COMPARATIVE SUSCEPTIBILITY OF TWO FORMS OF *ANOPHELES SINENSIS* WIEDEMANN 1828 (DIPTERA : CULICIDAE) TO INFECTION WITH *PLASMODIUM FALCIPARUM, P. VIVAX, P. YOELII* AND THE DETERMINATION OF MISLEADING FACTOR FOR SPOROZOITE IDENTIFICATION

Yupha Rongsriyam[1], Atchariya Jitpakdi[2], Wej Choochote[2], Pradya Somboon[2], Benjawan Tookyang[2] and Wannapa Suwonkerd[3]

[1]Department of Medical Entomology, Faculty of Tropical Medicine, Mahidol University, Bangkok 10400, Thailand; [2]Department of Parasitology, Faculty of Medicine, Chiang Mai University, Chiang Mai 50200, Thailand; [3]Malaria Center, Region 2, Chiang Mai 50200, Thailand

Abstract. Two karyotypic forms of laboratory-raised *Anopheles sinensis, ie* Form A (XY1) and Form B (XY2), were experimentally infected with various indigenous strains of *Plasmodium falciparum* and *P. vivax* using an artificial membrane feeding technique, and a rodent malaria, *P. yoelii*, using a direct feeding technic and dissected 7-9 days and 10-15 days after feeding for oocyst and sporozoite rates, respectively. The results revealed that two forms of *An. sinensis* were refractory vectors for *P. falciparum* and *P. yoelii* since 0% of oocyst and sporozoite rates were obtained, but poor vectors for *P. vivax* since 0.00-85.71% and 0.00-5.88% of oocyst and sporozoite rates were recovered. The sporozoite-like crystal found in the median lobe of the salivary gland of *An. sinensis* which could be a misleading factor in identification of true sporozoites in the salivary glands is reported for the first time.

INTRODUCTION

So far, at least 18 anopheline species have been incriminated as primary, secondary and suspected vectors of malaria in Thailand. The primary vectors are *Anopheles dirus* Peyton and Harrison, *An. minimus* Theobald and *An. maculatus* Theobald, while *An. aconitus* Donitz and *An. sundaicus* (Rodenwaldt) are considered as secondary vectors (Gould *et al*, 1967; Scanlon *et al*, 1968; Harrison, 1980; Rosenberg *et al*, 1990; Ketrangsee *et al*, 1991). The three intra-taxa of primary vectors are a species complex, which cannot be easily distinguished from each other (Baimai *et al*, 1984, 1993; Green *et al*, 1990). *An. pseudowillmori* (Theobald), a member species of the *maculatus* complex has been incriminated as an important vector (Green *et al*, 1991). The remaining 12 species, *ie An. annularis* Van der Wulp, *An. barbirostris* Van der Wulp, *An. campestris* Reid, *An. karwari* (James), *An. kochi* Donitz, *An. nigerrimus* Giles, *An. nivipes* (Theobald), *An. peditaeniatus* (Leicester), *An. philippinensis* Ludlow, *An. sinensis* Wiedemann, *An. tessellatus* Theobald and *An. vagus* Donitz were considered as suspected vectors since they were found to be positive for oocysts in the midgut and/or circumsporozoite (CS) antigens by an ELISA method (Gingrich *et al*, 1986; Baket *et al*, 1987; Harbach *et al*, 1987; Gingrich *et al*, 1990).

An. sinensis, was incriminated as the natural and experimental vectors of *Plasmodium vivax* in other countries, *ie* in Indonesia during the period 1931-1947 (O'Connor, 1980), in Korea (Chow, 1970), in Japan (Otsuru and Ohmori, 1960) and in China (Ho *et al*, 1962). In Thailand, *An. sinensis* seems to be of little medical importance (Harrison and Scanlon, 1975). However, an experiment of highly susceptible of wild-caught *An. sinensis* from Mae Sariang District, Mae Hong Son Province to indigenous strains of *P. vivax* was recently reported by Somboon *et al* (1994). In addition, two forms of *An. sinensis, ie*, Form A (XY1) and Form B (XY2), have also been incriminated from this area (Baimai *et al*, 1993). In view of the recent report of *An. sinensis* in northern Thailand, we feel these two mosquito forms need to be defined more thoroughly. Accordingly, the susceptibility of two forms of *An. sinensis* to indigenous *P. falciparum* and *P. vivax* and the rodent malaria, *P. yoelii*, are presented herein.

