CHANGES IN MALARIA VECTOR DENSITIES OVER A TWENTY-THREE YEAR PERIOD IN MAE HONG SON PROVINCE, NORTHERN THAILAND

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Abstract. Mae Hong Son Province in northwestern Thailand has a long history of malaria. During the last two decades the province has had one of the highest malaria incidences of all provinces in Thailand. Data were analyzed to determine whether the vector populations were stable or increasing during the last two decades and to determine the seasonal prevalence of the main vectors, and whether or not they were related to the malaria transmission peak, in the wet season. We compiled and analyzed accumulated entomological records from 1977 to 1999. The aim was to investigate long-term changes in mean densities of malaria vectors between two periods (1977-1989 and 1990-1999), and the differences in vector densities between two seasons (wet and dry). A total of 141,144 adult anophelines of 29 species were collected on indoor and outdoor human baits and animal baits during the study period. Of the main malaria vectors, the densities of *Anopheles minimus* s.l. and *Anopheles maculatus* complex increased significantly. *Anopheles dirus* s.l., however, was stable between the two periods. These vector populations were associated with consistently high malaria incidence in the province during the last two decades. *An. minimus* s.l. density was not significantly different between seasons. However, in the second period, both *An. dirus* s.l. and *An. maculatus* complex showed a tendency for higher wet season densities. This can explain the high malaria incidence in the rainy season in Mae Hong Son. Environmental and climatic factors seem to have been favorable for supporting a consistently high vector population in the province, and consequently a high malaria transmission rate during the period of study.

INTRODUCTION

Long-term studies on mosquito abundance are not common, partly because regular mosquito control and surveillance activities in many countries are quite recent innovations. If standard sampling protocols have not been used, the data may be difficult to interpret and analyze. In Thailand, however, such long-term mosquito data do exist. Entomological surveillance was first described and established as a regular part of malaria control programs in the early 1950s. It became more organized in the 1970s and has continued to the present time. This entomological surveillance followed standard collection protocols, reporting the number of adult mosquitos collected on indoor and outdoor human baits, as well as on animal bait when cows or buffalos were available (Bhatia and Notananda, 1953). All collected anopheline mosquitos were counted and morphologically identified to species.

In several studies, the effects of climatic, environmental and socioeconomic factors have been used to explain long-term changes in numbers of malaria cases (Bouma *et al*, 1996; Van

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der Hoek *et al*, 1997; Hu *et al*, 1998; Mouchet *et al*, 1998). However, none of these studies used detailed data on vector populations as a causal factor, although the probability of malaria transmission largely depends on the vectorial capacity of the mosquito population (Onori and Grab, 1980). Information from long-term entomological studies is valuable for analyzing the effect of temporal changes in vector densities on malaria incidence. Our intention was to describe changes in anopheline mosquito densities over 23 years in Mae Hong Son, a malaria hyper-endemic province in northwest Thailand.

The malaria history in Mae Hong Son Province shows that transmission occurs throughout the year with a major peak during the early part of the wet season (May to August) and a smaller one in the dry season (November to January), and this general pattern did not change between the last two decades (OVBD2, 2000). This transmission coincides with intense agricultural activity after the first monsoon, when farmers stay in farm huts which rice cultivating and are exposed to vectors (Somboon *et al*, 1998). Furthermore, transmission is closely related to forest locations where there is an abundance of malaria vector breeding sites (Ketrangsee *et al*, 1991), various occupational factors encourage population movement or the influx of refugees (Singhanetra-Renard, 1986; Butraporn *et al*, 1995; Stern, 1998), socioeconomic factors affecting malaria (Fungladda *et al*, 1987), and a high degree of drug resistance (Wernsdorfer *et al*, 1994).

Mosquito collection data from northern Thailand have accumulated at the Office of Vector-Borne Disease Control No.2, Chiang Mai, without any formal analysis. We constructed a database of records from 1977 to 1999, with the objective of retrospectively studying temporal and seasonal variations in anopheline densities in Mae Hong Son Province. We investigated whether stable or increasing densities of the main malaria vectors during the study period were associated with the consistent high malaria incidence in the province. Malaria transmission in northern Thailand usually takes place during the wet season (Ismail *et al*, 1974). Therefore, we hypothesized that malaria vector density would be higher in this season than in the dry season.

