions of parous mosquitoes in each period are shown as shaded areas. (A) *An. minimus*. (B) *An. dirus*

Mean abundance of *An. minimus* was similar among sites AB and CD (SQRT $\bar{x}$ = 1.2 and 1.1/man per night, respectively, P = 0 69). Mean abundance of *An. minimus* was greatest in postmonsoon and least in monsoon seasons (SQRT $\bar{x}$ = 1.4 and 0 5/man per period, respectively), but in other seasons it was similar to one or the other of the extreme seasons (F = 8 5; df = 4, 31; P < 0 001; LSD = 0 33). Mean abundance of *An. minimus* was highest from 1800 to 2100 hours (SQRT $\bar{x}$ = 1 3/man per period) and least from 0300 to 0500 hours (SQRT $\bar{x}$ = 0 7/man per period), whereas from 2100 to 2400 and 2400 to 0300 hours, it approximated the first or last periods, respectively (F = 8.1, df = 3, 31, P < 0 001, LSD = 0 15). Overall, parity rates of *An minimus* did not differ by sites or seasons. Proportions of the total of parous mosquitoes caught (arc sine transformed and normalized) decreased in consecutive periods from 1800 to 2100 hours ($\bar{x}$ = 0 37), 2100 to 2400 hours ($\bar{x}$ = 0 27), and 2400 to 0300 hours ($\bar{x}$ = 0.24), with the proportion from 0300 to 0500 hours ($\bar{x}$ = 0.12) significantly lower than the first period (F = 3 9, df = 3, 31, P = 0.02, LSD = 0.16) (Fig 2). Parity rates generally increased in consecutive periods up to 2400 hours (not significant), but varied after 2400 hours.

Anopheles peditaeniatus (Leicester) were caught primarily in late monsoon and postmonsoon seasons, usually before 2400 hours. Abundance and parity rates of *An. peditaeniatus* did not differ among sites, but parity rates generally increased in consecutive biting periods.

ELISA-Positive Mosquitoes. A total of 29 CS antigen-positive mosquitoes was collected Of these, 13 of 1,120 (1 2%) *An. dirus* and 2 of 176 (1.1%) *An. minimus* collected from Octobr 1985 to December 1986 were HT-positive. From January to

Table 3. Sporozoite equivalent values of *P. falciparum* and *P. vivax* circumsporozoite antigen-positive mosquitoes in bisected heads-thoraces or dissected salivary glands

Anopheles species	*Plasmodium falciparum*[a]				*Plasmodium vivax*[a]			
	n	HT	*n*	SG	*n*	HT	*n*	SG
dirus	7	1,281 (245–3,941)	9	1,272 (168–5,775)	6	365 (12–1,316)	3	2,311 (350–11,200)
minimus	1	210	1	168	2	63 (42–84)	—	—
peditaeniatus	1	5,600	—	—	—	—	—	—

Values are as geometric means (range).
[a] HT, bisected heads-thoraces, SG, dissected salivary glands.

November 1987, SG-positive mosquitoes included 10 of 768 (1.3%) *An. dirus*, 1 of 212 (0.5%) *An. minimus*, and 1 of 53 (1.9%) *An. peditaeniatus*. One each of *An. dirus* and *An. minimus* also were HT-positive in 1987, but they were not dissected because they appeared nulliparous. Of 10 *An. dirus* which were ELISA SG-positive in 1987, sporozoites were seen in only four specimens. Sporozoites were not seen in the *An. minimus* and *An. peditaeniatus* that were SG-dissected and positive by ELISA. However, an *An. minimus* from a light trap collection was PF-positive by ELISA and exhibited sporozoites. A man-biting *An. barbirostris* exhibited sporozoites in SG but was ELISA-negative.

No differences in sporozoite equivalent values of PF and PV in SG of positive *An. dirus* were seen. *An. dirus* appeared to have higher sporozoite equivalent values for PF and PV than *An. minimus*. These values also appeared higher for PF than PV in HT of *An. dirus* and *An. minimus*. However, high variability among specimens precluded statistical corroboration of these apparent differences (Table 3).