MATERIAL AND METHODS

Laboratory-raised *An. sinensis*

Five laboratory-colony strains of *An. sinensis* were established based on metaphase karyotype and geographical differences. Since the investigation of F_1-progenies of 27, 28 and 30 isolined colonies of *An. sinensis* collected from Mae Sariang, Muang Districts, Mae Hong Son Province and San Sai District Chiang Mai Province using the modified technic as described by Baimai (1977) and Choochote *et al* (1992) revealed the two forms of metaphase karyotypes, *ie.* From A (XY1) of which the Y1-chromosome was subtelocentric or acrocentric with only a small portion of the short arm present (Fig 1-A) and Form B (XY2) of which the Y2- chromosome was clearly submetacentric with the short arm approximately one-half the length of the long arm (Fig 1-B). These have been found in the same locality and 13/14, 1/27 and 4/26 of Form A/Form B were obtained from Mae Sariang District, Muang District, Mae Hong Son Province and San Sai District, Chiang Mai Province respectively.

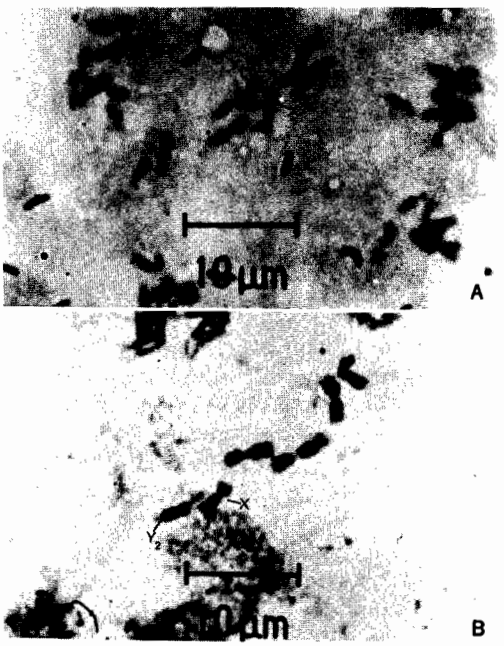

Fig 1–Metaphase karyotype from male testes of *An. sinensis* (Giemsa staining). (A) Form A showing X and Y1-chromosomes. (B) Form B showing X and Y2- chromosomes.

Thus, the laboratory colonies of *An. sinensis* were established into two categories, the isolined and mixed colonies. For isolined colony : Form A-colony was established by using single wild-caught female from Mae Sariang District Mae Hong Son Province (Ai), whereas Form B-colony was established from single wild-caught female from Muang District, Mae Hong Son Province (Bi). For Mixed colony : Form A-colony was established by using 13 wild-caught females from Mae Sariang District, Mae Hong Son Province (Am); Form B-colonies were established from 27 wild-caught females from Muang District, Mae Hong Son Province (Bm1) and 26 wild-caught females from San Sai District, Chiang Mai Province (Bm2). Metaphase karyotypes of all above five strain, Ai, Bi, Am, Bm1 and Bm2, were consecutively confirmed up tilll the 10th generation.

P. falciparum and *P. vivax* gametocytes

The gametocytes of *P. falciparum* and *P. vivax* were obtained from malaria patients. These patients were infected from the same or different areas of the mosquito collections. *P. falciparum* cases were infected from Fang District, Chiang Mai Province (PF1) and Mae Sariang District, Mae Hong Son Province (PF2). *P. vivax* cases were infected from Muang District (PV1) and Mae Sariang District (case I : PV2.1, case 2 : Pv2.2, case 3 : PV2.3), Mae Hong Son Province, Wieng Hang District, Chiang Mai Province (PV3) and Mae Sod District, Tak Province (PV4). Ten ml of blood containing gametocytes of the above malarial species and strains were collected by venepuncture into a heparinized syringe.

P. yoelii

P. yoelii (PY) was obtained from the London School of Tropical Medicine and Hygiene. It was experimentally infected to albino mice and maintained in the animal house of the Department of Parasitology, Faculty of Medicine, Chiang Mai University, since 1994.

Infection of mosquitos with *P. falciparum* and *P. vivax*

Three of five-day-old adult females of five strains of laboratory-raised *An. sinensis* and a control mos-

Table 1

The oocyst rates of *An. dirus* A and five strains of laboratory-raised *An. sinensis* after feeding on blood containing gametocytes of *P. falciparum*, *P. vivax* and *P. yoelii*, all dissected 7-9 days after feeding.

