LONGITUDINAL EVALUATION OF MALARIA EPIDEMIOLOGY IN AN ISOLATED VILLAGE IN WESTERN THAILAND: I. STUDY SITE AND ADULT ANOPHELINE BIONOMICS

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Abstract. This is the first in a series of papers describing the epidemiology of malaria in an isolated village in western Thailand. The study site was the village of Kong Mong Tha, located in Sangkhla Buri District, Kanchanaburi Province, Thailand. In this paper we present an overview of the study site and results from our adult anopheline mosquito surveillance conducted over 56 consecutive months from June 1999 until January 2004. The collection site, indoor/outdoor location, parity, biting activity and *Plasmodium falciparum* (Pf) and *P. vivax* (Pv) infection rates were used to calculate seasonal entomological inoculation rates for the predominant four *Anopheles* species. A total of 21,566 anophelines representing 28 distinct species and 2 groups that were not identified to species were collected using human bait, with almost 95% of the collection consisting of *Anopheles minimus*, *An. maculatus*, *An. sawadwongporni* and *An. barbirostris/campestris*. Mosquitoes generally peaked during the wet season, were collected throughout the night, and were collected most often outside (ca. 75%) versus inside (ca. 25%) of houses. Approximately 50% of collected mosquitoes were parous. Overall *Plasmodium* infection rates were 0.27%, with a total of 16 and 42 pools of Pf- and Pv-positive mosquitoes, respectively. Annual EIRs were 2.3 times higher for Pv than for Pf, resulting in approximately 5.5 and 2.6 infective bites per person per year, respectively. The results suggest *An. minimus* and *An. maculatus* are the primary and secondary vectors of Pf and Pv transmission in Kong Mong Tha, while *An. sawadwongporni* and *An. barbirostris/campestris* also appear to play a role based on the presence of circumsporozoite protein (CSP) in the head/thorax of the specimens tested.

Keywords: malaria epidemiology, anopheline bionomics, western Thailand

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INTRODUCTION

Malaria remains one of the most important infectious diseases in Thailand, with over 100,000 cases reported each year (Chareonviriyaphap *et al*, 2000, 2003). Over half of the reported cases occur along the mountainous Thai border with Myanmar, extending from Tak Province in the north to Kanchanaburi Province in the west. These provinces are particularly vulnerable to malaria due to the uncontrolled movement of refugee tribal populations across the Thai-Myanmar border (Rowland and Nosten, 2001) and the high prevalence of occupational activities such as gem mining, hunting and logging that increase exposure to infected anopheline mosquitoes (Chareonviriyaphap *et al*, 2003).

Kanchanaburi Province is the third most malarious of the 76 provinces in Thailand (Chareonviriyaphap *et al*, 2003), with a total of 54,512 (8.1%) of 669,841 blood smears collected from 1994-1998 positive for *Plasmodium* parasites (Vector-borne Disease Control Center 53, 1998). Of the approximately 73 species of *Anopheles* that occur in Thailand (Rattanarithikul *et al*, 2006), only *Anopheles dirus* Peyton and Harrison, *An. minimus* Theobald, and *An. maculatus* Theobald are important malaria vectors (Pinichpongse and Bullner, 1967; Chareonviriyaphap *et al*, 1999). All three species are commonly found in Kanchanaburi Province and have been incriminated as important malaria vectors in the province (Sucharit *et al*, 1988; Green *et al*, 1990; Walton *et al*, 1999; Coleman *et al*, 2002d; Kengluecha *et al*, 2005a; Rongnoparut *et al*, 2005; Muenworn *et al*, 2006; Sungvornyothin *et al*, 2006).

We conducted a 4-1/2 year longitudinal study to investigate the epidemiology of malaria transmission in the village of Kong Mong Tha in western Kanchanaburi Province, an area characterized by low levels of *Plasmodium falciparum* (Pf) and *P. vivax* (Pv) malaria transmission. In this paper we i) provide background information on the study site, to include habitat descriptions and demographics of the human population, and ii) report on our entomological assessment of adult anopheline populations, including biting activity, parity status, *Plasmodium* infection rates and the relative roles of the predominant human-biting *Anopheles* spp in the transmission of Pf and Pv. In subsequent papers we will report on our entomological assessment of larval anopheline populations as well as results of our malaria surveillance.

MATERIALS AND METHODS

Study site

The study was performed from June 1999 through January 2004 in the village of Kong Mong Tha, Laivo Tambon (Sub-District), Sangkhla Buri Amphoe (District), Kanchanaburi Province, western Thailand (Fig 1). The village is located on the Maenam Ran Ti River within the 3,360 km^2 Thung Yai Wildlife Reserve. The village is located approximately 160 meters above sea level and is surrounded by mountains up to 800 meters in elevation. The center of the village is located at 98^{o}33'16" E and 15^{o}10'17" N. At the time of the study, Kong Mong Tha was an isolated village accessible only by boat or foot for 10 months of the year. At the height of the hot, dry season (March-April), the village could be reached in 30 minutes from the main road in an all-terrain vehicle. Pf and Pv are the predominant parasite species in this village, followed by *P. malariae* and *P. ovale*. The majority of malaria cases are asymptomatic (Coleman *et al*, 2002a,b,c, 2004, 2006). In order to facilitate the collec-

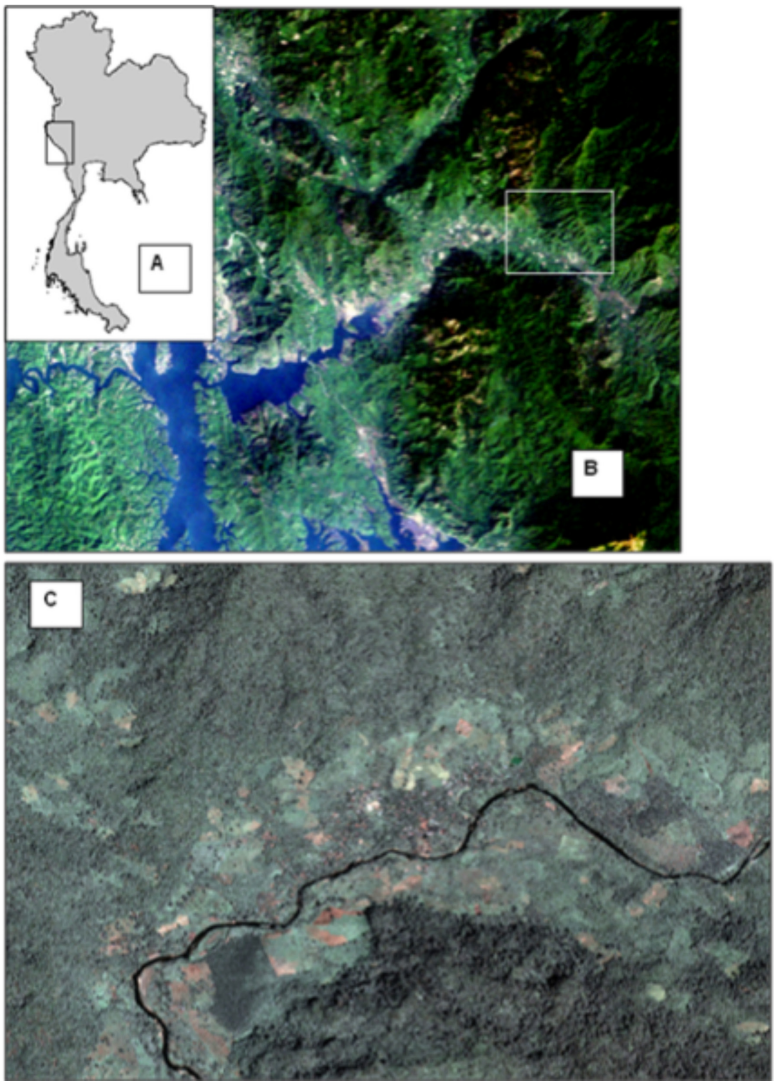

Fig 1–(A) Map of Thailand (ESRI) showing location of study area; (B) Landsat 7 ETM+ 30-meter resolution image of Sangkhla Buri District; and (C) IKONOS pan-enhanced 1-meter resolution image of the Kong Mong Tha study area.

tion of data, the village was divided into nine sectors (A-I) that served as separate sites for monitoring of the various factors affecting the transmission of malaria (Fig 2). A total of 114 houses were located in the study area, to include 7, 18, 13, 14, 10, 18, 10, 18 and 6 houses in sectors A-I, respectively. The majority of village houses are raised off the ground (height approximately 1-2 m) and constructed of rough wooden or bamboo support beams, with split bamboo lashed together for floors and walls.

