SEASONAL ABUNDANCE AND BITING ACTIVITY OF *ANOPHELES ACONITUS* (DIPTERA: CULICIDAE) IN CHIANG MAI, NORTHERN THAILAND

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Abstract. The seasonal abundance and nocturnal biting activity of *Anopheles aconitus*, a secondary vector of malaria in Thailand, were investigated from January 2005 to February 2006. Seasonal changes in abundance were mainly influenced by monthly rainfall, with a major peak occurring from August to November. This species preferred to bite animals rather than humans, and favored biting humans outdoors rather than indoors. The biting activity was highest at dusk and/or in the evening, but was low throughout the remaining night-hours. A unimodal pattern of biting, with a peak occurring at 06:00-08:00 PM was seen in *An. aconitus*. The overall parous rate was 51.19% (35.85-75.00%). During peak abundance (August to November), a parous rate ranging from 42.86 to 61.57 was observed. Of 1,198 dissected specimen stomachs, 0.17% (2/1,198) were found to be infected with oocysts. No sporozoites were detected in any of the 1,198 specimen salivary glands.

INTRODUCTION

Anopheles (*Cellia*) *aconitus* has been incriminated as a natural vector of *Plasmodium vivax* in central Thailand (Gould *et al*, 1967; Scanlon *et al*, 1968). It has also been reported as a vector for malaria in other countries, such as Indonesia (Kirnowardoyo, 1985; Kirnowardoyo and Supalin, 1986), Bangladesh (Maheswary *et al*, 1992) and Malaysia (Rahman *et al*, 1993). Recently, three karyotypic forms of *Anopheles aconitus*, Form A (X_1, X_2, Y_1), Form B (X_1, X_2, Y_2) and Form C (X_1, X_2, Y_3), have been reported sympatrically from Mae Taeng District, Chiang Mai Province, northern Thailand (Baimai *et al*, 1996). Subsequently, the latter two karyotypic forms were confirmed as efficient laboratory vectors for both *P. falciparum* and *P. vivax* (Junkum *et al*, 2005a). Based on the results of ovarian nurse cell polytene chromosome and isoenzyme investigations, and hybridization experiments, DNA sequences of ITS1, ITS2, D3, COI and COII regions of *An. aconitus*, Forms B and C are considered as conspecific cytological races in the Thai population (Jariyapan *et al*, 2005; Junkum *et al*, 2005b). Even though the above results indicate an extensive population genetic study of *An. aconitus*, very little concerning the biological and/or ecological aspects is known for strains found in northern Thailand. In view of its important role as a natural vector for *P. vivax*, and the lack of biological and/or ecological information, a more thorough study of *An. aconitus* is needed. Therefore, the seasonal abundance and nocturnal biting activity of *An. aconitus* in Mae Taeng District, Chiang Mai Province, northern Thailand are described below.

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MATERIALS AND METHODS

Study site

The study site was Ban Pang Mai Daeng, Mae Taeng District, Chiang Mai Province, northern Thailand (endemic for malaria, where Baimai *et al* (1996) determined three karyotypic forms of *An. aconitus*) was chosen as the study area (Fig 1).

Fig 1- Map of Thailand showing Chiang Mai Province (CM), where mosquito collections were performed at Mae Taeng District. Chiang Mai is situated at a latitude of 18° 47′N and longitude of 98° 59′E in northern Thailand, approximately 581 km away from the capital city, Bangkok (BK), central Thailand.