Experiments	Malaria strains	*An. dirus* A	*An. sinensis* strains				
			Ai	Bi	Am	Bm1	Bm2
Oocyst rates		%(Pos O/Dis) Avr (Range)	%(Pos O/Dis) Avr (Range)	%(Pos O/Dis) Avr (Range)	%(Pos O/Dis) Avr (Range)	%(Pos O/Dis) Avr (Range)	%(Pos O/Dis) Avr (Range)
P. falciparum	PF1	86.67 (13/15)	ND	0 (0/24)	0 (0/18)	0 (0/19)	0 (0/16)
		5.15 (1-11)		–	–	–	–
	PF2	100 (18/18)	ND	ND	0 (0-15)	0 (0-36)	ND
		10.39 (1-22)			–	–	
P. vivax	PV1	100 (6/6)	ND	40.00 (2/5)	ND	ND	0 (0/6)
		59.67 (31-98)		2.50 (1-4)			–
	PV2.1	100 (11/11)	ND	0 (0/6)	ND	ND	7.69 (1/13)
		28.82 (3-51)		–			1.00 (1)
	PV2.2	50.00 (6/12)	ND	0 (0/3)	13.33 (2/15)	35.71 (5/14)	25.00 (2/8)
		16.83 (6-26)		–	1.50 (1-4)	3.40 (1-6)	2.50 (2-3)
	PV2.3	100 (10/10)	ND	ND	30.77 (4/13)	27.27 (3/11)	33.33 (4/12)
		24.60 (5-50)			2.00 (1-3)	2.00 (1-3)	3.40 (1-4)
	PV3	100 (14/14)	60.00 (3/5)	ND	60.00 (3/5)	ND	ND
		17.00 (5-31)	8.00 (3-13)		4.67 (4-6)		
	PV4	100 (7/7)	28.57 (2/7)	11.11 (1/9)	85.71 (6/7)	85.71 (6/7)	ND
		95.00 (32-155)	32.50 (3-44)	1.00 (1)	19.17 (1.31)	15.17 (3.37)	
P. yoelii	PY	95.00 (19/20)	ND	ND	ND	ND	0 (0/23)
		45.53 (1-136)					-
	PY	88.24 (15/17)	ND	ND	0 (0/17)	0 (0/18)	ND
		58.33 (1-185)			–	–	
	PY	88.24 (15/17)	0 (0/22)	0 (0/23)	ND	ND	ND
		13.53 (4-25)	–	–			
	PY	100 (6/6)	0 (0/7)	ND	0 (0/23)	ND	ND
		21.00 (3-66)	–		–	–	
	PY	100 (7/7)	0 (0/7)	ND	ND	0(0/4)	ND
		71.14 (7-236)	–			–	

Pos O = Number of mosquitos positive for oocysts in midguts, Dis = Number of mosquitos dissected,
Avr = Average number of oocysts per infected midgut, Range = Range of oocysts recovered per infected midgut,
ND = not done

quito, *An. dirus* A (fasted for 12 hours) were allowed to feed on heparinized blood containing gametocytes using artificial membrane feeding technic as described by Chomcharn *et al* (1980). The fully engorged females were separated to small paper cups (diameter 6 cm, depth 7 cm) with 6-8 mosquitos per cup and maintained in the incubator at 27 ± 2°C and 70-80% relative humidity (RH). Seven to nine days after feeding, approximately one third of the live mosquitos were dissected for oocyst rates and the remaining were dissected for oocyst and sporozoite rates on day 10-15.

Infection of mosquitos with *P. yoelii*

The detailed technic for infection of mosquitos with *P. yoelii* was mainly followed the technic as described above, except the mosquitos were directly fed on anesthetized infected mice and the fully engorged mosquitos were maintained at 24 ± 2°C and 70-80% RH.