Maps and satellite images

The most recent topographical map of the region was prepared in 1999 by the National Imagery and Mapping Agency (USA) in cooperation with the Royal Thai Survey Department. Digitized data from the map (Title: "Ban Kong Mong Tha, Thailand"; Scale: 1:50,000; Map Identifiers: Map Sheet 4739 III, Series L7018, Edition 1-RTSD) was used to provide base layers (*eg*, topography, land use, roads, rivers, surface water) on which other data were overlaid (Clarke *et al*, 1996). A 1:50,000 aerial photograph of the site was taken on 28 February 1997 by the Royal Thai Survey Department. IKONOS and LANDSAT images were obtained from Space Imaging, Thornton, Colorado, and EROS Data Center, Sioux Falls, South Dakota, respectively. A RADARSAT image was obtained from the Alaska Synthetic Aperture Radar (SAR) Facility, in Fairbanks, Alaska. PCI

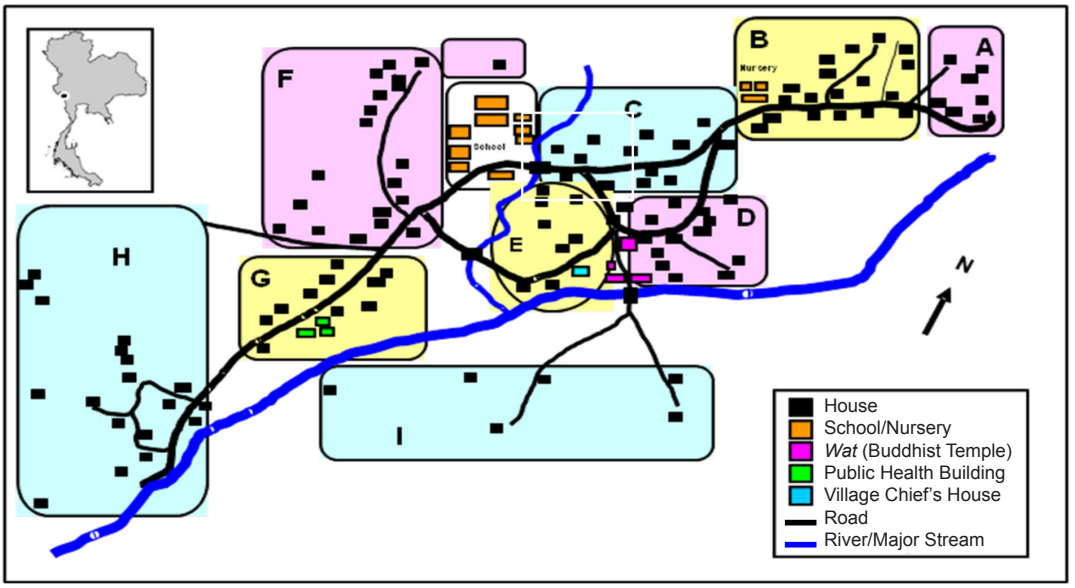

Fig 2–Diagram (not to scale) showing the nine administrative divisions (A-I) used within Kong Mong Tha Village. For administrative purposes, Division I includes the school and temple (*Wat)* buildings.

Geomatica, Version 8.2 software (PCI Geomatics, Richmond Hill, Ontario, Canada), was used for image processing. All images were geo-referenced using ground control points (*eg*, houses, roads, school, and river) collected with Garmin GPS III Plus (Garmin Corporation, Olathe, KA) or Trimble GeoExplorer 3 (Trimble Navigation Limited, Sunnyvale, CA) hand-held global positioning systems (GPS).

Geographic information system (GIS)

The mapping and geographic analyses of adult mosquito data in this paper (and of larval mosquito and human malaria data in subsequent papers) were performed using ArcGIS Version 8.3, including the ArcGIS 3D Analyst and Spatial Analyst extensions (Environmental Systems Research Institute, Redlands, CA). A contour map was digitized in PCI Geomatica and imported into ArcGIS. A digital elevation model (DEM) was interpolated from the digitized contours in ArcINFO Version 7.3 (Environmental Systems Research Institute, Redlands, CA). The DEM was used to assign base heights to create three-dimensional images of the village to better understand the topography of the area.

Climatic conditions

Sunrise and sunset are at approx. 06:00 AM and 06:00 PM, respectively. Daily climate variables (temperature, relative humidity, and rainfall) were obtained from battery-powered sensors located at ground level near the health clinic in Kong Mong Tha. Sampling was undertaken from January 2001 (May 2001 for rainfall) through the end of January 2004. Temperature and relative humidity measurements (accuracy = ±0.5°C and ±3%, respectively) were sampled and stored hourly by a HOBO Pro RH/Temp datalogger [Model H08-032-08, Onset Computer Corporation (OCC), Pocasset, MA]. A tipping-bucket rain gauge (Model RG2,

Table 1
Study design for the two phases of adult mosquito collections in Kong Mong Tha, Thailand.

	Phase of study	
Parameter	Phase I	Phase II
Phase period	June 1999 - April 2002	May 2002 - January 2004
No. of months	35	21
No. of nights/month	4 (Monday - Thursday)	4 (Monday - Thursday)
Sectors collections made	8 (A-H)	4 (B, D, F, H)
No. of houses/sector	1 (8 total)	6 (24 total)
No. of houses/night	2	12
Replicates for each house	1	2
Collection sites	Indoor and outdoor	Outdoor
Collection hours	06:00 PM-06:00 AM (12 hours)	06:00-12:00 PM (6 hours)
No. of collectors/house	4	2

OCC) stored an hourly time and date stamp for each 0.01" (25.4 mm) tip event. All climatic data were offloaded monthly onto a HOBO shuttle (Model H09-003-08, OCC) and stored on a laptop PC.

Mosquito landing collections

Mosquitoes were collected over 56 consecutive months that were broken into two phases. Phase I lasted from June 1999 until April 2002 (35 months). During this phase the study site was established, houses were selected for inclusion in the study and intensive adult mosquito surveillance was conducted in eight of the sectors (A-H). Phase II lasted from May 2002 until January 2004 (21 months). During this phase collection efforts focused on four of the sectors (B, D, F and H). Details on the study design of each phase are presented in Table 1. The study was approved by the Ethics Committee of the Ministry of Public Health (MOPH), Bangkok, Thailand, and by the Human Subjects Research Review Board (HSRRB) of the United States Army, Fort Detrick, Maryland, USA.

Each month during Phase I, mosquitoes were collected from one house each in sectors A through H (eight houses total), with collections made at two houses per night over four consecutive nights (Monday through Thursday). Prior to traveling to the field site, each house was randomly assigned a collection night and team of collectors. We attempted to use the same houses throughout the study; however, on several occasions we had to change the houses that were used (these changes primarily resulted from families leaving the village and tearing their houses down). Replacement houses were adjacent to the original houses and of similar construction. Mosquitoes were collected for 12 hours (06:00 PM-06:00 AM) at each house, with 50 minute collections followed by a 10-minute break each hour. Two teams of four individuals each collected mosquitoes at each house, with one team collecting from 06:00-12:00 PM and another from 00:00-06:00 AM. Two individuals from each team collected mosquitoes inside

the house and two collected mosquitoes outside. Collectors sat in chairs and captured mosquitoes individually in glass vials as they landed on their exposed legs. Outdoor collections were made within 2 m of the exterior wall of each house. Immediately after being collected in the glass vials, mosquitoes were transferred to 1-pint screened cartons labeled with the date, house number, location of collection (indoor or outdoor) and hour of collection. At the end of each night, mosquitoes were returned to the field laboratory in Kong Mong Tha where they were provided with 10% sucrose until they were returned on Friday to the main laboratory in Bangkok.

Each month during Phase II, mosquitoes were collected over four consecutive nights (Monday through Thursday) from six houses each in sectors B, D, F and H (24 houses total). The same 24 houses were used over the entire course of Phase II. On the first night of the study, collections were made at three randomly selected houses in each of the four sectors (12 houses total), while on the second night of the study collections were made at the three remaining houses in each sector (12 houses total). On nights three and four of the study we repeated these collections so that two collections were made at each of the 24 houses over the four-day period. Mosquitoes were collected outside of each house for six hours (06:00-12:00 PM) by two volunteers using the procedures described above.

All processing of mosquitoes took place at the Armed Forces Institute of Medical Sciences (AFRIMS) in Bangkok, Thailand. On the Monday following the Friday return to Bangkok, all mosquitoes were identified to species using morphological keys to adult anophelines in Thailand (Harrison, 1980; Rattanarithikul and Panthusiri, 1994; Rattanarithikul *et al*, 2006). Some mosquitoes could only be identified to species complexes (*eg*, *An. dirus* and *An. minimus*) that can only be further speciated using molecular techniques. *Anopheles barbirostris* and *An. campestris* were combined into "Barbirostris Group," because the separation of adult specimens based on external morphology is difficult (Limrat *et al*, 2001), and even the best preserved adult specimens may only be distinguished based on the presence of a few white scales on the abdominal sterna (Rattanarithikul *et al*, 2006).

