BITING BEHAVIOR AND SEASONAL VARIATION IN THE ABUNDANCE OF *ANOPHELES MINIMUS* SPECIES A AND C IN THAILAND

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Abstract. We measured the seasonal abundance and bloodfeeding behavior of species A and C of the mosquito *Anopheles minimus* Theobald 1901 in an endemic malarious area of western Thailand. *An. minimus s.l.* is a major vector of human malarial and filarial parasites in Southeast Asia. Mosquitos were collected once a month for one year using four collection methods: human-baiting indoors, human-baiting outdoors, human-baiting in the forest, and cow-baiting. We found that both species A and C tend to feed from cows rather than humans; we did not find any preference for indoor, outdoor or forest-biting in either species. Both species had a peak biting density in October/November, at the end of the rainy season, and species C showed a second, smaller peak at the end of the cool season. These findings are discussed in relation to previous reports of the behavior of *An. minimus s.l.*, particularly in light of suggestions that *An. minimus s.l.* has changed its feeding behavior in response to DDT spraying.

INTRODUCTION

Anopheline behavior is important in the epidemiology of disease transmission and in vector control. Detailed information on feeding and host-seeking behavior can be used to improve malaria control projects: mosquitos that prefer to feed on animals are less important in transmitting human disease than those that prefer to feed on humans; mosquitos that prefer to rest indoors are potentially vulnerable to residual spraying with insecticides such as DDT and pyrethroids (Ismail *et al*, 1974, 1978; Rowland *et al*, 2000), whereas those that rest outdoors are not.

We studied the seasonal abundance and bloodfeeding behavior of cryptic species of *Anopheles minimus* Theobald 1901 in an endemic malarious area of western Thailand near the Myanmar border. *Anopheles minimus sensu lato* is an important vector of both human malarial and filarial parasites in Southeast Asia (Harrison, 1980). In Thailand, it has been virtually eliminated from the peninsula and central plains, but remains abundant in forested hilly areas (Harrison, 1980). Population genetic evidence has shown *An. minimus* to comprise at least two morphologically similar species in Thailand and in Vietnam (Green *et al*, 1990; Sharpe, 1997; Van Bortel *et al*, 1999). The species have been given the informal names of *An. minimus* A and *An. minimus* C. Both differ morphologically from 'form A' and 'form B' from Southern China, described on the basis of morphological variations (Yu and Li, 1984; Yu, 1987).

Species A has been recorded throughout Thailand and in Vietnam, whereas species C has been recorded from only three provinces in northern and western Thailand, and in Vietnam (Green *et al*, 1990; Sharpe *et al*, 1999; Van Bortel *et al*, 1999). Identities throughout the

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rest of southeast Asia remain unknown.

An. minimus s.l. has long been characterized by its biting humans indoors and in some cases resting indoors too (Harrison, 1980). However, further investigations of this feeding behavior are needed for two reasons. First, the behavior of *An. minimus s.l.* has been noted to change in response to the spraying of houses with residual insecticides. The frequency of indoor biting (Ismail *et al,* 1974; 1975) and human biting (Suthas *et al*, 1986a) both decreased following DDT spraying. Second, the cryptic species may differ in their biting behaviors.

There is indirect evidence to suggest that species A and C may differ in their feeding behaviors. A mark-release-recapture experiment in northern Thailand showed host-preference heterogeneities: *An. minimus s.l.* caught on humans or bovids tended to return to these same host species (Suthas *et al,* 1986b). One explanation for this finding is that two species were present, one which prefers to bite humans and one which prefers to bite bovids. Additionally, there is evidence of feeding preference heterogeneities between the cryptic species of *An. minimus* in Vietnam (Van Bortel *et al,* 1999). 'Form I' mosquitos were half as likely as 'form II' mosquitos to be caught outdoors on humans or cows, but five times as likely to be caught by indoor resting collection methods than by all other collection methods combined.

The seasonal abundance and host preference of *An. minimus* species A and C are of fundamental epidemiological importance. Previous measurements of the seasonal abundance and host preference of *An. minimus* in Thailand have not distinguished between the two known cryptic species. However, given the widespread geographical distribution of species A, and the locations where the work was done, it is likely that those studies relate to populations either entirely or mainly composed of species A. As far as we are aware, this study represents the first report about the seasonal abundance and host preference of species C.