The proportion of CS antigen-positive mosquitoes in vector species differed by site and season (Table 2). *An. dirus* constituted 83% of the 29 positive mosquitoes (89 and 70% of PF- and PV-positive mosquitoes, respectively). Positive mosquitoes were most abundant during postmonsoon seasons (14 of 29, 48%). Thereafter, the number of ELISA-positive mosquitoes declined until early rainy seasons, when a secondary peak of six positive mosquitoes (four PF-positive) was detected. Only one positive mosquito was caught during two monsoon seasons (Fig. 3). Seasonal distribution of PF- and PV-positive mosquitoes was similar, with 68 and 55% of PF- and PV-positive mosquitoes, respectively, caught in postmonsoon or cool dry seasons. Sites CD yielded 83% of all positive mosquitoes, including 88 and 89% of positive *An. dirus* and PF-positive mosquitoes, respectively. Mosquito positivity rates were inversely proportional to mosquito abundance in all but monsoon seasons (Table 2). Most positive mosquitoes (21 of 29, 72%) were caught before 2400 hours, whereas only two (7%) were caught after 0300 hours.

The EIR and VC of *An. dirus* and *An. minimus* also were compared by site and season (Table 2). *P. falciparum* EIR usually far exceeded PV EIR, except in hot dry and monsoon seasons, when few positive mosquitoes were collected. The EIR for PF at sites CD far exceeded those at sites AB, except for hot, dry seasons (sites AB slightly higher) and monsoon seasons (both zero). The EIR for PV at sites CD also exceeded those at sites AB in postmonsoon and cool dry seasons but were otherwise equal or nearly so (monsoon seasons). High EIR for PF and PV were found in the same seasons, i.e., postmonsoon, cool dry, and early rainy seasons.

The VC of *An. dirus* and *An. minimus* across five seasons were correlated highly with each other ($r = 0.99$), but VC for *An. dirus* at sites CD always exceeded that at sites AB, whereas VC of *An. minimus* varied among sites (Table 2). Across 11 seasons of *An. dirus* collections, VC at sites AB and CD correlated poorly with one another ($Sr = -0.09$). The EIR at sites AB were usually zero, so correlation with VC was not attempted. Including only sites CD, some correlation between VC and EIR was observed (with PF and PV EIR, $Sr = 0.33$ and 0.45, respectively). Correlations of VC and EIR were much higher when both hot dry seasons were excluded from the 11 seasons of comparisons (with PF and PV EIR, $Sr = 0.53$ and 0.67, respectively), but VC among sites AB and CD still correlated poorly ($Sr = -0.22$). Overall correlations between EIR and VC of *An. minimus* were not performed because its EIRs were usually zero. The VC of *An. minimus* was highest during hot dry seasons at sites CD, coinciding with a high VC for *An. dirus* (Table 2).

Human Malariometry. Prevalence of PF at sites CD exceeded that at sites AB in all seasons, with significant differences ($P < 0.05$) during postmonsoon and cool dry seasons (Fig. 3). The monthly incidence of PF was similar at sites AB and CD ($r = 0.88$) and closely paralleled prevalence at sites AB and CD ($r = 0.73$ and 0.83, respectively). Peak PF incidence occurred in cool dry and hot dry seasons, lagging 4–8 wk behind relative abundance of PF-positive mosquitoes in postmonsoon and cool dry seasons. The incidence of PF also increased in early rainy seasons at sites CD, coinciding with a secondary peak of PF-positive mosquitoes. Prevalence of PV at sites AB and CD was similar but peaked during the monsoon seasons when PF prev-

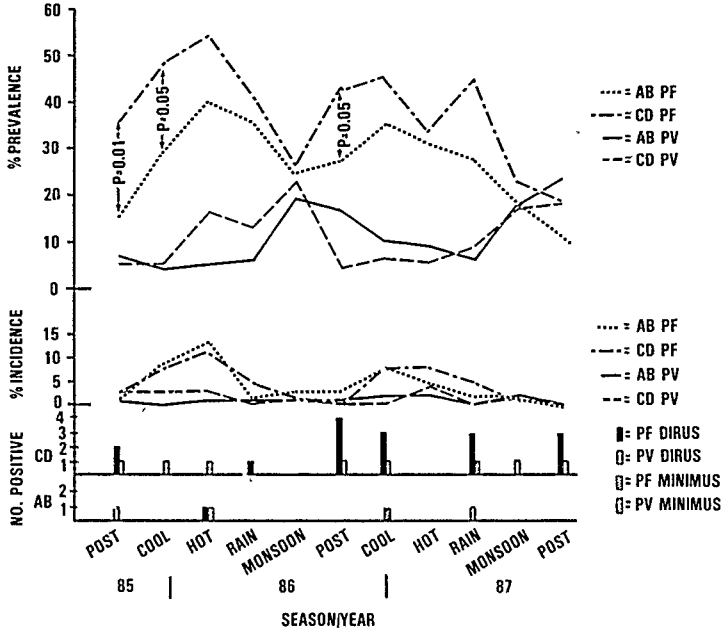

Fig. 3. Prevalence and incidence of *P. falciparum* (PF) and *P. vivax* (PV) by season (see Fig 1) at inner village (sites AB) and outer village (sites CD) from 1985 to 1987. The number of *An. dirus* and *An. minimus* positive for PF or PV sporozoite antigen also are shown by season and sites.