RESULTS

Oocyst rates of *An. dirus* A and five strains of *An. sinensis*

Details of oocyst rates are shown in Table 1. Observations on dissected midguts 7-9 days after feeding revealed that all strains of *An. sinensis* were not susceptible to *P. falciparum* and *P. yoelii*, but susceptible to *P. vivax* except in the experimental feeding of Bi *sinensis* strain on PV2.1 and PV2.2 *vivax* strains and Bm_2 *sinensis* strain of PV1 *vivax* strain of which the oocyst rates were 0 %. The oocyst rates and average number of oocysts per infected midgut of all strains of *An. sinensis* were lower than those of the efficient vector, *An. dirus* A, in all experimental studies. For isolined colonies, statistical analysis of the oocyst rates between *dirus* A and Ai *sinensis* strain; *dirus* A and Bi *sinensis* strain using Fisher's exact test manifested significant differences only in PV4 *vivax* strain ($p < 0.05$ in both *dirus* A/Ai and Bi *sinensis* strains). For mixed colonies, statistical analysis of the oocyst rates between *dirus* A and Am *sinensis* strain; *dirus* A and Bm1 *sinensis* strain; *dirus* A and Bm2 *sinensis* strain using Fisher's exact test manifested significant differences only in the experiments using PV2.3 *vivax* strain ($p < 0.05$ in all tests, *ie*, *dirus* A/Am, Bm1 and Bm2, respectively). Comparative analysis of oocyst rates between two isolined and among three mixed colonies were also investigated. The

Table 2

The oocyst and sporozoite rates of *An. dirus* A and five strains of laboratory-raised *An. sinensis* after feeding on blood containing *P. vivax* gametocytes, all dissected 10-15 days after feeding.

Experiments	Malaria strains		
	PV2.2	PV2.3	PV4
Oocyst rates	%(Pos O/Dis)	%(Pos O/Dis)	%(Pos O/Dis)
	Avr (range)	Avr (range)	Avr (range)
An. dirus A	28.57 (4/14)	82.35 (14/17)	68.42 (13/19)
	12.75 (1-22)	4.71 (1-20)	20.70 (2-52)
An. sinensis			
Ai	30.77 (4/13)	ND	52.94 (9/17)
	1.50 (1-3)		12.44 (2-33)
Bi	0 (0/14)	ND	0 (0/3)
	-		-
Am	34.21 (13/38)	65.22 (15/23)	ND
	1.62 (1-6)	3.47 (1-9)	
Bm1	14.28 (2/14)	52.38 (11/21)	40.00 (4/10)
	1.00 (1)	6.00 (1-12)	7.75 (1-17)
Bm2	35.71 (5/14)	52.94 (9/17)	ND
	2.40 (11-18)	3.44 (2-6)	
Sporozoite rates	%(Pos S/Dis)	%(Pos S/Dis)	%(Pos S/Dis)
An. dirus A	80.95 (17/21)	86.67 (13/15)	83.33 (15/18)
An. sinensis			
Ai	0 (0/13)	ND	0 (0/16)
Bi	0 (0/12)	ND	0 (0/4)
Am	0 (0/37)	0 (0/16)	ND
Bm1	0 (0/7)	5.88 (1/17)	0 (0/6)
Bm2	0 (0/16)	0 (0/18)	ND

Pos O = Number of mosquitos positive for oocysts in midguts, Pos S = Number of mosquitos positive for sporozoites in salivary glands, Dis = Number of mosquitos dissected, Avr = Average number of oocysts per infected midgut, Range = Range of oocyst recovered per infected midgut, ND = not done

results indicated that two forms of *An. sinensis*, From A (XY1) and Form B (XY2), had similar oocyst rates [$p > 0.05$ in all tests, *ie* Ai and Bi(PV4 *vivax* strain) for isolined colonies; Am and Bm1 (PV4 *vivax* strain) and Am, Bm1 and Bm2 (PV2.2, PV2.3 *vivax* strains) for mixed colonies].

Oocyst and sporozoite rates of *An. dirus* A and five strains of *An. sinensis*

The dissection of midguts of *An. dirus* A and five strains of *An. sinensis* during 10-15 days after feeding revealed that the oocyst rates of *An. dirus* A were 28.57 - 82.35% and those of *An. sinensis* were 14.28 - 65.22% (Table 2). Statistical analysis of the oocyst rates between *An. dirus* A and *An. sinensis* were not done because at this period (10-15 days of postblood meal) the majority of mature oocysts from the midgut of an efficient vector, *An. dirus* A, ruptured and yielded unreliable results. However, a difference in stage of oocyst development in *An. dirus* A and *An. sinensis* could be observed. Most of the oocysts recovered from *An. dirus* A showed maturity stage, while in *An. sinensis*, the maturity rate of oocysts from infected mosquitos (72) was very low (11.11%, 8/72) and nearly all of the investigated oocysts were abnormal in divelopment, retaining stages and some forming melanin inside cysts.