MATERIALS AND METHODS

Study location

The study was conducted in January to December 1997 in Ban Phu Toei, a village of approximately 350 inhabitants in Sai Yok district, Kanchanaburi Province, western Thailand. Ban Phu Toei is located 30 km from the border with Myanmar and lies at 100 m above sea level, latitude 14.3ºN, longitude 98.5ºE. Total annual rainfall is 1.5 m with distinct wet and dry seasons. The village rests in a small valley between forested hills which rise to elevations of approximately 400 m. Surrounding vegetation is primarily agricultural land of rice and maize, abandoned cropland and dense secondary forest. The region is highly malarious with both *Plasmodium falciparum* and *P. vivax* infections that are resistant to many anti-malarial drugs including chloroquine and mefloquine. The principal malaria vectors are the *An. minimus* and *An. dirus* species complexes.

Mosquito collection

Adult *An. minimus* A and C were collected once a month from three fixed locations in the village, using human landing techniques: 1) indoor; 2) peridomestic, approximately 20 m from human dwellings; 3) forest fringe, approximately 300 m from human dwellings. Collections were made between 18.00 and 00.00 hrs on two consecutive nights by two persons at each site. A fourth collection was made using a cow-baited trap 4 m x 4 m x 3 m at a fixed location approximately 30 m from human dwellings. Cow trap collections were made on the same nights as the human-landing collections, between the hours of 18.00 and 00.00. Resting mosquitos were aspirated from inside the net for 10 minutes of every hour.

Mosquitos were identified using the morphological keys of Peyton and Scanlon (1966), Rattanarithikul and Panthusiri (1994) and Harrison (1980). Species A and C were distinguished using a morphological character: presence or absence of the humeral pale (HP) spot on the costal vein of the wing. Past surveys in the same region, using molecular methods to identify each species, showed that 95% of species A lack HP spots, whereas 73% of species C possess HP spots (Green *et al*, 1990).

Environmental data

Monthly climate data including rainfall, humidity and temperature were obtained for Kanchanaburi Province (for a village 40 km from Ban Phu Toei) from the Meteorological Department in Bangkok. The year was divided into three seasons, based on recorded rainfall and temperature values. Dry cool season months, from November through February, had less than five days of rain and mean temperatures less than 28ºC. Hot season months, from March through June, had mean temperatures over 29ºC; rainy season months, from July through November, had more than five days of rain and mean temperatures less than 29ºC.

Statistical analyses

The differences in mean numbers of *An. minimus* A and C captured by each collection method and in each season were compared by ANOVA, using the GLM procedure in SAS for Windows, version 6.12. Count data were adjusted per collector (1 cow *vs* 2 humans), and log-transformed. F-tests given use partial sums of squares and represent the significance of each variable after the effects of the other variables in the model have been accounted for. Probability values <0.05 were considered significant. Tukey-Kramer tests were used for multiple comparisons of means; significance was evaluated at the level of 0.01.

Multiple regression analysis was used to investigate the association between adult mosquito abundance and environmental variables. Climate data for the month of adult capture and time-lagged by one and two months before the collection month were included in the analysis.

RESULTS

Mosquito abundance and seasonal distribution

A total of 1,616 *An. minimus* A and 6,269 *An. minimus* C were obtained by the four collection methods during the one-year study period (Table 1). Data for species A were not obtained for January and data from the cow

Table 1

Total number of adult *An. minimus* species A and species C collected from four locations in Ban Phu Toei village, Kanchanaburi Province, Thailand.

Month	Season	Collection site											
		Indoor			Peridomestic			Forest			Cow trap		
		A	C	%A	A	C	%A	A	C	%A	A	C	%A
Jan	Cool	-[a]	50	-	-	62	-	-	278	-	-	270	-
Feb	Cool	7	133	5.0	18	286	5.9	71	211	25.2	94	359	20.8
Mar	Hot	17	87	16.3	22	177	11.1	23	115	16.7	28	348	7.4
April	Hot	23	111	17.2	8	74	9.8	17	123	12.1	23	121	16.0
May	Hot	4	145	2.7	10	83	10.8	12	113	9.6	7	136	4.9
June	Hot	4	30	11.8	5	31	13.9	6	26	18.8	-	-	-
July	Rainy	11	60	15.5	13	59	18.1	19	68	21.8	-	-	-
Aug	Rainy	19	85	18.3	4	72	5.3	12	81	12.9	-	-	-
Sept	Rainy	26	58	30.9	14	52	21.2	26	99	20.8	29	88	24.8
Oct	Rainy	57	156	26.8	36	108	25.0	109	239	31.3	68	166	29.1
Nov	Cool	79	194	28.9	51	132	27.9	241	510	32.1	126	192	39.6
Dec	Cool	53	77	40.8	50	91	35.5	70	134	34.3	104	179	36.7
Total		300	1,186	19.5	231	1,227	16.8	606	1,997	21.4	479	1,859	22.4