alence was lowest. Over five seasons, mean PV and PF prevalence were correlated negatively (at AB, $r = -0.95$, at CD, $r = -0.88$). Incidence of PV was low throughout the study, with only slight elevations in dry and monsoon seasons.

Larval Mosquito Collections. *Anopheles dirus* larvae were found first in October 1985 in a tire rut near site A but were not found again for 8 mo because its larval habitats still were unrecognized. From July 1986, *An. dirus* larvae were found regularly in similar habitats, with the first *An. dirus* larval collections of each monsoon season taken from isolated stream pools or tire ruts near site D (Table 4). Thereafter, larval collections of *An. dirus* increased in consecutive months through each October, when they were taken at all sites (Table 4). By November or December, ground pools were few, and *An. dirus* larvae were found only in isolated stream pools (site D) and a large water storage vessel (site C). The only *An. dirus* larvae found in the hot dry season (April 1987) were from two large, shaded stream pools isolated in a dry stream bed near site D (Table 4). No systematic search was conducted for *An. minimus* because its habitats (moderately shaded stream margins, often in leaf litter) are well known (Reid 1968). However, larval *An. minimus* were collected at all sites, and its habitats were widely available in all but monsoon seasons.

Discussion

Anopheles dirus was the primary vector species in Ban Phluang, comprising 83% of ELISA-positive mosquitoes. High PF EIR appeared most dependent on the elevated abundance of *An. dirus* in the postmonsoon seasons of high infection rates in the cool, dry seasons. This finding agreed with those of Rosenberg et al. (1990), who also found infected *An. dirus* mostly in postmonsoon and dry seasons. However, our detection of a secondary peak of positive *An. dirus* in early rainy seasons (seasons of elevated adult and larval mosquito abundance in the outer village) indicated an additional period of elevated malaria transmission. *An minimus*, with

Table 4. Monthly collections of *A. dirus* larvae according to site and type of habitat from 1985 to 1987[a]

Site[b]	Habitat[c]	1985		1986										1987									
		Oct.	Dec.	Feb.	April	June	July	Aug.	Sept.	Oct.	Nov.	Dec.	Jan.	Feb.	March	April	May	June	July	Aug.	Sept.	Oct.
AB	Grndpool	X	—	—	—	—	—	X	—	X	—	—	—	—	—	—	—	—	—	X	X	X
	Artcont	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	X	—
	Strmpool	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
CD	Grndpool	—	—	—	—	—	—	—	X	—	X	—	—	—	—	—	—	—	X	X	X	X
	Artcont	—	—	—	—	—	—	—	—	—	X	—	—	—	—	—	—	—	—	X	X	X
	Strmpool	—	—	—	—	—	X	—	—	X	X	X	—	—	—	X	X	—	—	—	—	—

[a] X, larvae present; —, larvae absent or not found.
[b] AB, inner village sites, CD, outer village sites.
[c] Grndpool, ground pool, Artcont, artificial containers, Strmpool, stream pool.

three HT-positive (PV) and one SG-positive (PF) mosquitoes, was considered to be a secondary vector, as suggested by Rosenberg et al (1990). Elevated abundance and VC of *An. minimus* (relative to *An. dirus*) in seasons of comparatively high PV gametocyte prevalence, coupled with its PV/PF positivity ratio of 3:1, suggested its greater role as a PV than a PF vector in Ban Phluang High abundance of *An. minimus* in dry seasons was likely due to increased availability of larval habitats Other anopheline species, including *An. peditaeniatus*, exhibited much lower parity rates than *An. dirus* or *An minimus* (possibly because of a longer gonotrophic cycle or shorter longevity), yielding low VC estimates. The sporozoite-positive, ELISA-negative specimen of *An. barbirostris* may have been due to a CS protein variant not detected by our monoclonal antibodies, a possibility shown by Rosenberg et al. (1989).