Once mosquitoes were identified, the ovarian tracheoles were examined for parity using the procedures of Detinova (1962). Mosquitoes were graded as nulliparous, parous or gravid (gravid mosquitoes were not assessed further to determine if they had previously developed eggs). The head/thorax and abdomen of each mosquito were then tested separately by ELISA for the presence of circumsporozoite protein (CSP) to Pf and Pv variants 210 and 247, according to the procedures of Wirtz *et al* (1985, 1987, 1992). Briefly, the head/thorax was bisected from the abdomen, placed in separate 1.5-ml centrifuge tubes containing 50µl of blocking buffer, and frozen at -70°C. Most mosquito specimens were tested individually by ELISA; however, *An. minimus* mosquitoes were routinely tested in pools of 2-5 mosquitoes, while other species were occasionally pooled. All pools consisted of mosquitoes with the same parity status that were collected at the same date, time and location. If a pool tested positive for CSP, it was assumed that only one mosquito in the pool was positive, and hence the minimum infection rate was calculated (Coleman *et al*, 2002d).

Vector data, calculations and analyses

Species, collection site (village sec-

tor), location (indoor or outdoor), parity status, biting period and the presence or absence of CS antigen was determined for each specimen in human landing collections. Using these data, the sporozoite antigen-positive rate and entomological inoculation rate (EIR) were calculated for each predominant vector species per season. EIR describes the risk of human exposure to bites from potentially infectious mosquitoes (Draper and Davidson, 1953). EIR values were calculated by multiplying the mosquito biting rate by the sporozoite antigen-positive rate (Beier, 2002) to calculate the estimated number of infective bites per season (assuming 91.25 nights per season).

Statistics

Differences in mosquito number (log_{10}-transformed) were assessed for season, month or sector using an *F*-test (ANOVA) or Student's *t*-test for differences between means for individual factors. For collections performed during Phase I, differences in the proportions of mosquitoes in different sectors and collection locations (indoors or outdoors) were assessed using a chi-square (χ^2) goodness-of-fit test. For both study phases, the effect of season on the percentage of parous mosquitoes (arcsin-transformed) was estimated using ANOVA. All statistical tests were performed using SPSS Version 14 (IBM, Armonk, NY) and assessed at the 0.05 level of significance. Means are expressed with the standard error unless indicated otherwise.

RESULTS

Climatic conditions

We used a combination of mean monthly temperature and rainfall data to define four distinct seasons (cool, hot, wet and mixed) of equal length. The cool season (15 November-14 February) was characterized by temperatures <25°C and <20 mm rainfall, the hot season (15 February-14 May) by temperatures >27°C and <60 mm rainfall, the wet season (15 May-14 August) by temperatures of 25-27°C and >120 mm rainfall, and the mixed season (15 August-14 November) by temperatures of 25-27°C and rainfall of 20-120 mm. The wet and mixed seasons (rainy period) are characterized by the southwest monsoon and tropical cyclones from the Intertropical Convergence Zone (ITCZ) (Thailand Meteorological Department, http://www.tmd.go.th/en/archive/climateconditions.php). The cool season is characterized by the northeast monsoon from the anticyclone in China, and the hot season is the transitional period from the northeast to southwest monsoons.

The average annual daily temperature is 26.1°C (range = 12-42°C), and the average daily rainfall is 7.7 mm. During the wet season, rainfall averages 13.7 mm (daily maximum = 126 mm in May 2002). Mean relative humidity varies between 60% and 80% (100% in the rainy season). Mean daily and monthly temperature and relative humidity are presented in Fig 3a and 3b, respectively, with average monthly rainfall presented in Fig 3b. The average daily temperature was similar between seasons, reflecting the comparable intensity of solar radiation during the day. Minimum nightly temperatures, however, were considerably lower during the cool season. Daily relative humidity values showed a similar pattern between seasons; higher mean values during the rainy season were linked to increased rainfall.

Species composition

A total of 21,566 female mosquitoes were collected from 7 June 1999 through 15 January 2004 (Table 2). Of these, 11,565 (54%) were collected in Phase I of the study

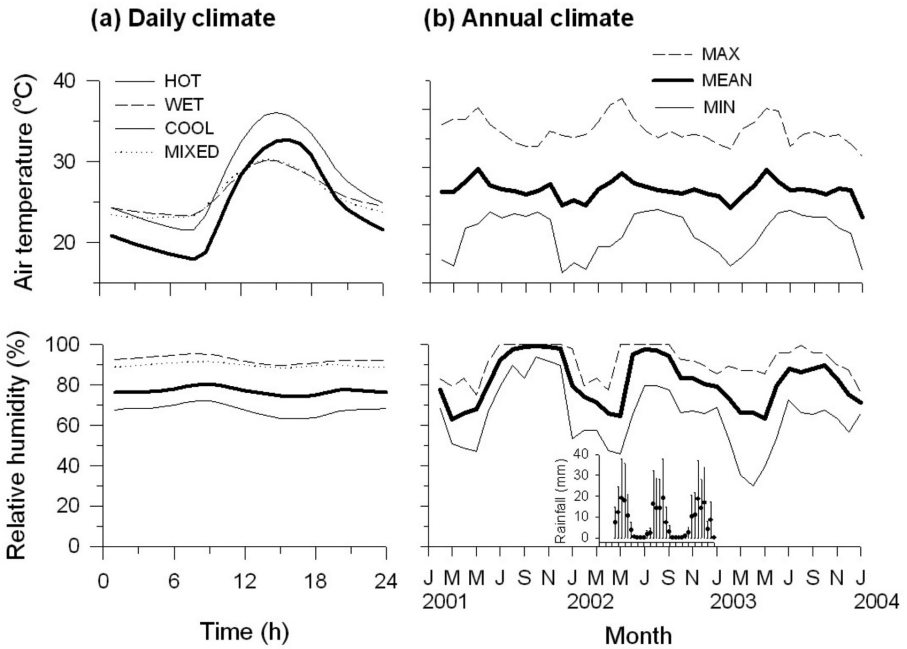

Fig 3–Mean (a) daily and (b) monthly air temperature and relative humidity in Kong Mong Tha, Thailand, January 2001-January 2004. Inset shows mean (± SD) monthly rainfall, May 2001-January 2004.

and 10,001 (46%) were collected in Phase II. The 21,566 mosquitoes comprised 28 distinct *Anopheles* species and two groups (*An. hyrcanus* group and *An. umbrosus* group) that were not identified to species. The 28 species included two (*An. barbirostris* and *An. campestris*) that could not be differentiated morphologically. Because of this, they are henceforth referred to as *An. barbirostris/campestris*. Four species (*An. minimus* Theobald, *An. maculatus* Theobald, *An. sawadwongporni* Rattanarithikul & Green and *An. barbirostris/campestris* Reid) accounted for almost 95% of the total collection, with eight additional species (*An. philippinensis* Ludlow, *An. dirus* Peyton & Harrison, *An. kochi* Doenitz, *An. varuna* Iyengar, *An. aconitus* Doenitz, *An. nivipes* Theobald, and *An. umbrosus* group.) accounting for an additional 5%. The remaining 17 species cumulatively accounted for less than 1% of the total collection. Due to the large number of species collected, most of the later analyses in the present paper will focus on the four most common anophelines (*An. minimus, An. maculatus, An. sawadwongporni* and *An. barbirostris/campestris*).

Temporal distribution

The monthly abundance of the four most common anophelines in human landing catches is presented in Fig 4. In general, each species was characterized by fairly low numbers over most of the study, interspersed by occasional surges in the population. Populations of *An. minimus* peaked once each year during either the wet or mixed season (June 1999, October 2000, October 2001, June 2002, September 2003), whereas *An. barbirostris/campestris* populations peaked once a year during

Table 2
Total number and parity status of *Anopheles* species collected in the village of Kong Mong Tha, Thailand, June 1999 - January 2004. The percentage of gravid mosquitoes is not shown but can be calculated by subtracting the nulliparous and parous rates from 100%.