[a]Data not available

trap were not obtained from June through August. For all collection methods, species A was less abundant than species C, averaging 19.8% of the monthly capture.

An initial model comparing mean abundance between species, collection method and season found significant interaction effects between season and species. Both species decreased in abundance during the hot season, but the magnitude of decrease was greater for species A. The mean abundance of species C decreased from 129.94 (SD = 98.08) in the cool season to 77.50 (SD = 82.90) in the hot season, a decline of 40%; that of species A decreased from 53.67 (SD = 44.32) in the cool season to 8.90 (SD = 7.58) in the hot season, a decline of 83%. Subsequent analyses were therefore stratified by species.

Both members of the *An. minimus* complex varied significantly in mean abundance between seasons (species A: $F_{2,29}$ = 14.24; p = 0.0001; species C: $F_{2,33}$ = 4.58; p = 0.018; by two-way ANOVA of season and collection method). Mean abundance of both species was significantly higher in the cool season than in the other seasons (Tukey-Kramer test at p = 0.01). Peak biting densities for species A were in November; abundance was low from May through August (Fig 1). Two peaks in species C abundance were observed: one during the early cool season in October and November, and the second in February, at the end of the cool season (Fig 1). Biting densities of species C declined in June and stayed low until September.

The median month of capture, defined as the month by which half the annual total capture had been collected, was earlier for species C than species A. Half of the annual total capture for species C had been collected by March from cow trap, by May from the peridomestic collection, by July from the indoor collection, and by August from the forest. These months correspond to the hot season and early part of the rainy season. In contrast, the median month of capture for species A was not until October from the indoor, peridomestic site and cow-trap collections, and November from the forest. These months are at the end of the rainy season and the beginning of the dry season.

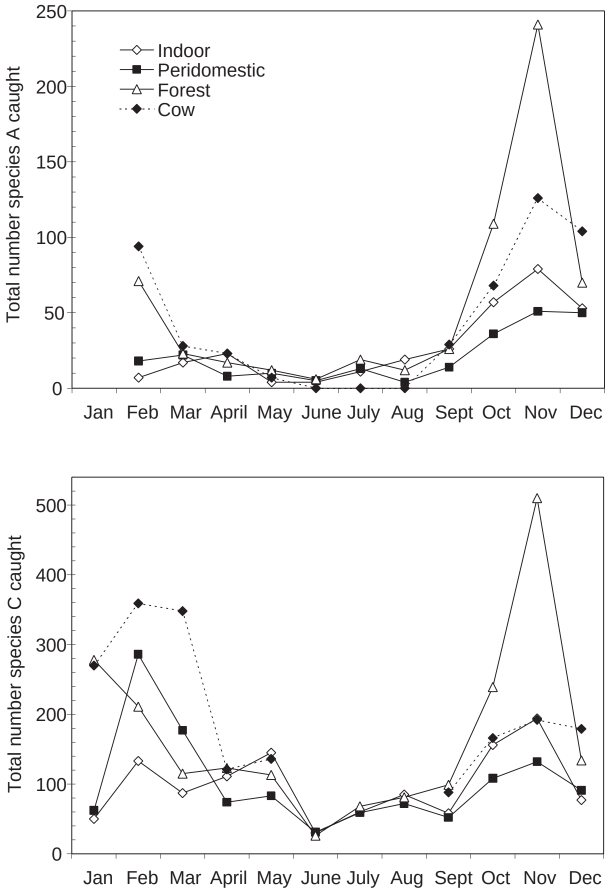

Fig 1–Number of *An. minimus* species A (top) and species C (bottom) collected by four methods over a one-year period.