Risk of receiving a potentially infected mosquito bite varied with site and season as indicated by the EIR. Highest risk occurred at sites CD in postmonsoon and early rainy seasons. However, highest mosquito positivity rates occurred in hot dry seasons when mosquito abundance was low, also a finding of Rosenberg et al. (1990). Positive mosquitoes were fewer in cool dry and hot dry seasons, but the EIR still indicated a substantial risk of infection. Lowest risk and lowest mosquito positivity rates occurred consistently during monsoon seasons.

Plasmodium falciparum- and PV-positive mosquitoes were detected concurrently, except for one monsoon season (1987). However, PF-positive mosquitoes usually dominated, comprising 63% of positive mosquitoes, somewhat less than the 76% PF-positive mosquitoes reported by Rosenberg et al (1990). *P. falciparum*-positive mosquitoes dominated in postmonsoon and early rainy seasons, when they constituted 74% of positive mosquitoes.

Seasonal differences in mosquito parity rates should affect positivity rates and VC because high parity rates indicate increased longevity (Davidson 1954). In postmonsoon seasons, high (and nearly equal) parity rates of *An. dirus* and *An. minimus*, coupled with elevated abundance, would explain high vectorial capacities. In cool dry seasons, parity rates were so low at sites CD (45%) that few positive mosquitoes were expected. However five positive mosquitoes were found, including a nulliparous *An. dirus* with both PF and PV antigen. Although gonotrophic dissociation or discordance cannot be excluded, miscoding of the specimen appears more probable. In any case, the unusual combination of relatively high EIR and low VC suggests that parity rates in the cool dry season (and hence VC) may have been reduced artificially by the increased recruitment of nulliparous females. The significantly higher parity rate of *An. dirus* at sites CD than at sites AB in hot dry seasons was unexplained because exhaustive surveys yielded larvae only at site CD.

Good correlation of VC with the EIR only when hot dry seasons were excluded from calculations was attributed to highly variable parity rates High proportions of parous *An dirus* from 2100 to 0300 hours suggested that older females feed later than nulliparous females, except after 0300 hours. In *An. gambiae*, this finding was attributed to early evening oviposition by parous females followed by host-seeking (Molineaux & Grammicia 1980), also a probable explanation for *An. dirus*. Early peak biting activity of *An. dirus* during cool dry seasons appeared related to low mean minimum temperatures (sometimes reaching 12–13°C after 2400 hours), curtailing mosquito activity. Although studies relating biting behavior to human activity were not performed, the fact that most positive mosquitoes fed before 2400 hours suggests the potential value of such research.

Seasonal peaks of PF-positive mosquitoes (and PF EIR) in postmonsoon, cool dry, and early rainy seasons were related in time to peaks in PF incidence and prevalence, especially in the outer village. However, abundance of PV-positive mosquitoes (and PV EIR) appeared unrelated to PV prevalence and incidence. This event could result from prolonged latency in exoerythrocytic PV infections (Cooper et al. 1947) or interspecific dominance of PF over PV in concurrent infections (Boyd & Kitchen 1937).

Forest foci of *An. dirus* at sites CD appeared essential to sustaining PF transmission through dry seasons. Abundance of *An. dirus* only in outer village areas (sites CD) in dry seasons was well supported by adult and larval collection data. Abundance of *An dirus* only in heavily forested areas in dry seasons also was observed by Wilkinson et al. (1978). Absence of *An. dirus* larvae and low parity rates in monsoon seasons was ascribed to flushing of larval habitats by heavy rains and intensive residual pesticide spraying during the fruit-growing season. Abundance of *An. dirus* increased first at outer village sites, radiating toward the inner village as larval habitat increased late in monsoon and early rainy seasons.

Considering the dominant role of *An. dirus* in malaria transmission in Ban Phluang and the few, highly focal dry-season habitats found, malaria hyperendemicity appeared highly dependent on seasonal repopulation of *An. dirus* from forest habitats in the outer village The role of *Anopheles minimus* as a secondary vector might have been missed without the ELISA, which is more sensitive in detecting antigen when sporozoite numbers are low than is microscopic examination (Esposito et al 1986) Although other anophelines occasionally may become infected, they probably play a minor role in malaria transmission in the village. However, because there are short periods when species from the *An. barbirostris* and *An. hyrcanus* groups (including *An peditaentatus*) might become infective, these groups should be studied further Ultimately, hyperendemic malaria in Ban Phluang and similar villages may be most dependent on adaptation of *An. dirus* to steady depletion of forest habitats and more intensive agricultural practices observed throughout the region.

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