Species	Number collected	% of total collection	No. examined for parity	% Nulliparous	% Parous
An. aconitus	94	0.44	74	54.1	40.5
An. annularis	5	0.02	5	60.0	40.0
An. barbirostris/campestris	1,568	7.27	1,480	52.1	40.9
An. barbumbrosus	24	0.11	23	52.2	39.1
An. dirus	185	0.86	142	45.8	47.9
An. donaldi	4	0.02	4	75.0	25.0
An. dravidicus	2	0.01	2	0.0	100.0
An. greeni	8	0.04	7	71.4	28.6
An. hodgkini	4	0.02	4	0.0	75.0
An. hyrcanus group[a]	7	0.03	4	50.0	50.0
An. jamesii	24	0.11	21	47.6	52.4
An. jeyporiensis	1	<0.01	1	100.0	0.0
An. karwari	15	0.07	14	28.6	57.1
An. kochi	173	0.80	136	35.3	61.0
An. maculatus	5,064	23.48	4,597	56.4	40.9
An. minimus	11,439	53.04	9,724	41.5	54.4
An. nigerrimus	1	<0.01	1	100.0	0.0
An. nitidus	1	<0.01	1	0.0	100.0
An. nivipes	72	0.33	63	54.0	36.5
An. notanandai	1	<0.01	0	ND	ND
An. peditaeniatus	32	0.15	31	54.8	41.9
An. philippinensis	367	1.70	342	40.1	56.4
An. pseudojamesii	1	<0.01	1	0.0	100.0
An. pseudowillmori	2	0.01	2	50.0	50.0
An. sawadwongporni	2,299	10.66	2,117	56.8	38.6
An. tessellatus	11	0.05	7	57.1	14.3
An. umbrosus group[a]	35	0.16	31	54.8	32.3
An. vagus	7	0.03	5	60.0	20.0
An. varuna	120	0.56	104	45.2	51.9
Total	21,566	100.00	18,943	47.8	48.1

[a]Not identified further to species; ND, not determined.

the cool season (December 1999, December 2000, November 2001, October 2002, November 2003). In contrast, *An. maculatus* populations peaked only twice during the study (June 2002, June 2003) while *An. sawadwongporni* populations generally peaked twice a year (February and October 2000, March and September 2001, May and December 2002 except in 2013 which peaked once in May 2003). Regarding the seasonal abundance, the majority of the species were most abundant in the wet

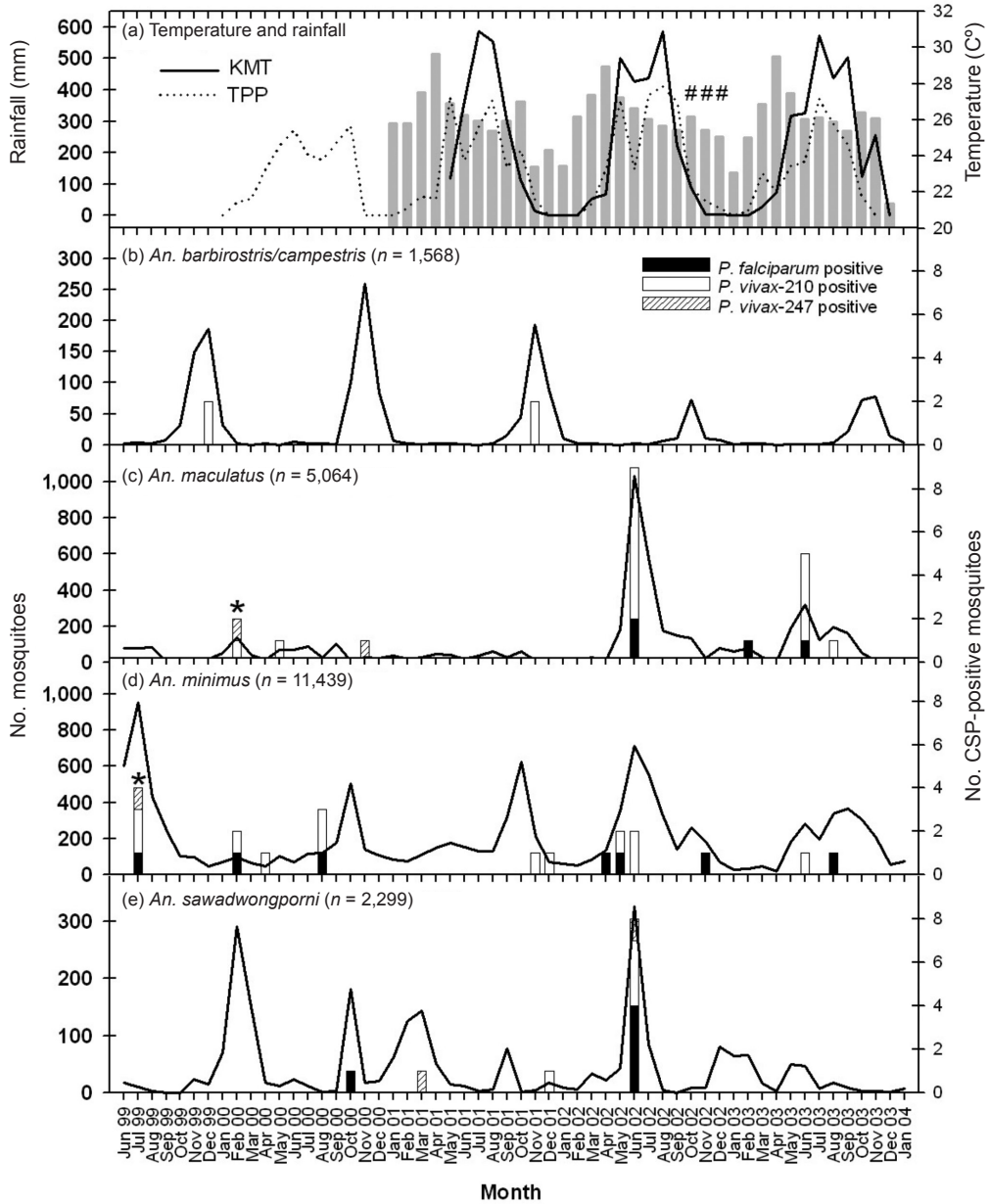

Fig 4–(a) Mean daily temperature (°C; bars) and rainfall (mm; lines), and (b-e) total numbers of *An. barbirostris/campestris*, *An. maculatus*, *An. minimus* and *An. sawadwongporni* mosquitoes (lines) and number of CSP-positive mosquitoes (bars) collected monthly in Kong Mong Tha, Thailand, June 1999 to January 2004. Temperature and rainfall were sampled January 2001-December 2003 in both Kong Mong Tha (KMT) and Thong Pha Phum (TPP; rainfall only), a town located approximately 40 km away. Hatches (#) in (a) indicate months (October-December 2002) when gravid F0 mosquitoes were collected in cow-baited traps and used to estimate the oviposition interval and gonotrophic cycle. Asterisks (*) in (c) and (d) indicate mixed Pv-210/247 infections detected in a single *An. maculatus* mosquito or in a pool of five *An. minimus* mosquitoes previously described by Coleman *et al* (2002d).

season (*eg*, *An. dirus*, *An. maculatus*, *An. varuna*), the mixed season (*An. umbrosus* group) or both seasons (*An. aconitus*, *An. kochi*, *An. minimus*). Two species (*An. barbirostris/campestris* and *An. philippinensis*) were primarily collected in the cool season, and two species (*An. nivipes* and *An. sawadwongporni*) were distributed evenly throughout the year.

Nightly activity

The nightly landing activity of the four most abundant species is presented in Fig 5. *Anopheles minimus* and *An. barbirostris/campestris* were collected throughout the night, along with the majority of other species, including *An. aconitus*, *An. dirus*, *An. umbrosus* group and *An. varuna*. *Anopheles maculatus* and *An. sawadwongporni* were most abundant early in the evening, along with *An. kochi*, *An. nivipes* and *An. philippinensis*. The peak biting period for both *An. maculatus* and *An. sawadwongporni* was one hour earlier in the mixed and cool seasons (06:00-07:00 PM) compared to the hot and wet seasons (07:00-08:00 PM), but this temporal shift was not observed for *An. barbirostris/campestris* (abundant only during the mixed and cool seasons) or *An. minimus*. During the cool season, the tendency for *An. maculatus* and *An. sawadwongporni* to bite earlier in the evening (Fig 5) might have coincided with lower nightly temperatures compared to the hot and rainy seasons.

Geographic distribution

For collections of the four predominant *Anopheles* species during Phase I of the study, most species including *An. minimus* and *An. sawadwongporni*, appeared to be randomly distributed throughout the village. However, unexpectedly high proportions (39%) of *An. barbirostris/campestris* were collected in sector H near fruit orchards (χ^2 = 85.1 for cool and mixed seasons only, df = 7, $p < 0.001$), and high proportions (46%) of *An. maculatus* were collected in sector F near the forest border (χ^2 = 524.0, df = 21, $p< 0.001$). During Phase I (when both indoor and outdoor collections were made), mosquitoes were collected in significantly greater numbers outdoors than indoors, with 73% (7,889/10,830) captured outdoors ($\chi^2 = 414$ for all species combined, df = 3, $p < 0.001$). Certain species were almost exclusively exophagic; for example, 87% (1,244/1,433) of *An. maculatus* and 88% (1,275/1,454) of *An. sawadwongporni* mosquitoes were collected outdoors compared with 69% (859/1,250) of *An. barbirostris/campestris* and 67% (4,511/6,693) of *An. minimus* mosquitoes (Table 4). In general, the degree of exophagy was lowest during the mixed season and highest during the cool season for *An. minimus* ($\chi^2 = 40.2$, df = 3, $p < 0.001$) and *An. sawadwongporni* (χ^2 = 14.2, df = 3, $p < 0.01$), and during the hot season for *An. maculatus* (χ^2 = 13.5, df = 3, $p < 0.01$).