Mosquito host preference

Both species varied significantly in mean abundance between the four collection methods (species A: $F_{3,29}$ = 8.04; p = 0.0005; species C: $F_{3,33}$ = 11.85; p = 0.0001; two-way ANOVA of collection method and season). For both species, the mean number captured in the cow trap was significantly greater than that with other methods (Tukey-Kramer test at p = 0.01). No significant difference in mean abundance was detected between the three human landing collections, for either species.

Mean monthly biting densities from the cow trap collections were 29.9 adult mosquitos (SD = 22.1) per cow-night for species A, and 103.3 mosquitos (SD = 48.8) per cow-night for species C. The lowest biting densities were recorded from the peridomestic collection for species A (5.3 adult mosquitos per human-night, SD = 4.3), and from the indoor collection for species C (24.7 mosquitos per human-night, SD = 12.4). Although mean abundance was not significantly different between the three human landing collections during the dry cool season, the biting densities of both species at the forest site were on average three times higher than at the other human-landing locations.

Relationship with environmental variables

For each species, data from all collections were combined in order to investigate the relationship between environmental variables and abundance. The absence of a significant interaction between season and method in our ANOVA models indicated that seasonal effects were independent of collection method. Since cow-trap collections were significantly larger than the human landing collections, initial regression analyses were stratified between mosquitos caught on humans and those caught on the cow. Similar results were found for both collection types and therefore subsequent analyses combined data from all the collection methods

Overall biting densities of species A showed a strong positive linear relationship with the mean relative humidity recorded one and two months before the collection month ($r^2 = 0.44$; $p < 0.001$ and $r^2 = 0.62$; $p < 0.001$, respectively; linear regression). Biting densities were positively associated with the total rainfall recorded two months before the collection month ($r^2 = 0.23$; $p = 0.002$). In addition, species A biting densities showed a negative relationship with mean minimum temperatures recorded in the month of capture ($r^2 = 0.49$; $p < 0.001$), and with mean maximum temperatures recorded one ($r^2 = 0.41$; $p < 0.001$) and two ($r^2 = 0.54$; $p < 0.001$) months before the collection month. In a multiple linear regression model, only the mean relative humidity recorded two months before the collection month ($p < 0.001$) and mean minimum temperature in the month of capture ($p = 0.008$) remained significantly associated with species A abundance ($r^2 = 0.69$).

Species C biting densities showed a strong positive relationship with the mean maximum humidity recorded one and two months before the collection month ($r^2 = 0.24$; $p < 0.001$ and $r^2 = 0.27$; $p < 0.001$, respectively). Biting densities were negatively associated with the number of rainy days in the month of capture ($r^2 = 0.19$; $p = 0.003$), as well as with mean minimum temperatures recorded one ($r^2 = 0.30$; $p < 0.001$) and two ($r^2 = 0.15$; $p = 0.010$) months before the collection month. The only environmental variables that remained significantly associated with species C abundance in a multivariate regression model were the mean maximum humidity recorded two months before the collection month ($p = 0.021$) and mean minimum temperature in the month before collection ($r^2 = 0.38$; $p = 0.010$).

DISCUSSION

To the best of our knowledge, this study is the first attempt to measure the seasonal abundance and bloodfeeding behavior of the cryptic species A and C of *An. minimus*. We identified the two known species of the complex using a morphological criterion, the presence or absence of the HP spot. By this method, 22% of the mosquitos identified as species A (*ie* without HP spots) may have been misclassified; only 6% of those identified as species C would have been misclassified. It is therefore likely that we overestimated the density of species A and underestimated the density of species C. However, unless the feeding behavior of mosquitos varies according to the presence or absence of wing-spots, this source of error will not affect the interpretation of the bloodfeeding behaviors of the species.

An. minimus A and C were prevalent throughout the year, with both species appearing in high densities in October-November and remaining abundant through February, during the dry cool season. Biting densities of both members of the species complex were lowest at the end of the hot season in June. These observations are consistent with those of previous surveys in northern Thailand, which noted high *An. minimus s.l.* densities at the end of the rainy season and through the cool season, with decreasing abundance during the hot season (Ismail *et al,* 1974; Ratanatham *et al,* 1988).

Our data also showed a second peak in species C abundance at the end of the cool season in February. Studies in Cambodia and northern Thailand have also found that the major peak in *An. minimus s.l.* abundance occurs around November, with a second smaller peak in the rainy season (Chow, 1970; Ismail *et al,* 1974, 1978). We did not observe a similar pattern of increase during the rainy season at Ban Phu Toei; however, variation in rainfall or local environmental differences affecting larval habitat availability may account for this.