Specimen collection

Collections were carried out twice a month (two consecutive nights) from 06:00 PM to 06:00 AM, from January 2005 to February 2006. For human bait, two persons at each site [one inside a house (indoors) and one outside a house (outdoors)] sat and exposed their legs as bait. One catcher at each site caught all anopheline mosquitoes attempting to bite using an aspirator. For animal bait, one calf was tethered approximately 10 m from the human bait, and all anopheline mosquitoes attempting to land and bite the calf were collected using a battery-powered aspirator. The collection time for each hour was divided into 50 minutes for catching and 10 minutes resting. Adults caught hourly were preserved in separate cups, supplied with 5% sucrose solution and transported to the insectary. The species were identified using the morphological keys of Harrison (1980). The stomach and salivary glands for all identified *An. aconitus* specimens were dissected for oocyst and sporozoite investigations, and 2 ovaries for parity identification (Detinova, 1962). Climatic data, *ie*, temperature and relative humidity, were measured every 10 minutes from 06:00 PM to 06:00 AM using a handy-typed digital thermometer and hygrometer, whereas rainfall data were obtained from the Northern Meteorological Center. The year was divided into three seasons following the Thai Meteorological Department, which bases its records on rainfall and temperature values: hot season (mid February to mid May), rainy season (mid May to mid October), and dry-cool season (mid October to mid February).

RESULTS

The numbers of captured *An. aconitus* females on human and water-buffalo bait are shown in Table 1. In total, 1,334 adult *An. aconitus* females were captured indoor from humans (8), outdoor on humans (165) and on water-buffalo bait (1,161), (Table 1).

The mosquitoes were more abundant during the rainy season (August) and the dry-cool season (November), with peaks obtained during the transitional month of October in the late rainy season and beginning of the dry-cool season from both human bait positioned outdoors (24.50/night) and water-buffalo bait (150.50/night) (Fig 2). Statistical analysis revealed a positive association between the monthly number of mosquitoes and rainfall ($r = 0.96$, $p < 0.05$); but no significant relationship between monthly number and temperature ($r = -0.02$, $p > 0.05$), or monthly number and relative humidity ($r = 0.55$, $p > 0.05$) on human bait collection was found. Similar results were also seen with water-buffalo bait collections, *ie*, the monthly number was positively associated with rainfall ($r = 0.58$, $p < 0.05$), but it did not correlate with temperature ($r = -0.07$, $p > 0.05$) or humidity ($r = 0.55$, $p > 0.05$).

The nocturnal biting of *An. aconitus* on human bait outdoors and water-buffalo bait from 06:00 AM - 06:00 PM during a major peak

Table 1
Number of *An. aconitus* females collected on human and water-buffalo bait from January 2005 to February 2006; collections were performed from 06:00 AM - 06:00 PM for two consecutive nights each month.

Months	Human bait		Water-buffalo bait	Total no.	Parous rates (no.)	Mean temperature in °C (range)	Mean % humidity (range)	Mean rainfall in mm
	Indoors	Outdoors	Outdoors					
Jan 05	0	3	17	20	40 (8/20)	22.56 (17.10-29.43)	68.27 (51.67-78.00)	0.0
Feb 05	0	1	11	12	41.67 (5/12)	25.58 (20.47-29.07)	49.15 (28.33-64.33)	4.9
Mar 05	0	1	5	6	50 (3/6)	27.76 (23.97-31.40)	45.56 (38.00-51.00)	24.7
Apr 05	0	0	4	4	75 (3/4)	28.16 (25.73-31.90)	61.01 (44.33-70.00)	57.2
May 05	0	2	9	11	60 (6/10)	28.45 (25.93-31.37)	62.71 (45.33-76.00)	104.7
Jun 05	0	2	12	14	42.86 (6/14)	27.52 (26.10-29.87)	76.47 (59.33-86.67)	193.5
Jul 05	1	3	25	29	37.04 (10/27)	25.14 (23.07-27.53)	90.31 (79.33-96.67)	179.1
Aug 05	1	22	136	159	56.69 (72/127)	26.13 (24.80-27.80)	85.01 (74.00-90.00)	155.2
Sep 05	3	41	294	338	42.86 (129/301)	26.93 (24.10-29.80)	79.08 (70.00-88.67)	436.3
Oct 05	1	49	301	351	56.10 (161/287)	25.78 (24.47-27.13)	77.78 (70.00-83.00)	192.0
Nov 05	0	32	241	273	61.57 (141/229)	23.92 (19.63-26.50)	80.72 (72.00-87.67)	22.8
Dec 05	2	7	53	62	35.85 (19/53)	20.79 (19.47-23.07)	66.06 (49.67-80.00)	27.9
Jan 06	0	2	29	31	42.86 (12/28)	22.13 (16.97-25.17)	61.28 (43.00-73.67)	0.0
Feb 06	0	0	24	24	38.10 (8/21)	24.65 (23.30-26.37)	59.13 (45.00-70.33)	0.0
Total	8	165	1,161	1,334	51.19 (583/1,139)	-	-	-