Parity

The overall parity status of each *Anopheles* species collected is presented in Table 2. Almost half (47.8%) of all mosquitoes evaluated were nulliparous, with rates ranging from 35.3% (*An. kochi*) to 56.8% (*An. sawadwongporni*), for species with >100 specimens collected throughout the study. The remaining mosquitoes were either parous (48.1%) or gravid (4.1%) at the time of collection. The relationships between parity status and season, month, hour of collection and sector were examined further for the four predominant anophelines in Kong Mong Tha (Fig 6). Overall parity rates (by season and year) were highest for *An. minimus* (mean = 58.7 ± 2.8%; 95% CI: 53.0-64.5%) compared to *An. barbirostris/campestris* (40.3 ± 4.8%; 30.2-50.4%), *An. maculatus* (44.0

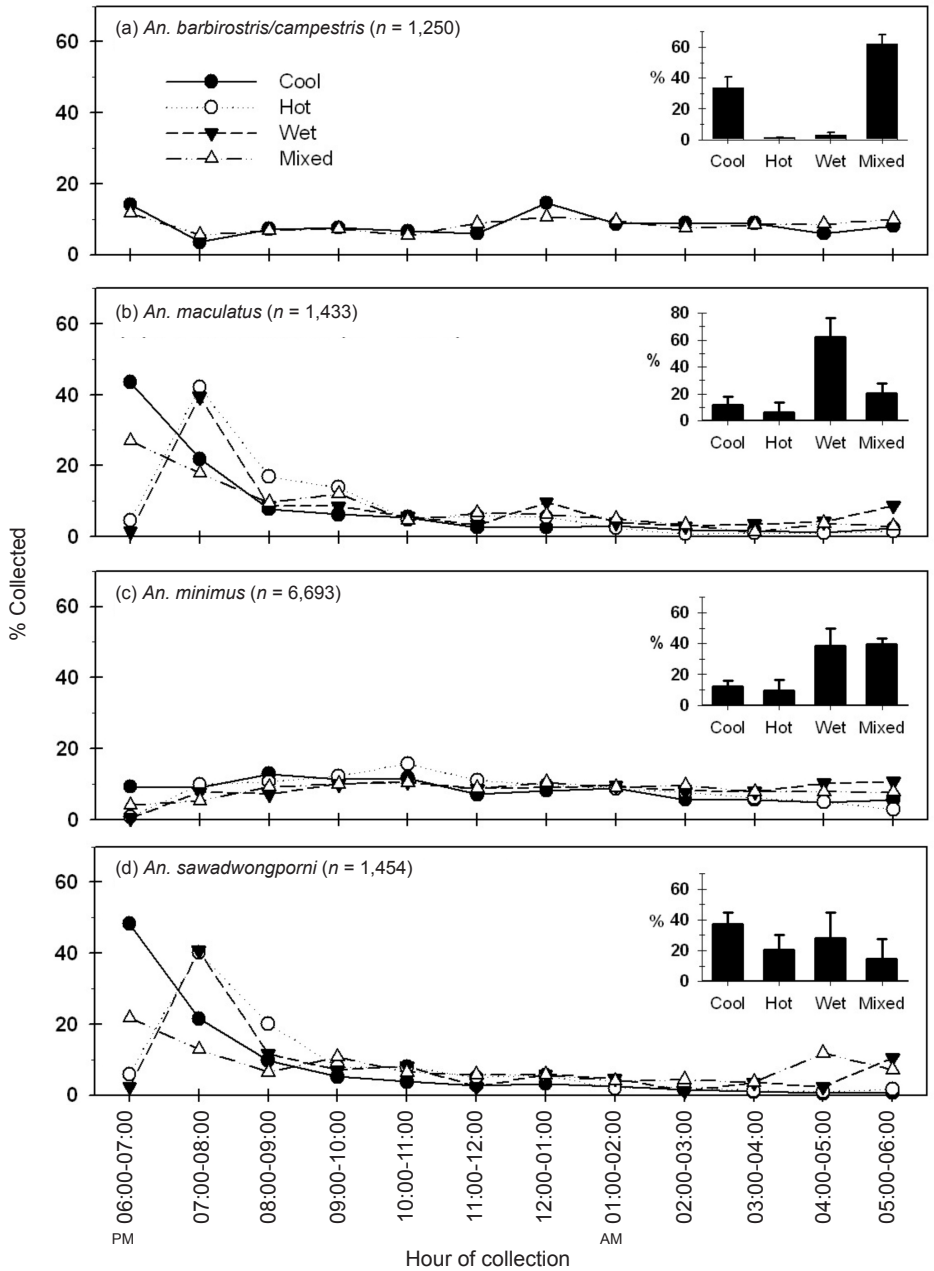

Fig 5–Nightly activity (06:00 PM - 06:00 AM) of (a) *An. barbirostris/campestris*, (b) *An. maculatus*, (c) *An. minimus* and (d) *An. sawadwongporni* in Kong Mong Tha, Thailand during Phase I (June 1999-April 2002). Values represent the mean percentages of mosquitoes collected per hour during the cool, hot, wet and mixed seasons (*An. barbirostris/campestris* not shown for hot and wet seasons due to low numbers caught). Insets show mean percentages (+SE) of mosquitoes collected per season for Phases I and II (2000-2003; *ie*, 16 discrete seasons). All figures are shown on a vertical scale of 0-70% for clarity (note different vertical scale for *An. maculatus* inset).

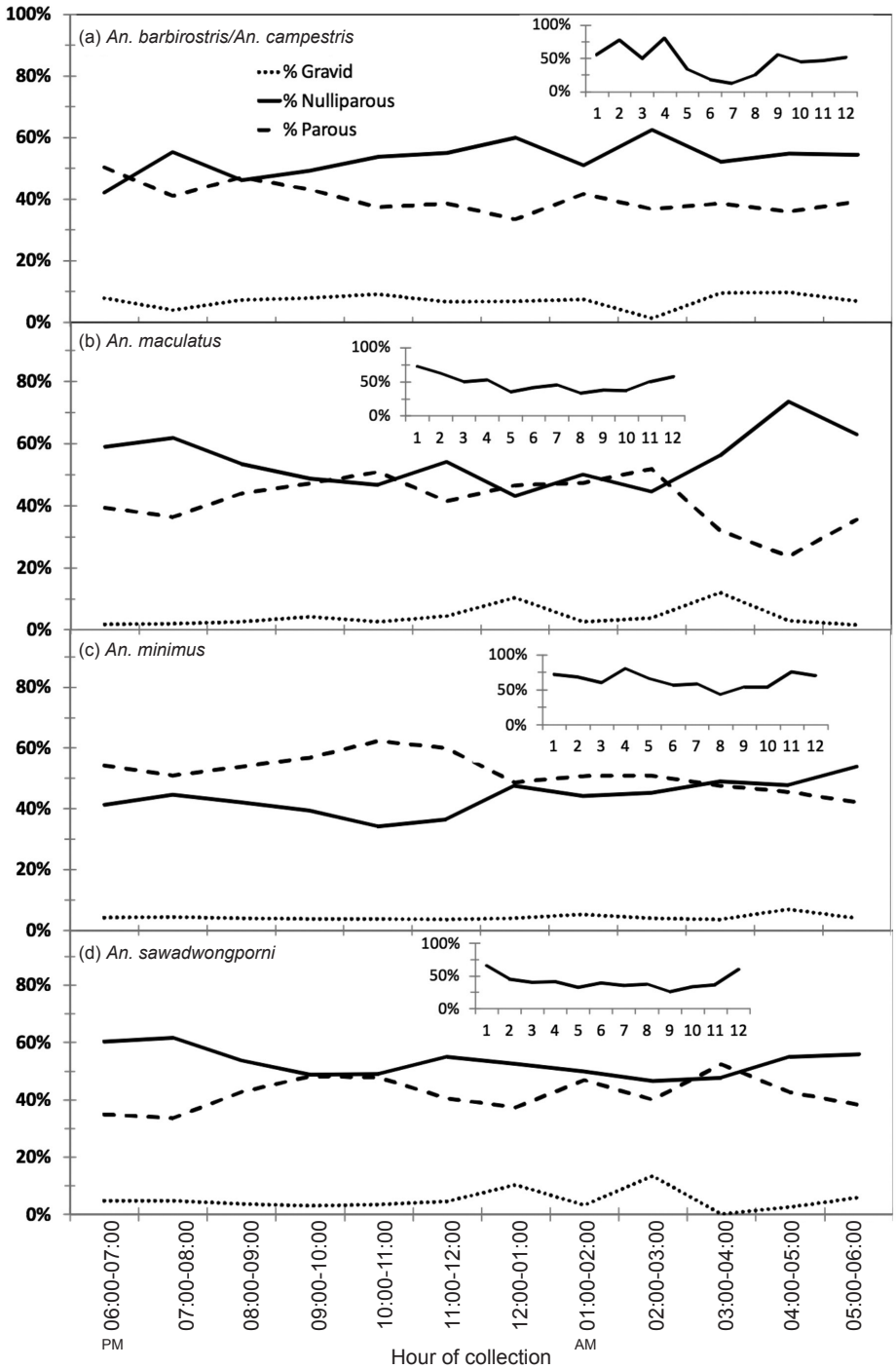

Fig 6–Percentage of gravid, parous and nulliparous mosquitoes collected during 06:00 PM - 06:00 AM for (a) *An. barbirostris/campestris*, (b) *An. maculatus*, (c) *An. minimus* and (d) *An. sawadwongporni* in Kong Mong Tha, Thailand during Phase I (June 1999-April 2002). Insets show percentage of gravid and parous mosquitoes collected in each month (*eg*, 1 = January, 12 = December).