The dissection of salivary glands of *An. dirus* A and five strains of *An. sinensis* showed that all strains of *An. sinensis* were non-efficient vectors to *P. vivax* since they yielded 0-5.88% sporozoite rates when compared with an efficient vector, *An. dirus* A, for which 80.95 - 86.67% sporozoite rates were obtained. It is pertinent to note that only 11 sporozoites were found in squashed salivary glands of one infected *An. sinensis* (Bm1 strain).

The interesting point in the present study is the sporozoite-like crystal found in the salivary glands of some strains of *An. sinensis*, *ie* 16.67% (2/12) from Bi *sinensis* (PV2.2 *vivax* strain); 30.76% (4/13) from Ai *sinensis* (PV2.2 *vivax* strain) and 37.50% (6/16) from Ai *sinensis* (PV4 *vivax* strain); 67.57% (25/37) from Am *sinensis* (PV2.2 *vivax* strain) and 43.75% (7/16) from Am *sinensis* (PV2.3 *vivax* strain). The sporozoite-like crystal rather resembles a true sporozoite, particularly when it is inside a non-squashed salivary glands; the latter has regular spindle-shaped while the former has irregular, long or short with blunt or taper end(s) (Fig 2). It was stable in 0.85 % normal saline for at least 1/2 hour and after that the aggregration and/or precipitation of the crystal could be clearly seen and could be easily distinguished from sporozoites. The confirmations of some crystal positive salivary glands using standard stain for sporozoite (Giemsa staining) demonstrated that all of crystal was lost during the staining procedures compared with the staining of true sporozoites. Another distinctive point was the distribution of the sporozoite-like crystal in salivary glands: it was entirely located only in the smallest or median lobe of salivary glands, whereas the true sporozoites were randomly distributed throughout the three lobes of salivary glands.

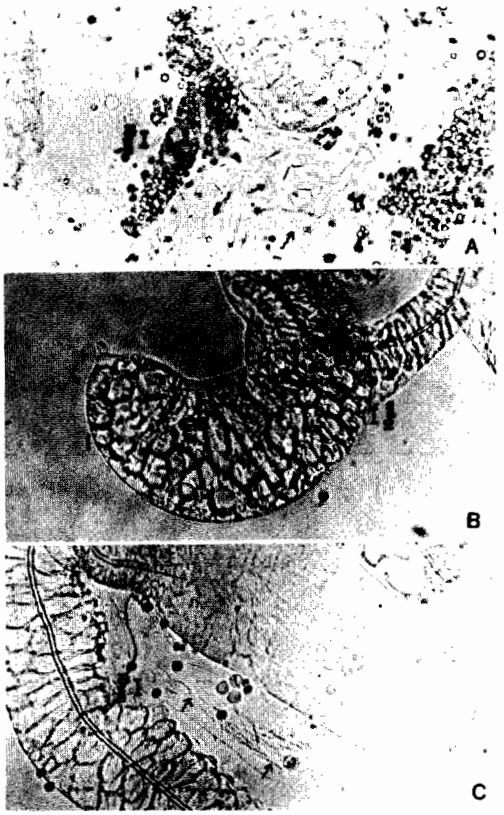

Fig 2–(A), showing free flow *P. vivax* sporozoites from the squashed salivary gland. Note the regular spindle-shaped sporozoite (small arrow). (B), Showing sporozoite-like crystals inside the median lobe of salivary glands (small arrow). (C), Showing free flow sporozoite-like crystals from the squashed salivary glands. Note the irregular, long or short with blunt or taper end(s) of crystals (small arrow).