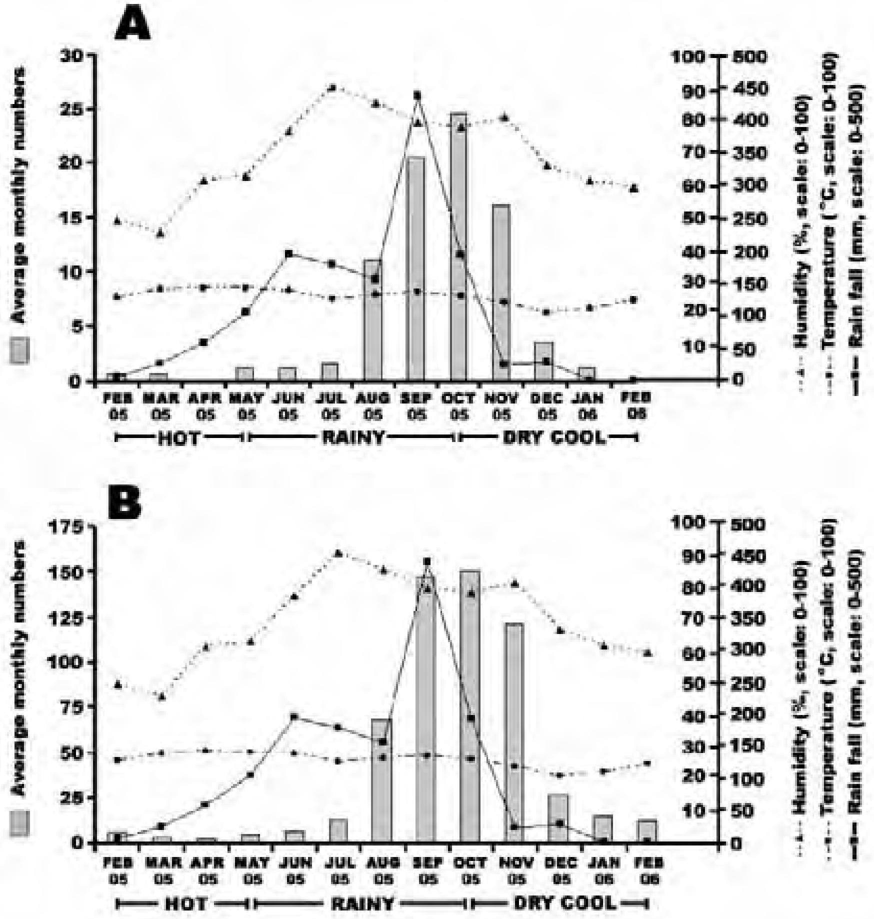

Fig 2- Seasonal changes in abundance of *An. aconitus*; A: biting on human bait positioned outdoors; B: biting on water-buffalo bait.

of abundance (total number of females caught over two consecutive nights was greater than 20 in humans and greater than 50 in water-buffalo bait) in August, September, October, November and December (Table 2, Figs 3 and 4).

A unimodal pattern, with a peak at dusk and/or in the evening (06:00 - 08:00 PM) was seen on both humans and water-buffalo bait, although the temperature gradually decreased and humidity gradually increased during the night, but this did not appear to be a main cause of bites in the evening, since both temperature and humidity were high enough for mosquito activity. Nevertheless, variations in hourly peak from month to month were plainly observed (Fig 4 A, B, C).

Dissections stomach and salivary glands for oocysts and sporozoites in 146 females caught from human bait yielded zero positive samples, whereas 2 stomachs (one caught in October, and the other in November) from a total of 1,052 females dissecter [0.19% (2/1,052) oocyst rate] captured from water-buffalo bait were positive for 2 and 5 immature oocysts, respectively.