± 3.9%; 35.9-52.0%) and *An. sawadwongporni* (38.1 ± 4.5%; 28.7-47.5%) (ANOVA, $F_{3,76} = 5.04$, $p < 0.01$). Parity rates were highest during the cool and hot seasons (November-April) and lowest during the wet and mixed seasons (May-October) for all species, except *An. barbirostris/campestris* which was abundant only during the mixed and cool seasons. The highest monthly parity rates were observed for *An. minimus*, with significant variation in parity status between months ranging from 42.7 ± 8.6% in December (cool season) to 73.7 ± 6.0% in August (mixed season) (ANOVA, $F_{11,44} = 2.09$, $p < 0.05$). For *An. maculatus*, parity rates ranged from 27.1 ± 6.2% in April (hot season) to 66.8 ± 3.1% in September (mixed season) (ANOVA, $F_{11,44} = 2.38$, $p < 0.05$). For *An. sawadwongporni*, parity rates ranged from 28.1 ± 6.5% in May (wet season) to 65.0 ± 6.8% in January (cool season) (ANOVA, $F_{11,44} = 1.70$, $p > 0.05$). The low parity rates during the wet season were likely due to rainfall that caused a dilution of the parous mosquito population with nulliparous mosquitoes that had not yet taken a blood meal. Throughout the night, the proportion of parous mosquitoes increased from approx. 07:00 PM to 03:00 AM and decreased until 06:00 AM for *An. maculatus* (ANOVA, $F_{11,153} = 4.26$, $p < 0.001$) and *An. sawadwongporni* (ANOVA, $F_{11,121} = 2.08$, $p < 0.05$), which suggested that nulliparous mosquitoes sought a bloodmeal earlier in the evening than parous mosquitoes (this trend was not significant for *An. barbirostris/campestris* or *An. minimus*). Parity rates were similar across the administrative sectors in which the mosquitoes were collected, which suggested that host-seeking anophelines probably traversed the village in search of a bloodmeal.

Mosquito infection rates

A total of 21,029 mosquitoes were divided into 15,246 pools and tested for the presence of CSP for Pf, Pv-variant 210 (Pv-210) and Pv-variant 247 (Pv-247) (Table 3). For all species except for *An. minimus*, most of the pools contained a single mosquito. For *An. minimus*, a mean of 2.1 ± 1.3 (median = 5) mosquitoes were tested per pool. A total of 56 pools (0.27%) tested positive for *Plasmodium* CSP. Sixteen pools tested positive for Pf CSP (0.08% infection rate), to include four pools ($n = 4$ mosquitoes) of *An. maculatus*, seven pools ($n = 17$) of *An. minimus* and five pools ($n = 9$) of *An. sawadwongporni* mosquitoes. Of the 16 pools, head/thorax samples tested positive in nine pools (56%), to include three pools (75%) of *An. maculatus*, three pools (43%) of *An. minimus* and five pools (56%) of *An. sawadwongporni* mosquitoes. A total of 42 pools tested positive for Pv CSP (0.2% infection rate). Of these, 35 pools tested positive for Pv-210 and seven pools tested positive for Pv-247, including a pool containing one *An. hodgkini* mosquito. Most of the CSP-positive pools were positive for head/thorax subsamples, indicative of a disseminated sporozoite infection, possibly of the salivary glands. For Pv-210, head/thorax samples tested positive in 3/4 of *An. barbirostris/campestris*, 12/14 of *An. maculatus*, 9/12 of *An. minimus* and 1/1 of *An. varuna* mosquitoes. For Pv-247, head/thorax samples tested positive in all *An. kochi* (1/1), *An. maculatus* (2/2), *An. minimus* (1/1) and *An. sawadwongporni* (2/2) mosquitoes. Only the abdomen sample tested positive in the single *An. hodgkini* mosquito infected with Pv-247. One pool of *An. maculatus* and one pool of *An. minimus* tested positive for both Pv-210 and Pv-247 (Table 3).

Of the 23 CSP-positive pools ($n = 40$ mosquitoes) collected during Phase I of the study, 14 pools (61%) represented 24 mosquitoes that were collected outdoors.

Table 3
Evaluation of anopheline mosquitoes collected in the village of Kong Mong Tha, Thailand for *Plasmodium falciparum* (Pf), *P. vivax* variant 210 (Pv-210) and *P. vivax* variant 247 (Pv-247) using CSP-ELISA.

Species	Number tested (%)	Number of pools	Number of ELISA-positive pools of mosquitoes (% positive)			
			Pf	Pv-210	Pv-247	All *Plasmodium*
An. aconitus	94 (99)	93	0	0	0	0
An. barbirostris/campestris	1,552 (99)	1,547	0	4 (0.26)	0	4 (0.26)
An. dirus	177 (95)	175	0	0	0	0
An. kochi	170 (98)	159	0	0	1 (0.59)	1 (0.59)
An. maculatus	5,061 (99)	4,949	4 (0.08)	14 (0.28)	2 (0.04)	19 (0.38)[a]
An. minimus	10,981 (96)	5,350	7 (0.06)	12 (0.11)	1 (0.01)	19 (0.17)[a]
An. nivipes	72 (99)	71	0	0	0	0
An. philippinensis	367 (99)	365	0	0	0	0
An. sawadwongporni	2,265 (99)	2,247	5 (0.22)	4 (0.18)	2 (0.09)	11 (0.49)
An. umbrosus group	29 (83)	29	0	0	0	0
An. varuna	113 (94)	113	0	1 (0.88)	0	1 (0.89)
18 other species	148 (99)	148	0	0	1 (0.68)	1 (0.68)[b]
Total	21,029 (97)	15,246	16 (0.08)	35 (0.17)	7 (0.03)	56 (0.27)[a]

[a]One pool of *An. maculatus* and one pool of *An. minimus* were positive for both Pv-210 and Pv-247 (Coleman *et al,* 2002d).
[b]One of four (25%) *An. hodgkini* mosquitoes tested positive for Pv-247.

Table 4
Seasonal entomological variables of malaria transmission related to *An.barbirostris/campestris* (Bar Grp), *An. maculatus*, *An. minimus* and *An. sawadwongporni* (*sawad*) mosquitoes in Kong Mong Tha. Values represent the observed daily biting and parous rates, and estimated entomological inoculation rates (EIR) and infective bites for *P. falciparum* (Pf) or *P. vivax* (Pv).

Anopheles sp	Season	Percent collected outdoors (%)	Daily biting rate (ma)[a]	Parity rate (m)[b]	EIR[c]		Infective bites[d]	
					Pf	Pv	Pf	Pv
Bar Grp	Cool	76.9	1.5	44%	0.000	0.004	0.0	0.3
	Hot	0.0	0.04	60%	0.000	0.000	0.0	0.0
	Wet	87.5	0.06	21%	0.000	0.000	0.0	0.0
	Mix	60.0	2	38%	0.000	0.002	0.0	0.2
maculatus	Cool	86.6	0.7	59%	0.002	0.002	0.2	0.2
	Hot	92.0	0.9	47%	0.000	0.003	0.0	0.3
	Wet	87.0	2.4	38%	0.005	0.018	0.4	1.6
	Mix	81.0	1.1	37%	0.000	0.003	0.0	0.3
minimus	Cool	72.5	2	67%	0.002	0.004	0.2	0.3
	Hot	72.0	2.8	64%	0.003	0.003	0.3	0.3
	Wet	68.4	7.1	50%	0.005	0.013	0.4	1.2
	Mix	62.8	7	54%	0.003	0.002	0.3	0.2
sawad	Cool	89.8	1.2	46%	0.000	0.004	0.0	0.3
	Hot	89.2	1.3	36%	0.000	0.000	0.0	0.0
	Wet	84.2	0.5	34%	0.006	0.006	0.6	0.6
	Mix	81.7	0.8	31%	0.002	0.000	0.2	0.0

[a] *ma*, daily biting rate; *ie*, number of mosquitoes landing per person per night; [b] m, parity rate (% parous); [c] EIR, number of CSP-positive mosquitoes ÷ *ma*; [d] Estimated number of infective bites per season = EIR × 91.25 nights/season.