MATERIALS AND METHODS

Study area

Mae Hong Son Province is located on the Thai-Myanmar border in northwestern Thailand (Fig 1). The province is geographically homogenous with 90% of the area covered with mountains and about 70% with mainly mixed deciduous and dry dipterocarp forests. The rapid socioeconomic changes in Thailand of recent decades has mainly taken place in larger population centers and focal areas in the central valley of Mae Hong Son Province. Many villages are remotely situated, with no public transport. Approximately 40% of the province has poor accessibility, and during the rainy season accessibility is reduced even more. In a large part of the province, there is limited land for paddy fields and the average size of farms is small. The province is administratively divided into 7 districts (amphoe), 45 cantons (tambon), and 395 villages. The population consists of Thai nationals and many hill tribes of various ethnic groups. Many refugee camps are situated along the border, housing a large number of displaced people from conflict areas within Myanmar. The population in 1977 was 123,816 and in 1999 it had increased by 58% to 195,209 (Office of Vector Borne Disease Control No.2, 1978-2000).

Available records and data analysis

Accumulated mosquito collection data at the Office of Vector Borne Disease Control No. 2 in Chiang Mai (OVBD2), from 1977 to 1999, were compiled and analyzed. Data from before 1977 were few and incomplete and were therefore not used. Each record included the name of the village where mosquitos had been collected, the number of adult anopheline species collected by indoor human bait, outdoor human bait and animal bait, number of collection nights, number of collectors, village population, and number of recorded malaria cases. In human bait collections, two persons collected mosquitos indoors and outdoors, respectively. Half-night collections were undertaken, *ie* from sunset to midnight. Simultaneous animal bait collections were conducted using one cow or buffalo, if available. Each record was standardized by calculating the density of mosquitos, given as the number of mosquitos per bait per half-night.

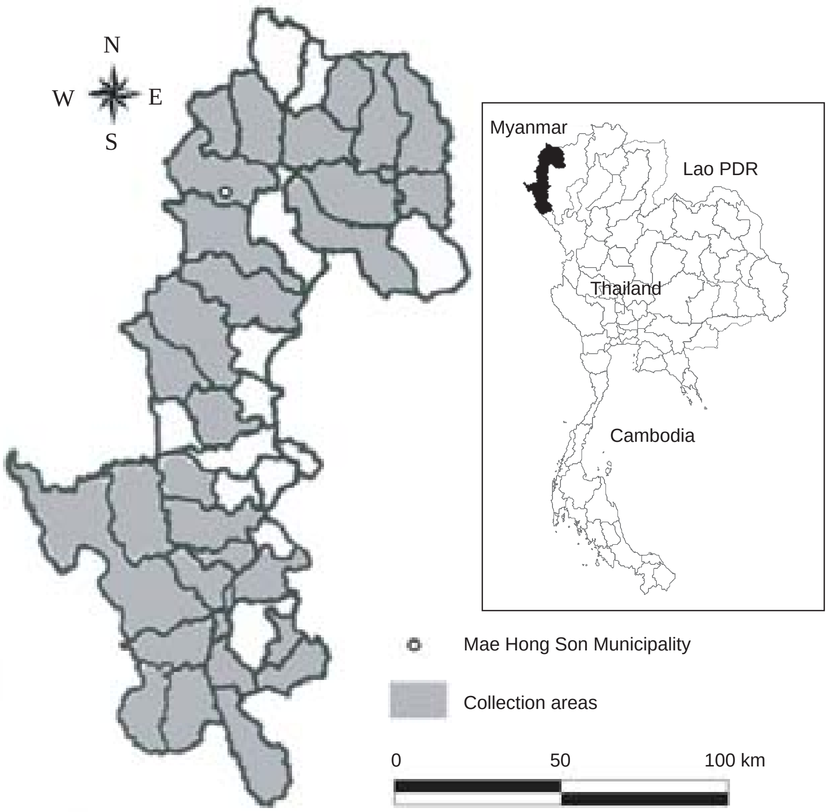

Fig 1–Location of Mae Hong Son Province and cantons (in gray) where entomological data were collected from 1977-1999.