Crystal formation rates in *An. dirus* A and five strains of *An. sinensis*

In connection with the sporozoite-like crystal fund in the dissected salivary glands of *An. sinensis* as described before, thus the experiments to determine capability of *An. dirus* A and *An. sinensis* were carried out using unfed and fed blood females of both mosquito species. Their salivary glands were dissected and examined at time-intervals. Details of crystal formation rates in salivary glands of *An. dirus* A and five strains of *An. sinensis* are shown in Table 3. In *An. dirus* A, crystal formation was not observed in all ages of one to twenty-day-old females in all experiments. Similar results were also found in unfed blood of all strains of one-day-old *An. sinensis*. For unfed blood *An. sinensis*, the crystal formation rates of four-day-old females were 5% (Ai strain) and 35% (Am strain) in *sinensis* Form A; 5% (Bi strain), 5% (Bm1 strain) and 0% (Bm2 strain) in *sinensis* Form B. The rates were markedly increased, but patternless in eight to twenty-day-olds. For *An. sinensis* Form A, the rates ranged from 20-65% and 45-85%, in Ai and Am strains, respectively. For *An. sinensis* Form B, the rates were ranged from 0.15%, 0-5% and 0.10% in Bi, Bm1 and Bm2 *sinensis* strains, respectively. Similar results were also found in fed blood female *An. sinensis* of which the crystal formation rates of eight to twenty-day-old females ranged from 20-55% (Ai strain) and 35-90% (Am strain) in *sinensis* Form A and 5-15% (Bi strain), 0-15% (Bm1 strain) and 0.5% (Bm2 strain) in *sinensis* Form B. When, regardless of mosquito ages, statistical analysis could be reliably performed (Table 3, Summation). The results of analysis indicated that *An. sinensis* Form A yielded more percentages of crystal formation than *An. sinensis* Form B on most particulars, *ie* $X^2 = 16.79$, $p < 0.05$ (Ai *sinensis* Form A/Bi *sinensis* Form B), $X^2 = 77.30$, $p < 0.05$ (Am *sinensis* Form A/Bm1 *sinensis* Form B) and $X^2 = 74.05$, $p < 0.05$ (Am *sinensis* Form A/Bm2 *sinensis* Form B) in unfed blood females; $X^2 = 14.94$, $p < 0.05$ (Ai *sinensis* Form A/Bi *sinensis* Form B), $X^2 = 46.55$, $p < 0.05$ (Am *sinensis* Form A/Bm1 *sinensis* Form B) and $X^2 = 55.70$, $p < 0.05$ (Am *sinensis* Form A/Bm2 *siensnsis* Form B) in fed blood females. Statistical analysis between two strains, Bm1 and Bm2 of *An. sinensis* Form B gave non-significant differences, *ie* $X^2 = 0.15$, $p > 0.05$ and $X^2 = 1.34$, $p > 0.05$ of unfed and fed blood females, respectively.

Table 3

Crystal formation rates in salivary glands of *An. dirus* A and five strains of laboratory-raised *An. sinensis*.

Age of mosquitos dissected (days)	Type of meals	Percentages of mosquitos positive for crystals (No.)*					
		An. dirus A	*An. sinensis* strains				
			Ai	Bi	Am	Bm1	Bm2
1	Sugar	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
4	Sugar	0 (0)	5 (1)	5 (1)	35 (7)	5 (1)	0 (0)
8	Sugar	0 (0)	30 (6)	0 (0)	85 (17)	0 (0)	5 (1)
	Blood	0 (0)	20 (4)	5 (1)	45 (9)	0 (0)	0 (0)
12	Sugar	0 (0)	65 (13)	0 (0)	70 (14)	0 (0)	10 (2)
	Blood	0 (0)	55 (11)	10 (2)	35 (7)	10 (2)	5 (1)
16	Sugar	0 (0)	20 (4)	15 (3)	45 (9)	5 (1)	5 (1)
	Blood	0 (0)	25 (5)	5 (1)	55 (11)	15 (3)	5 (1)
20	Sugar	0 (0)	30 (6)	10 (2)	70 (14)	5 (1)	0 (0)
	Blood	0 (0)	35 (7)	15 (3)	90 (18)	0 (0)	0 (0)
Summation (4-20)	Sugar**	0 (0)	30 (30)	6 (6)	61 (61)	3 (3)	4 (4)
	Blood***	0 (0)	33.75 (27)	8.75 (7)	56.25 (45)	6.25 (5)	2.50 (2)

* Twenty mosquitos for each experiment, ** Total dissection = 100,
***Total dissection = 80

DISCUSSION

In order to delineate a mosquito vector in an endemic area of vector-borne diseases, it is necessary to comfirm the following evidence for a species of mosquitos. Firstly, naturally caught specimens of a species of mosquito contain infective stages of a parasite. Secondly, the same forms of infective stages develop in a laboratory-bred, clean colony of the same mosquito species after being fed on carrier blood containing parasites, and thirdly, the same mosquito species fed on the blood of humans in the endemic area (Sasa, 1976). Of these criteria, therefore, the susceptibility test in an experimental laboratory is one useful parameter to incriminate the potential vector of a certain species. Nevertheless susceptibility one does not imply an important role in transmission of disease in nature, whereas a refractory result can entirely rule out its significance.