We found that both species showed a significant preference for feeding from cows rather than from humans. Several studies in northern Thailand have found that *An. minimus s.l.* exhibits a marked zoophily, with three to six times more mosquitos being captured from cattle than from human-baited traps (Suthas *et al,* 1986a; Ratanatham *et al,* 1988) whereas a study in southern Thailand found that *An. minimus s.l.* prefers human bait (Rattanarithikul *et al,* 1996). It has been proposed that feeding behavior may change in response to DDT selection pressure. Suthas *et al* (1986a) found no host preference amongst *An. minimus s.l.* in villages in northern Thailand where pesticides had not been applied, but a pronounced zoophily in two villages that had been subjected to annual DDT spraying. The houses in Ban Phu Toei had not been sprayed with DDT for more than one year before our study began, but the possible influence of pesticides on the zoophily that we noted cannot be discounted.

No significant difference in abundance was observed among the three human-landing sites, indicating no clear preference for indoor, outdoor, domestic, or forest feeding. Our observations differ from those of previous studies, some of which have noted *An. minimus s.l.* in Thailand to be distinctly exophagic (Ismail *et al,* 1974, 1978; Ratanatham *et al,* 1988) while others have found *An. minimus s.l.* in Thailand and Myanmar to be endophagic (Suthas *et al,* 1986a; Tun-Lin *et al,* 1995). Van Bortel *et al* (1999) showed that *An. minimus* C in Vietnam was obtained twice as often as *An. minimus* A from outdoor collections, compared with all other collection methods. Ismail *et al* (1974) noted a relative increase in outdoor biting in *An. minimus s.l.* during the cool dry season in northern Thailand. We observed a similar trend at Ban Phu Toei, with landing densities from October to February being highest at the forest site. The differences among the results of previous studies may be a consequence of cryptic species being present, or of changes in *An. minimus* feeding behavior over time in response to, for example, DDT spraying.

Species A was less abundant than species C, averaging 20% of each collection, and declining further in the hot season when it accounted for less than 12% of collections at all locations. These results are consistent with previous reports from Ban Phu Toei and a nearby village, where *An. minimus* C was found to be predominant, comprising 73% to 95% of the *An. minimus* captured (Green *et al,* 1990; Sucharit *et al,* 1988).

Overall biting densities of *An. minimus s.l.* at Ban Phu Toei were much higher than have been reported for other parts of Thailand. In north central Thailand, Ratanatham *et al* (1988) recorded maximum biting densities of 8.9 adult *An. minimus s.l.* per human-night and average densities of 2.1 adult mosquitos per human-night, in collections made from indoor and peridomestic locations. Studies in northern Thailand have noted maximum densities as high as 22.9 *An. minimus s.l.* per human-night, with average densities between 5.5 and 8.6 adult mosquitos per human-night (Ismail *et al,* 1978; Harbach *et al,* 1987). These studies all used ten- to twelve-hour biting collections. We report average biting densities of 8.6 adult mosquitos per human-night for species A and

30.6 mosquitos per human-night for species C from the human landing locations, based on six-hour collections. Previous studies did not distinguish between the two members of the species complex, although given what is known about both species' distributions and relative abundances, it is likely that these observations were mainly of species A.

An. minimus A and C abundances were positively associated with the humidity two months before capture, and inversely associated with the minimum temperature in the month, or previous month, of capture. Rainfall did not show a significant association with adult mosquito abundance, when other environmental variables were accounted for. *An. minimus s.l.* has been reported to oviposit primarily in clear, running streams in forested areas (Harrison, 1980). In Ban Phu Toei, such oviposition sites may be present throughout the year and may not depend on seasonal rainfall. The positive correlation between humidity and abundance for each species is likely to be caused by increased adult longevity, rather than increased availability of oviposition sites: this finding may have implications for control methods that are aimed at reducing habitat availability.

An. minimus A is known to be a major disease vector in the region, despite its propensity for feeding on cattle rather than humans. However, the vectorial status of *An. minimus* C with respect to either malarial or filarial parasites has not yet been determined. Furthermore, the human landing densities that we noted for species C are among the highest recorded for *An. minimus s.l.* We therefore conclude that host preference does not preclude either species from being a significant vector of human disease; we feel that it is important that the vectorial status of *An. minimus* C, now known about for a decade be elucidated.

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