Mosquito collections were undertaken in 97 villages (about 25% of all villages in the province) in 32 cantons (71% of all cantons) (Fig 1). We separated all records into two time periods, the first from 1977 to 1989 and the second from 1990 to 1999, and into two seasons, a wet season (May-September) and a dry season (October-April). Because of relatively few data from the early 1980s, we added available data from the end of the 1970s to the first period, so that replications were as much as possible comparable for each period. It was difficult to find long-term continuous records from single villages during the whole period. For this reason, in our analysis, villages were pooled into the next larger administrative unit, the canton. Thus, a total of 321 records of indoor human bait collections, 501 records of outdoor human bait collections, and 271 records of animal bait collections were available.

Mosquitos were morphologically identified to species using the keys of Peyton and Scanlon (1966); Sawadwongporn (1972); Rattanarithikul and Harrison (1973); Harrison and Scanlon (1975); Peyton and Harrison (1979); Harrison (1980); Rattanarithikul and Green (1986); Rattanarithikul and Panthusiri (1994). Recently, *An. minimus* and *An. dirus* were genetically found to be species complexes of at least three and five sibling species, respectively (Baimai, 1988; Baimai *et al*, 1988; Sucharit *et al*, 1988; Green *et al*, 1990; 1992; Sharpe *et al*, 1999, Walton *et al*, 1999). However, in this study these two species were morphologically identified as *An.minimus* s.l. and *An. dirus* s.l., respectively. Before 1989, all species belonging to the *An. maculatus* complex were collectively identified as *An. maculatus*. Since 1990, individual members of the complex have been morphologically identified as *An.*

Table 1
List of anopheline species collected in Mae Hong Son from 1977 to 1999, their mean densities (no. of mosquitos/bait/half-night) and ± SE.

Species	Mean densities ± SE		
	Human bait		Animal bait
	Indoor	Outdoor	
An. dirus s.l.	0.08 ± 0.02	0.15 ± 0.05	0.32 ± 0.01
An. minimus s.l.	2.11 ± 0.29	4.1 ± 0.27	6.9 ± 0.75
An. maculatus complex	0.55 ± 0.09	3.2 ± 0.18	12.3 ± 1.27
An. aconitus	0.19 ± 0.06	1.2 ± 0.18	8.4 ± 2.58
An. philippinensis	0.1 ± 0.05	0.5 ± 0.15	0.8 ± 0.33
An. barbirostris	0.1 ± 0.03	0.3 ± 0.06	9.3 ± 2.87
An. annularis	0.5 ± 0.14	2.6 ± 0.67	11.8 ± 2.32
An. barbumbrosus	<0.01	<0.01	<0.01
An. campestris	<0.01	0.01 ± 0.005	<0.01
An. culicifacies	0.01 ± 0.02	0.01 ± 0.003	1. 3 ± 0.81
An. hyrcanus group	0.21 ± 0.05	1.0 ± 0.15	9.6 ± 1.66
An. jamesii	<0.01	0.01 ± 0.003	<0.01
An. jeyporiensis	<0.01	0.06 ± 0.003	<0.1 ± 0.08
An. karwari	<0.01	<0.01	<0.01
An. kochi	0.1 ± 0.03	0.2 ± 0.03	9.8 ± 1.4
An. nivipes	<0.1	0.2 ± 0.05	2.2 ± 0.6
An. pseudojamesi	<0.01	<0.01	0.02 ± 0.02
An. splendidus	0.1 ± 0.03	0.3 ± 0.06	1.0 ± 0.47
An. tessellatus	<0.1 ± 0.004	0.1 ± 0.05	0.4 ± 0.07
An. vagus	<0.01	0.1 ± 0.01	15.4 ± 3.8
An. varuna	<0.01	0.1 ± 0.02	<0.01
An. umbrosus	<0.1±	<0.1±	<0.1±
Species of *An. maculatus* complex			
An. dravidicus	<0.01	0.1 ± 0.02	0.3 ± 0.06
An. maculatus s.s.	0.1 ± 0.02	1.2 ± 0.19	6.75 ± 1.13
An. notanandai	<0.01	<0.01	<0.01
An. pseudowillmori	0.05 ± 0.2	0.14 ± 0.04	0.2 ± 0.06
An. sawadwongporni	0.2 ± 0.04	0.7 ± 0.14	2.5 ± 0.47
An. willmori	0.12 ± 0.02	0.4 ± 0.05	2.01 ± 0.31
Species of *An. hyrcanus* group			
An. nigerrimus	<0.01	<0.01	<0.01
An. peditaeniatus	<0.01	<0.02 ± 0.01	0.61 ±0.19
An. sinensis	0	<0.01	<0.16 ± 0.12