Parity was determined for 1,139 females. Overall, 51.19% (35.85-75.00%) of the mosquitoes were parous. Based on the higher number of caught females during August to November compared to the other months, the high parous rates were 56.69, 42.86, 56.10 and 61.57% in August (rainy season), September (rainy season), October (transitional month of the

Table 2

Average nightly number of *An. aconitus* bites on humans positioned outdoors and water-buffalo bait collected from 06:00-08:00 PM in each representative month for evaluation of abundance.

Collection time	Rainy season						Dry-cool season			
	Aug 05		Sep 05		Oct 05		Nov 05		Dec 05	
	H	B	H	B	H	B	H	B	H	B
06:00-07:00 PM	3.5	23	8.5	40.5	12	63	14	96	2.5	12
07:00-08:00 PM	4.5	34.5	10.5	59.5	7.5	29.5	1.5	8.5	1	5.5
08:00-09:00 PM	2.5	6	1	17.5	4	7.5	0.5	3.5	0	4.5
09:00-10:00 PM	0.5	2	0	1.5	1	3.5	0	2.5	0	1.5
10:00-11:00 PM	0	1	0.5	2.5	0	4	0	0	0	2
11:00-12:00 PM	0	0	0	0.5	0	4.5	0	0	0	0.5
00:00-01:00 AM	0	0	0	1.5	0	0	0	0	0	0
01:00-02:00 AM	0	0	0	3.5	0	5.5	0	0	0	0
02:00-03:00 AM	0	0.5	0	5	0	3.5	0	1	0	0
03:00-04:00 AM	0	0	0	5.5	0	11	0	1	0	0
04:00-05:00 AM	0	1	0	4.5	0	8	0	2.5	0	0.5
05:00-0:600 AM	0	0	0	5	0	10.5	0	5.5	0	0
Biting activity pattern	U (07:00-08:00 PM)	U (07:00-08:00 PM)	U (07:00-08:00 PM)	U (07:00-08:00 PM)	U (06:00-07:00 PM)	U (06:00-07:00 PM)	U (06:00-07:00 PM)	U (06:00-07:00 PM)	U (06:00-07:00 PM)	U (06:00-07:00 PM)