In the present study, five strain of laboratory-raised *An. sinensis* (Ai, Bi, Am, Am1 and Bm2) were tested for susceptibility to various strains of *P. falciparum* (PF1, PF2) and *P. vivax* (PV1, PV2.1, PV2.2, PV2.3, PV3 and PV4). This experiment was carried out to find whether *An. sinensis* forms were susceptible to indigenous and non-indigenous strains of malaria. In addition, susceptibility to *P. yoelii* was investigated. The dissections of mosquitos 7-9 days after feeding indicated that all strains of *An. sinensis* were not susceptible to *P. falciparum* and *P. yoelii*. No oocyst was obtained from either form of *An. sinensis* when compared with a control vector, *An. dirus* A, for which the oocyst rates ranged from 86.67-100%. The results were in agreement with previous reports, *ie An. sinensis* strains from south China and northern Thailand were refractory to *P. falciparum* (Otsuru and Ohmori, 1960; Somboon *et al*, 1994). However, this mosquito species was infected with *P. vivax* at appropriate rates, but the oocyst rates and number of oocysts per infected midgut were still lower than for *An. dirus* A. Additionally, most of the oocysts observed from midguts of *An. dirus* A were mature, having "wheel pattern" of sporozoites inside cysts, whereas in *An. sinensis* the maturity rate of oocysts obtained from 72 infected mosquitos was very low, 11.11% (8/72), and nearly all of them had abnormal development, retaining the stages and some forming melanin inside cysts. The results were in agreement with Somboon *et al* (1994). They found 83.33% (5/6) oocyst rates in *An. sinensis* strains from Mae Sariang District, Mae Hong Son Province, but contrary stages of oocyst development. The previous investigators stated that the oocysts were normally developed, while most of them had abnormal development in the presented results. On the other hand, the results of dissection of midguts and salivary glands of all strains of *An. sinensis* 10-15 days postblood meal revealed that most of the recovered oocysts were also retained stages. Consequently, low sporozoite rates were obtained (0.00-5.88%), while in *An. dirus* A nearly all oocysts were mature and 80.95-86.69% sporozoite rate were recovered. The sporozoite rates of 61.54% (8/13) in *An. sinensis* as reported by Somboon *et al* (1994) were contrary to the present study although the same mosquito species, the same area of interest, and the same indigenous malaria parasite were investigated in these two studies.

It was fortunate in this study that the sporozoite-like crystal was found in the dissected salivary glands of *An. sinensis* (16.67-67.57%), an observation not previously reported. This sporozoite-like crystal was not found in all ages (1-20 days) of *An. dirus* A, while in *An. sinensis* it was observed in both unfed and fed blood females, particulary in the age range from 8-20 days. The remarkable point was that the sporozoite rate (61.54%, 8/13) in *An. sinensis* reported by Somboon *et al* (1994) was in the range of crystal formation rates (35.00-90.00%). According to the present study it could be confidently concluded that two forms of *An. sinensis* were refractory and/or non-efficient vectors of *P. vivax*.

Formerly, Gingrich *et al* (1986) reported the positive ELISA for circumsporozoite (CS) antigens of *P. falciparum* and/or *P. vivax* in *An. sinensis* by using the whole bodies of mosquitos. This diagnostic tool did not definitely incriminate the mosquito as the natural vector since it could detect only CS protein from the developing oocysts (Beier *et al*, 1987), soluble CS protein shed from oocysts and sporozoites (Verhave *et al*, 1988) and CS protein in various body parts (Robert *et al*, 1988). Additionally, false positive *P. falciparum* and *P. vivax* detection by ELISA was reported (Somboon *et al*, 1993). However, the mosquito species which were highly susceptible to malarial infections could not be incriminated as the potential vectors since sporozoites did not invade salivary glands (Rosenberg, 1985). Judged from the above results, the suscep-

tibility test using a laboratory-bred, clean mosquito colony should be an efficient and reliable diagnostic method to incriminated potentially malarial vectors.

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