maculatus s.s., *An. dravidicus*, *An. notanandai*, *An. pseudowillmori*, *An. sawadwongporni*, and *An. willmori* (Rattanarithikul and Green, 1986).

The three major malaria vectors, *An. dirus* s.l. (Scanlon and Sandhinand, 1965; Peyton and Harrison, 1979), *An. minimus* s.l. (Ayurakitkosol and Griffith, 1963), and the *An. maculatus* complex (Reid, 1968; Upatham *et al*, 1988; Rattanarithikul and Green, 1986) were subjected to statistical analysis. To determine differences in mean mosquito density, two-way analysis of variance was conducted. The variation of adult density found in indoor, outdoor, and animal bait collections was analyzed separately for each period (1st period: 1977-1989 or 2nd period: 1990-1999) and season (dry or wet). All statistical analyses were performed using SYSTAT statistical software (Wilkinson, 1996).

Table 2
Comparison of mean densities (number of mosquitos/bait/half-night) of three main malaria vectors in Mae Hong Son, between two periods (1977-1989 and 1990-1999) and between two seasons (dry and wet); pairs of means in boxes and in boxes with highlight are significantly different (p<0.05).

Anopheles species	Seasons	Human bait indoor		Human bait outdoor		Animal bait	
		1st period	2nd period	1st period	2nd period	1st period	2nd period
An.minimus s.l.	Dry	0.825	2.002	4.044	4.502	3.143	7.097
	Wet	0.845	2.766	2.680	4.417	4.337	8.484
An. dirus s.l.	Dry	0.017	0.018	0.019	0.059	0.004	0.050
	Wet	0.005	0.181	0.231	0.231	0.000	0.033
An. macualtus	Dry	0.004	0.218	3.435	1.621	14.494	8.743
complex	Wet	0.010	1.120	2.171	4.859	11.223	14.289

1st period = 1977-1989, 2nd period = 1990-1999; dry season = October-April, rainy season = May-September.

RESULTS

During the study period, a total of 141,144 anophelines of 29 species were recorded in Mae Hong Son Province, of which 64,469 were collected on outdoor human bait, 63,416 on animal bait, and 13,259 on indoor human bait. The mosquito indices are expressed as the mean number of mosquitos per bait per half night. Table 1 gives a list of all anopheline species, and the mean densities (±SE) by the three different collection methods. Of all anophelines collected, *An. minimus* s.l. was predominantly biting on humans with a mean density of 2.1 and 4.1 from indoor and outdoor collection, respectively (Table 1). On animal bait, the most abundant species was *An. vagus* (15.4), followed by *An. maculatus* complex (12.3), and *An. annularis* (11.8) (Table 1).

Malaria vectors

***An. minimus* s.l.** The results of the ANOVA on the whole data set for this species showed that there were significant differences between periods (1977-1989 and 1990-1999) ($F_{1, 1093}$=13.40, p<0.001) and there were no significant interactions between any of the factors (p>0.05). Also, for each season, there were significant differences between periods (dry: $F_{1, 525}$=4.69, p<0.05; and wet: $F_{1, 568}$=9.11, p<0.01), but when separated by collection method, only an increase in outdoor human bait in the wet season was significant (Table 2). No significant differences were found between seasons in the whole data set ($F_{1, 1093}$=0.27, p=0.60), nor in each period (p>0.2).