Of the 15 pools (n = 21 mosquitoes) representing CSP-positive head/thorax samples, nine pools containing 12 mosquitoes were collected outdoors. The majority of the mosquitoes (83%) in the 23 pools were collected during the initial six hours of landing collections (06:00-12:00 PM), whereas mosquitoes in the 15 pools containing positive head/thorax samples were collected throughout the night.

The seasonal distributions of CSP-positive pools containing *An. barbirostris/campestris*, *An. minimus*, *An. maculatus* and *An. sawadwongporni* mosquitoes are presented in Fig 4. Thirty-five of the 56 total CSP-positive pools (63%) were collected during the wet season (May - August), coinciding with peak mosquito populations, including 11 of the 16 total Pf-positive pools and 25 of the 40 total Pv-positive pools. A total of 22 pools (39%), including nine pools of *An. maculatus* and eight pools of *An. sawadwongporni*, coincided with the 2002 rainy season alone. For *An. minimus*, 57% (total n = 7) and 67% (n = 12) of Pf- and Pv-positive pools, respectively, were detected during the wet season. Similarly for *An. maculatus* and *An. sawadwongporni*, 78% (n = 9) and 76% (n = 21) of Pf- and Pv-positive pools, respectively, were detected during the wet season. The remaining infected

pools (25/56; 36%) were detected during the mixed season (8/56; 14%), cool season (10/56; 18%) and hot season (3/56; 5%). All four positive pools of *An. barbirostris*/*campestris* were collected in November and December (cool season).

Entomological inoculation rates

For all *Anopheles* species combined, overall entomological inoculation rates (EIRs) were approximately more than 2-fold higher for Pv than Pf (0.064 *vs* 0.028), resulting in an estimated 5.8 or 2.6 infectious bites (total 8.4 bites) received per person per year, respectively (Table 4). The EIRs and expected infectious bites were highest in the wet season for both Pv (EIR = 0.037; 3.4 infectious bites) and Pf (0.016; 1.4 bites), while they were lowest in the hot season (0.006 and 0.003, respectively; approx. 0.5 bites). The EIRs for *An. maculatus* and *An. minimus* (EIR = approx. 0.03 per species) suggested that inhabitants of Kong Mong Tha would be inoculated with both Pv and Pf from bites of approximately 3 mosquitoes of each species per year, most likely during the wet season. For *An. sawadwongporni*, the total EIR for all seasons combined (*ie*, 0.018) suggests that people would be exposed to 1.6 infectious bites per year for Pv and Pf combined. For *An. barbirostris*/*campestris*, the low EIR for *Pv* suggests this species would be capable of inoculating one person every two years.

DISCUSSION

The sheer diversity of the anopheline fauna collected within the relatively small (<5 km^2) geographic area in which this study was conducted was our most surprising result. A total of 28 distinct species were collected, along with specimens that likely represented two additional species in the *Anopheles* subgenus that were not identified beyond group (Hyrcanus and Umbrosus groups). Four species (*An. barbirostris*/*campestris*, *An. maculatus*, *An. minimus* and *An. sawadwongporni*) comprised almost 95% of the collection (Table 2). Most of the other species collected were relatively uncommon, with 14 species consisting of 15 or fewer adult specimens per species across 56 months of collections. These uncommon species are clearly of little importance in the transmission of malaria in Kong Mong Tha; however, their presence highlights the ecological diversity present in the village and surrounding area. In an entomological study conducted in five villages in southern Thailand, Rattanarithikul *et al* (1996b) described 21 species of anophelines (total n = 11,608), of which the five predominant species also consisted of *An. minimus* (47.4%), Barbirostris group. (16.0%), *An. sawadwongporni* (9.7%) and *An. maculatus* (8.0%). Similarly, Somboon *et al* (1998) described 23 species of anophelines (total n = 45,031) in 5 villages in Mae Hong Son Province, of which *An. minimus* (35.8%) and *An. sawadwongporni* (14.5%) predominated.

Anopheles minimus accounted for 54% of the adult anophelines collected during our study (Table 2). *Anopheles minimus* was also the most abundant species (i) in each sector except for sector F, where *An. maculatus* predominated, (ii) each month except for February and March (hot season) when *An. sawadwongporni* predominated and December (cool season) when *An. barbirostris*/*campestris* were the most abundant species, (iii) each hour of collection, except 06:00-08:00 PM when *An. maculatus* predominated and (iv) in all four seasons. *Anopheles minimus* was also the most abundant mosquito collected indoors, accounting for approximately 70% of the mosquitoes that were collected

inside houses. The proportion of *An. minimus* collected outdoors is consistent with the observed exophagy (approx. 60%) of *An. minimus* collected in Mae Sot District (Tisgratog *et al*, 2012). Populations of *An. minimus* generally peaked once during the rainy part of the year (*ie*, wet/mixed seasons), with peaks occurring in July 1999, October 2000, October 2001, July 2002 and September 2003. Despite having one of the lowest infection rates of the four predominant *Anopheles* species, *An. minimus* had the highest annual entomological inoculation rates (Tables 3 and 4). Over 60% of head/thorax samples tested positive, especially for mosquitoes infected with Pv. Based on these results, *An. minimus* is probably the primary vector of both Pf and Pv in Kong Mong Tha. *Anopheles minimus* has previously been incriminated as a primary vector of malaria in Thailand (Harbach *et al*, 1987; Ratanatham *et al*, 1988; Gingrich *et al*, 1990; Rattanarithikul *et al*, 1996a; Somboon *et al*, 1998; Singhasivanon *et al*, 1999; Chareonviriyaphap *et al*, 2000; Zollner *et al*, 2006) and is widely distributed throughout the country. *Anopheles minimus s.s.* (formerly *An. minimus* species A) and *An. harrisoni* (formerly *An. minimus* C) are both found in western Thailand (Rattanarithikul *et al*, 2006); however, we did not attempt to differentiate the members of the *An. minimus* complex in this study.

Anopheles maculatus is also considered an important malaria vector in Thailand (Green *et al*, 1990; Rattanarithikul *et al*, 2006) and was the second most abundant species collected each month during the wet and mixed seasons and the third most abundant during the cool and hot seasons. In contrast to *An. minimus*, which was active throughout the night, approximately 50% of *An. maculatus* mosquitoes were collected during the first two hours after sunset (06:00-08:00 PM). This early evening activity has been observed elsewhere in Thailand (Harbach *et al*, 1987) and northern Malaysia (Rahman *et al*, 1995) and may enhance its vector potential as most villagers are not using their mosquito bed nets during this 2-hour period. However, our results are inconsistent with observations of primary *An. maculatus* feeding activity during 08:00-10:00 PM in a village in Sai Yok District located approximately 90 km from KMT (Muenworn *et al*, 2009). *Anopheles maculatus* was highly exophagic, with almost 90% of mosquitoes collected outdoors. *Plasmodium* infection rates were twice as high for *An. maculatus* than for *An. minimus* (Table 3), and a higher proportion of head/thorax samples were CSP-positive for *An. maculatus* (84.2%) compared to *An. minimus* (63.2%). Rattanarithikul *et al* (1996a) reported that *An. maculatus* had the second highest vectorial capacity of all anophelines evaluated in five villages in southern Thailand (*ie*, total C = 1.94 compared with 4.27 for *An. minimus*), though Upatham *et al* (1988) reported that *An. maculatus* complex (including *An. maculatus* species B, forms E and F) was primarily zoophilic and of relatively minor importance as a malaria vector in Pak Chong District (ENE of Bangkok).

Anopheles sawadwongporni was the third most common anopheline collected during the study and had the highest overall infection rate (0.49%) of the four most common anophelines. Somboon *et al* (1998) reported an infection rate of 0.13% in *An. sawadwongporni* collected north-west Thailand, while Rattanarithikul *et al* (1996a) reported a rate of 1.1% in southern Thailand. Zollner *et al* (2006) conducted detailed studies that evaluated sporogonic development of Pv in *An. sawadwongporni* and clearly demonstrated that this mosquito was a competent vec-

tor. In contrast to other mosquitoes that were most abundant at certain times of the year (*eg*, *An. barbirostris/campestris* in the cool season and *An. maculatus* in the wet season), *An. sawadwongporni* was abundant throughout the year with populations peaking at different times. For example, over the course of this study six population peaks occurred – these peaks occurred in 7 different months (February, March, May, June, September, October and December). As with *An. maculatus*, *An. sawadwongporni* mosquitoes were collected most frequently early in the evening. These results are in agreement with those reported by Rattanarithikul *et al* (1996b).