H: human bait; B: water-buffalo bait, U: unimodal; ( ): major peak time

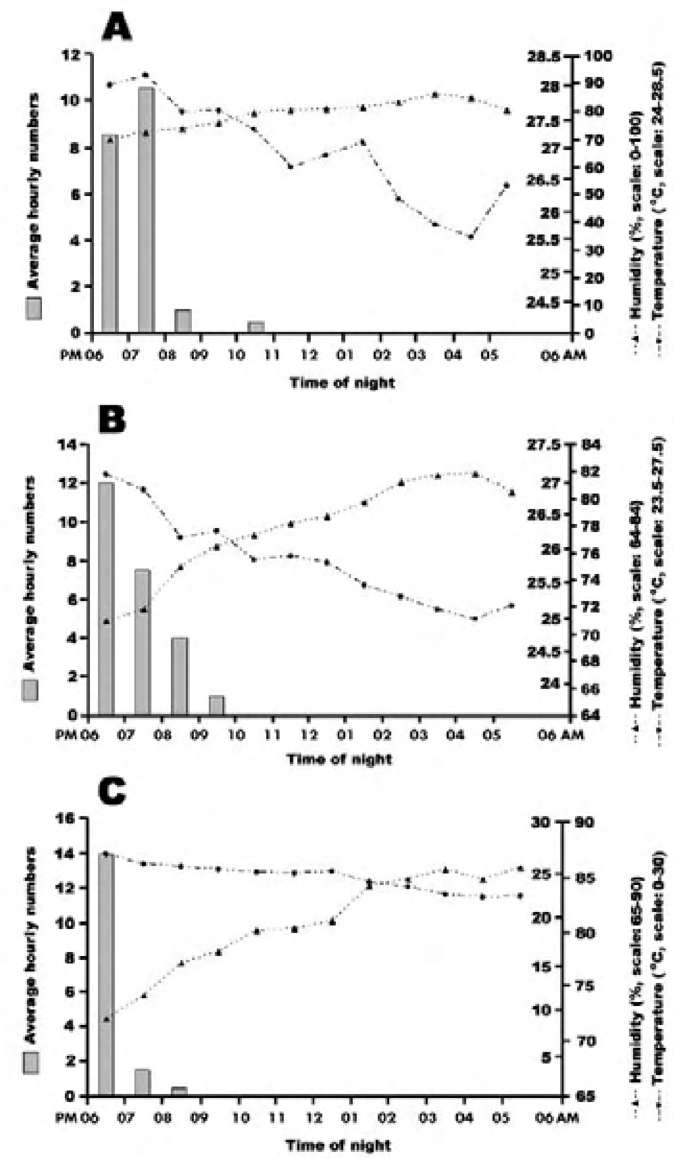

Fig 3- Variations in hourly biting activities of *An. aconitus* on human bait positioned outdoors from 06:00 PM – 06:00 AM. (A) September 2005, (B) October 2005, and (C) November 2005.

rainy season and dry-cool season), and November (dry-cool season), respectively (Table 1). The parous rate of 42.86% in September differed significantly from the other months ($\chi^2 = 20.97$, $p < 0.05$).

DISCUSSION

Previous studies of the seasonal abundance and nocturnal biting activity of adult *An. aconitus* from various types of geographical locations indicated the variations from one location to another, and these factors were presumably influenced by climatic conditions and types of bait (Chow *et al*, 1960; Reid, 1968; Harrison, 1980; Rahman *et al*, 1993). In the present study conducted in the rice plain of Mae Taeng District, Chiang Mai Province, northern Thailand, where