***An. dirus* s.l.** In two-way analysis of all the data, there were no significant differences in density between any of the factors analyzed (period, season). However, when the periods were analyzed for this species separately, in the second period, a higher indoor density during the wet season was found (Table 2).

***An. maculatus* complex.** For this species complex, there was no difference in density between the two periods ($F_{1, 1093}$=2.04, p>0.05). However, there was a significant interaction between periods and seasons in the whole data set ($F_{1,1093}$=5.05, p<0.05), which is reflected by a significant difference between periods in the dry season ($F_{1,525}$=9.61, p<0.01), but not in the wet season ($F_{1, 568}$=2.70, p>0.05). Furthermore, we found contrasting results when analyzing differences between periods for each collection method and each season. For the outdoor human bait collections, there were significant decreases in the dry season and significant increases in the wet season (Table 2). The human indoor collections increased significantly between periods in both seasons. There were no differences between seasons in the first period, but in the second period there were significantly higher densities in the wet season for all collection methods.

DISCUSSION

We found that the densities of the three main malaria vectors either increased or were stable in Mae Hong Son Province during the study period. The density of *An. dirus* s.l., a more efficient vector than *An. minimus* s.l. (Gould *et al*, 1966; Ismail *et al*, 1974; 1975), was stable throughout the period. However, the density of *An. minimus* s.l. and *An. maculatus* complex had significantly increased from the first period (1977-1989) to the second period (1990-1999). Specifically, the density increase in the latter two species was mainly observed in the wet season. These facts support our hypothesis that large or increasing vector populations could be a contributing factor for the consistent high malaria incidence in the province.

We also found significantly higher densities of *An. dirus* s.l. and *An. maculatus* complex in the wet season than in the dry season, especially in the second period. High wet season densities of *An. dirus* s.l. and *An. maculatus* complex have also been reported in other studies (Ismail *et al*, 1974; 1975; Rosenberg, 1982; Upatham *et al*, 1988; Rosenberg *et al.* 1990; Suwonkerd *et al*, 1995; Takagi *et al*, 1995). It is believed that *An. dirus* s.l. usually retreats to dense humid forest areas during the dry season and returns to the forest fringe and populated areas during the rainy season (Rosenberg *et al*, 1990), thus being largely responsible for wet season transmission (Ismail *et al*, 1974). *An. maculatus* complex has generally been thought to be an important vector in southern Thailand (Upatham *et al*, 1988), and of little or no importance elsewhere in the country (Harbach *et al*, 1987). However, *An. maculatus* s.s. and *An. sawadwongporni*, which are members of this complex, were incriminated in Mae Hong Son Province (Somboon *et al*, 1994). *An. pseudowillmori*, also a member of this complex, was incriminated in the neighboring province of Tak (Green *et al*, 1991). It appears, therefore, the species of the *An. maculatus* complex may have played a larger role in malaria transmission in northern Thailand than previously assumed. The density of *An. minimus* s.l. was not significantly different between seasons. Previous studies found that *An. minimus* s.l. was prevalent throughout the year, including a major part of the dry, cool season, as it remained at high density from November to February, and for a shorter duration in the early part of the rainy season (Ismail *et al*, 1974; 1975; 1978). Therefore, it is seems that *An. minimus* s.l. is responsible for transmitting malaria throughout the year, thus reinforcing wet season transmission by *An. dirus* s.l. and *An. maculatus* complex. These results partly support our hypothesis that the seasonal prevalence of the main malaria vectors was higher in the wet season, thus being responsible for the higher transmission rates in this season.

Rainfall has been suggested as an important climatic factor affecting both mosquito population and malaria transmission (Bouma *et al*, 1996; Hu *et al*, 1998; Mouchet *et al*, 1998). In Mae Hong Son, total rainfall varied between 1,025 and 1,650 mm/year during the years 1970-1998 (Meteorological Department, 1970-1999). About 70%-90% of the rainfall in Mae Hong Son Province was recorded in the wet season. However, no significant difference in wet season rainfall was observed between the two study periods (t=1.70, p=0.104) (Fig 2). Therefore, it seems that variation in rainfall did not have much effect on malaria vector density in the study area during the study period.