Anopheles barbirostris/campestris was the fourth most common anopheline collected in Kong Mong Tha and potentially plays a significant role in the transmission of Pv in western Thailand. The adult morphology makes it difficult to differentiate *An. campestris* from *An. barbirostris* (Limrat *et al*, 2001), though the unraveling of sibling species in the *An. barbirostris* complex using molecular methods has resulted in a clearer species delineation (Saeung *et al*, 2007, 2008; Paredes-Esquivel *et al*, 2009; Suwannamit *et al*, 2009; Otsuka, 2011; Thongsahuan *et al*, 2011). *Anopheles barbirostris/campestris* mosquitoes (96%) were primarily collected from October through January (mixed/cool seasons), with peak densities of mosquitoes collected in December. Adults were collected throughout the night without any clear activity period, though Apiwathnasorn *et al* (2002) have previously reported a peak biting period between 08:00 PM and 01:00 AM (peaking around 11:00 PM) in Sa Kaeo Province, Thailand. The majority of *An. barbirostris/campestris* mosquitoes were collected outdoors, in contrast to the mostly endophagic mosquitoes collected by Apiwathnasorn *et al* (2002). In the present study, *An. barbirostris/campestris* mosquitoes were predominantly collected on the western edge of the village, with 36% of specimens collected in Sector H. A total of four *An. barbirostris/campestris* mosquitoes were infected with Pv-210. Three of the specimens were thorax-positive, suggesting that this species is a potential vector in Kong Mong Tha. Although only 0.26% of all *An. barbirostris/campestris* mosquitoes tested were infected, the infection rates in December 1999 and November 2001 were approximately 1%. Kengluecha *et al* (2005b) reported that *An. campestris* was not a malaria vector; however, *An. barbirostris/campestris* has been incriminated as a probable vector in Thailand (Somboon *et al*, 1994; Frances *et al*, 1996; Limrat *et al*, 2001). Apiwathnasorn *et al* (2002) conclusively demonstrated that *An. barbirostris/campestris* could support the development of Pv and suggested its potentially important status as a vector. Furthermore, Thongsahuan *et al* (2011) demonstrated that three *An. barbirostris* species (A1, A2 and A3) and two *An. campestris*-like forms (B and E) are potential vectors of Pv.

Anopheles dirus accounted for only 0.85% of the overall catch in the present study and hence was not amongst the four predominant *Anopheles* species, but this species complex deserves mention due to its status as the primary malaria vectors throughout Thailand (Gingrich *et al*, 1990; Rosenberg *et al*, 1990; Zollner *et al*, 2005; Rattanarithikul *et al*, 2006; Zollner *et al*, 2006). As with the other species complexes, we did not distinguish between the sibling species in the *An. dirus* complex; however, previously *An. dirus* population cytogenetics studies suggests the possibility that our specimens consisted of *An. dirus* A, *An. dirus* D, and even *An. dirus* C (Baimai *et al*, 1984; Baimai, 1988). In the present study, virtually

all *An. dirus* mosquitoes were collected during the wet season, which is in accordance with the high densities of *An. dirus* larvae collected from wheel tracks, animal wallows or other temporary ground pools during the same time of year (Zollner *et al*, unpublished data). Somboon *et al* (1998) also described low proportions (2.1%) of *An. dirus* mosquitoes in human landing collections in Mae Hong Son Province. It is unknown whether the altitude or topography affects the distribution or vector status of *An. dirus* mosquitoes at these study locations.

Four species (*An. minimus*, *An. maculatus*, *An. sawadwongporni* and *An. barbirostris/ campestris*) appear to play a significant role in the transmission of malaria in Kong Mong Tha. These four species were relatively abundant in human landing collections performed throughout the village, and they were presumed to be capable of transmitting *Plasmodium* parasites based on the presence of CSP in the head/thorax of the specimens tested. Although it is known that CSP can be released by mature oocysts and circulate in the mosquito hemolymph (Ryan *et al*, 2002), and we did not confirm CSP-positive specimens by PCR, evaluate individual salivary glands for the presence of live sporozoites or conduct vector competence studies, all four species have previously been incriminated as vectors of Pf and/or Pv in Thailand (Ratanatham *et al*, 1988; Somboon *et al*, 1994; Clarke *et al*, 1996; Apiwathnasorn *et al*, 2002; Zollner *et al*, 2006).

Several additional species (*An. kochi*, *An. varuna*, *An. philippinensis* and *An. dirus*) were frequently collected in lower numbers and may also play a role in the transmission of malaria in the village. We detected Pv CSP in *An. kochi* and *An. varuna. Anopheles kochi* has been incriminated as a potential vector of both Pf and Pv (Somboon *et al*, 1994), and the presence of Pv-210 CSP in the thorax of a positive specimen suggests that Pv sporozoites might have been present, though there is no indication whether any sporozoites invaded the mosquito salivary glands (Verhave *et al*, 1988). Neither *An. varuna* nor *An. philippinensis* has been implicated as a malaria vector in Thailand; however, Yapabandara and Curtis (2004) incriminated *An. varuna* as a malaria vector in Sri Lanka, and Prakash *et al* (2004, 2005) incriminated *An. philippinensis* as a vector in India. No *Plasmodium*-infected *An. dirus* were collected during our study, although sympatric populations of *An. dirus* species A, C and D have been previously collected along the central Thai-Myanmar border (Walton *et al*, 2000).

The exophagic preference of the four predominant mosquito species (*An. barbirostris/campestris*, *An. maculatus, An. minimus* and *An. sawadwongporni*) has been observed in previous studies conducted in Thailand (Somboon *et al*, 1998). In Kong Mong Tha, it is likely that malaria transmission is linked to exophilic human behavior; throughout the study, we observed villagers sitting outdoors for approximately 3 hours after sunset (06:00-09:00 PM) while their youngest children (approximately 6 months to 5 years of age) were sleeping indoors. This behavior means that a constant supply of ready bloodmeals were available for anopheline vectors without the need to enter houses, though *An. minimus* was the least exophagic of all four predominant mosquito species in Kong Mong Tha. In addition, the Thai Ministry of Public Health (MOPH) conducted biannual mosquito control campaigns (usually in March and October) using pyrethroid thermal fogging or residual spray inside and out-

side all houses in Kong Mong Tha. It is unknown whether the exophagic behavior of the main vector species might have resulted from a behavioral shift following several years of spraying.

The relative roles of Barbirostris group, *An. maculatus*, *An. minimus* and *An. sawadwongporni* in malaria transmission can be described by the EIRs for each species. The low EIRs for all *Anopheles* species reflect the low incidence of malaria in Kong Mong Tha (Coleman *et al*, unpublished data). For all four predominant *Anopheles* species combined, the inhabitants of Kong Mong Tha might expect to receive just eight infective bites per year overall, particularly during the wet season or early mixed season based on the higher numbers of potentially infective mosquitoes collected during those times of year. EIRs and estimated infective bites for both Pv and Pf were consistently as high or higher for *An. minimus* than for any of the other key vectors during each of the four seasons, which confirms its status as the primary malaria vector in Kong Mong Tha. The 2-fold higher EIR values for Pv (0.064) compared to Pf (0.028) are likely due in part to a shorter Pv sporogonic cycle in susceptible anophelines (*ie*, 9 days *versus* 12 days for Pf) and a higher prevalence of new or relapsing Pv infections in Kong Mong Tha (Coleman *et al*, unpublished data).

The efficiency of the four predominant *Anopheles* species as malaria vectors depends on how frequently they feed on people and the likelihood that they will survive to transmit *Plasmodium* parasites in a subsequent infective bloodmeal. Mosquitoes that take more frequent bloodmeals and survive longer have greater potential for obtaining an infection that they might transmit to a susceptible person (Roberts *et al*, 1983). Vector survival rates are based on gonotrophic cycle and parity status, and a reduced survival rate has significant epidemiological implications since it decreases the number of blood-feedings and oviposition cycles (Klein *et al*, 1986). We did not calculate daily survival values for the four predominant vector species described in the present study, because we lacked directly observed values for different gonotrophic cycle lengths in accordance with temperature fluctuations in different seasons. In addition, we did not have some of the key epidemiological parameters (*ie*, human blood index and length of *P. vivax* and *P. falciparum* sporogonic cycles) to directly calculate vectorial capacity for the predominant *Anopheles* species. While vectorial capacity values have not been calculated for any vector species in Thailand based on direct measurements of all transmission parameters, Rattanarithikul *et al* (1996b) estimated that *An. minimus* had the highest vectorial capacity in peninsular Thailand, especially during the dry season for both Pv and Pf transmission. Based on the high parity rates for *An. minimus*, from which survival rates can be indirectly estimated (Davidson 1954; Trung *et al*, 2004), it is likely that *An. minimus* has a higher vectorial capacity compared with *An. maculatus* and other vector species in Kong Mong Tha.

In this paper, we have attempted to distill a large data set into definable trends that describe the key transmission parameters for the predominant vectors of Pf and Pv transmission in Kong Mong Tha. The data from the present study clearly indicate that *An. minimus* and *An. maculatus* are the primary vectors of both Pf and Pv in the village of Kong Mong Tha. Subsequent papers in the series will describe the bionomics of larval *Anopheles* mosquitoes, the epidemiology of Pf and

Pv transmission, and the efficacy of mosquito control campaigns in the village of Kong Mong Tha.

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