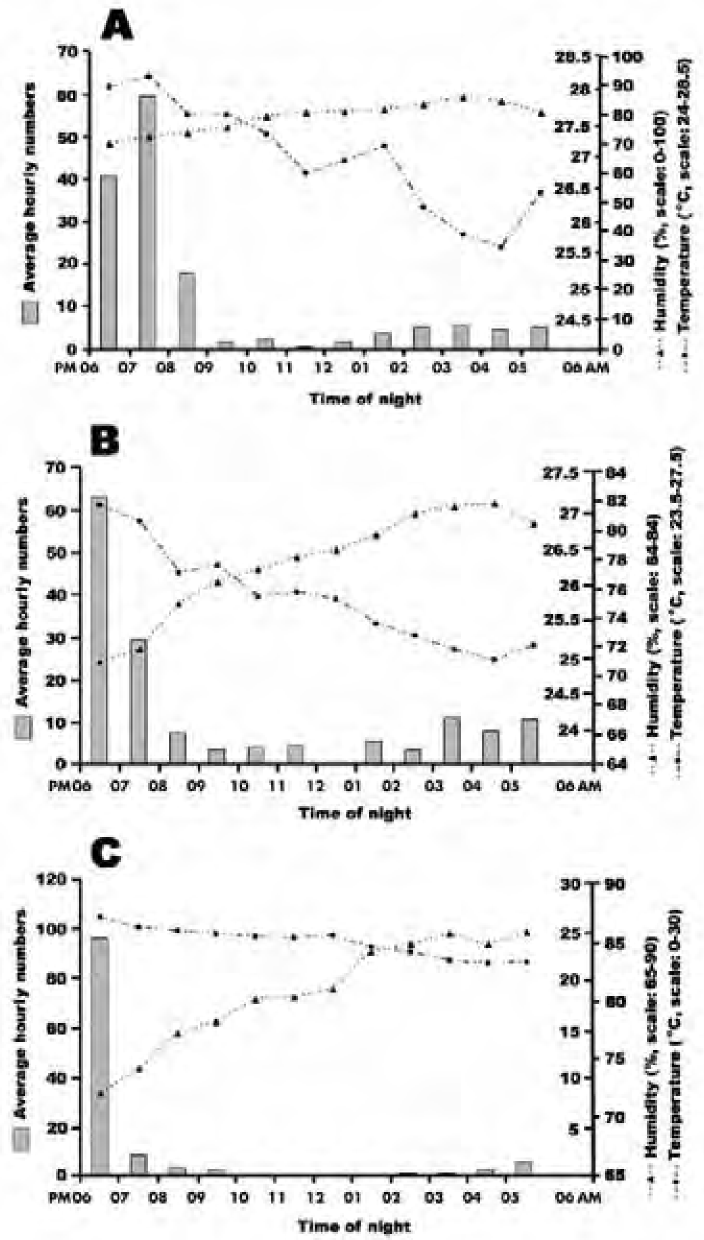

Fig 4- Variations in hourly biting activities of *An. aconitus* on water-buffalo bait from 08:00 PM-06:00 AM. (A) September 2005, (B) October 2005, and (C) November 2005.

a high volume of rainfall occurs between June to October (peaking in September), the population densities of *An. aconitus* obtained from both human and water-buffalo bait showed a greater abundance during the rainy season (August) to dry-cool season (November) (peaking in October). Additionally, seasonal changes in monthly number had a positive association with rainfall, but no correlation with monthly temperature or relative humidity. These results are in contrast to those found by Rahman *et al* (1993), who carried out during a seasonality study of *An. aconitus* in an endemic village, Kampong Bongor, located near the Malaysia-

Thailand border (longitude 101° 11′N, latitude 5° 30′ N). Although the monthly rainfall was similar to the present study, the population densities demonstrated were more abundant from March to May (peaking in May), when the rainfall was extremely low, indicating no correlation between monthly number and rainfall, and presumably, other factor(s) were involved.

The results of nocturnal biting activity exhibited that *An. aconitus* bit both humans and animals, with the animal biting densities significantly higher throughout the seasons of occurrence. Additionally, the *An. aconitus* bit humans outdoors approximately 20.63 times more often than indoors, and bit animals about 6.71 times more often than humans. Our study is generally in agreement with one in Pathum Thani Province, in the central plain of Thailand (Harrison, 1980). At this location, *An. aconitus* bit humans outdoors approximately 8.34 times more often than indoors, and bit animals about 8.10 times more often than humans. The results of biting patterns on both humans and animals revealed that *An. aconitus* had a unique unimodal pattern that peaked at dusk and/or in the evening (06:00-08:00 PM) throughout the rainy to dry-cool seasons. This situation increases the frequency of man-vector contacts, since these time periods are coincidental with various activities near human households.

Parous rates on a monthly basis were used to evaluate mortality and daily survival rates in mosquitoes (Samarawickrema, 1967; Upatham *et al*, 1988). In our study, the average parous rate was 51.2% (583/1,139), in comparison to 21.0% (12/57) in Pathum Thani Province, where *An. aconitus* was incriminated (positive for both oocysts and sporozoites on dissection) as a vector for *P. vivax* (Gould *et al*, 1967), and 65.7% (44/67) in Buri Ram Province (Harrison, 1980). The result of a very low oocyst rate (0.2% (2/1,052)), and no sporozoites from dissected salivary glands of *An. aconitus* during the 14-month study period, may indicate the effect of the antimalaria program in the area (Division of Malaria, 2005). Efficient laboratory vectors for *P. falciparum* and *P. vivax* (Junkum *et al*, 2005a) have a longer survival rate, as determined by a high parous rate, and the outdoor biting behavior at dusk, may increase the chances of transmitting malaria to man, particularly during outbreaks and/or re-emerging periods.

ACKNOWLEDGEMENTS

The authors sincerely thank the Thailand Research Fund (TRF: BRG/14/2545) and the Thailand Research Fund through the Royal Golden Jubilee PhD Program (Grant No. PHD/0044/2546) for financially supporting this research project; Niwes Nantachit, Dean of the Faculty of Medicine, Chiang Mai University, for his interest in this research; and the Faculty of Medicine Endowment Fund for Research Publication for its financial support in defraying publication costs.

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