Deforestation has been associated with either increases or decreases of vector transmitted diseases (Walsh *et al*, 1993). In Africa, where 90% of worldwide malaria morbidity and mortality occurs, deforestation is considered a factor in the increase in malaria, due to the development of anopheline larval sites exposed to the sun (Mouchet *et al*, 1988). By contrast, in Thailand, the ecological conditions in deforested areas become unfavorable for breeding of the main malaria vectors, suggesting a reduced malaria risk (Ismail *et al*, 1978; Rosenberg *et al*, 1990). This corresponds with a study in Yunnan, in southern China, where dense forests were associated with a high abundance of *An. minimus* s.l., and a high incidence of malaria (Hu *et al*, 1998). In Mae Hong Son Province, forest cover decreased from approximately 87% in 1976 to about 70% in 1998 (Royal Forest Department, 1999). However, since mosquito collections were mainly undertaken in more or less remotely located transmission areas, where, in general, little land use change has taken

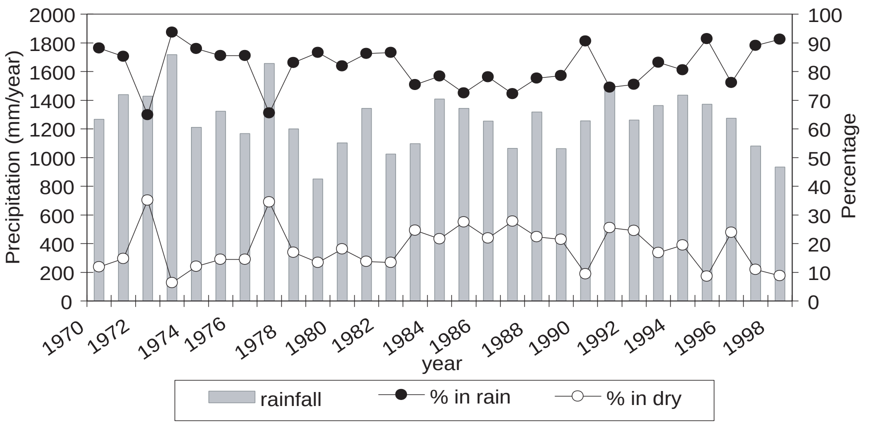

Fig 2–Yearly changes in precipitation and the proportion of rainfall recorded in wet and dry seasons from 1977-1998 in Mae Hong Son Province, northern Thailand.

place, it is reasonable to assume that there was a higher forest cover and a lower rate of deforestation in these locations. In a non-transmission area in the neighboring province of Chiang Mai, malaria vector density, especially that of *An. minimus* s.l., decreased between 1977 and 1999, and it was concluded that this might have been a result of increased landscape diversity and forest fragmentation (Suwonkerd *et al*, 2002). However, in transmission areas in Chiang Mai Province, forest cover was extensive, landscape diversity low, and malaria vector densities increased during the same period. In a comparative study of six areas in northern Thailand, of which two were situated in Mae Hong Son Province, Overgaard *et al* (in press), found that anopheline diversity and density were generally higher in forested areas with low landscape diversity than in agricultural areas with a high landscape diversity. Thus, it seems that the mosquito situation in Mae Hong Son over the last two decades resembles that of transmission areas in Chiang Mai Province.

However, apart from the density of malaria vectors, and climatic and environmental factors, there are other possible explanations for the observed high malaria rates in Mae Hong Son Province. In particular, much movement of refugees and others across the border to Myanmar, as well a high degree of multi-drug parasite resistance, may play an important role in malaria transmission in the province.

To conclude, the increases in malaria vector densities in Mae Hong Son Province suggest that entomological factors probably play a large role in malaria transmission in the province. Furthermore, environmental and climatic factors seem to have been favorable for a consistently high vector population and relatively stable malaria transmission rate. Understanding how different factors affect malaria transmission and mosquito populations will eventually lead to better planning of malaria control.

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