COMPARATIVE SUSCEPTIBILITY OF SOUTHEAST ASIAN *ANOPHELES* MOSQUITOES TO THE SIMIAN MALARIA PARASITE *PLASMODIUM CYNOMOLGI*[1]

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ABSTRACT. Seven *Anopheles* species/isolates were compared with *Anopheles dirus* (control) for susceptibility to *Plasmodium cynomolgi* B strain. The mean numbers of oocysts in paired replicates of *An. dirus* and *An. takasagoensis* were not significantly different. The remaining test species had significantly fewer mean numbers of oocysts than *Anopheles dirus* ($P < 0.01$). *Anopheles dirus* had the highest percentage of mosquitoes infected with *P. cynomolgi* sporozoites (82%). Of the test groups, *Anopheles dirus* B and *An. takasagoensis* had the highest percentage of mosquitoes with sporozoites, 77 and 78%, respectively. Fewer than 50% of *Anopheles maculatus* E and *An. maculatus* B (NN isolate) had sporozoites in the salivary glands. *Anopheles maculatus* B (HK isolate) and *Anopheles philippinensis* were the least susceptible, with fewer than 30% having sporozoites in the salivary glands.

INTRODUCTION

Recent biosystematic investigations have clarified the taxonomy of suspected and incriminated anopheline vectors of malaria parasites in Thailand. The Dirus Complex of *Anopheles*, for instance, is an assemblage of several nearly indistinguishable sibling species. *Anopheles balabacensis* Baisas was previously considered the primary vector of malaria in Thailand. It is now known that this species does not occur in Thailand and what was thought to be *An. balabacensis* in Thailand actually consists of at least 5 species (Baimai et al. 1988), of which the most common is *An. dirus* Peyton and Harrison. Cytogenetic and hybridization studies (Klein et al. 1984; Green et al. 1985a, 1985b; Rattanarithikul and Green 1987) demonstrated that other species, e.g., *An. maculatus* Theobald and *An. philippinensis* Ludlow, which have been incriminated or are suspected vectors of malaria (Anonymous 1980), are also species complexes in Thailand. The recognition of these sibling species warrants their evaluation as vectors of malaria parasites.

This study compared the susceptibility of *An. dirus*, a confirmed human malaria vector (Ketrangsee 1989, Gingrich et al. 1990), with 7 other Southeast Asian vector species/isolates to *Plasmodium cynomolgi* Mayer.

MATERIALS AND METHODS

Mosquito rearing: Mosquitoes were maintained in colonies at the Armed Forces Research Institute of Medical Sciences (AFRIMS), Bangkok, Thailand (Table 1). Females were kept in screened cages (32 cm on each side) and allowed to feed twice weekly on a hamster. Females that fed to repletion were force-mated (Ow Yang et al. 1963). Eggs were collected and immatures reared as described by Klein et al. (1982). Adults were housed in screened cages and provided a 5% multivitamin solution. The mean temperature and humidity of the insectary were 26 ± 2°C and 70 ± 5% RH, respectively. Both natural and fluorescent lighting were used, and the light cycle was maintained at 12 ± 2 h daylight.

Parasite maintenance: The B strain of *P. cynomolgi*, originally isolated in 1959 from *Macaca fasicularis* Raffles from Pahang, Malaysia (Eyles 1963), was used. *Plasmodium cynomolgi* was cycled in a colony of rhesus monkeys (*Macaca mulatta* Zimmerman) by the mosquito-sporozoite-monkey inoculation technique (Schmidt et al. 1963, 1982). Parasitemia, gametocyte rates and gametocyte sex ratios were determined microscopically from Giemsa stained blood films prepared daily from infected rhesus monkeys. *Anopheles dirus* (originally *dirus* A) was selected as the control, since previous studies had demonstrated that it was highly susceptible to *P. cynomolgi* [Collins et al. 1972 (as *An. balabacensis*), Klein et al. 1986]. The susceptibility of *An.*

[1] In conducting research described in this report, the investigators adhered to the "Guide for the Care and Use of Laboratory Animals," as promulgated by the Institute of Laboratory Animal Resources, National Academy of Sciences, National Research Council.

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Table 1. *Anopheles* species used in *Plasmodium cynomolgi* susceptibility studies.[1]

Anopheles species/(isolate)	Date colony colonized	Source	References
dirus	1964, suppl. w/eggs, 1971	Thailand	Esah and Scanlon 1966, Wilkinson et al. 1972
dirus B	1965–66	Peninsular Malaysia	
takasagoensis	1972	Taiwan	
maculatus E (Kuala Lumpur)	1966	Peninsular Malaysia	Ow Yang et al. 1963
maculatus B (Nakorn Nayok)	1979	Thailand	
maculatus B (Hui Kuum)	1979	Thailand	
philippinensis (Rayong)	1979	Thailand	Klein et al. 1982
nivipes (Korat)	1980	Thailand	Klein et al. 1982

[1] All colonies, except *An. dirus* B, which is self-mating, are maintained in the insectary at AFRIMS by forced-mating methods (Ow Yang et al. 1963).

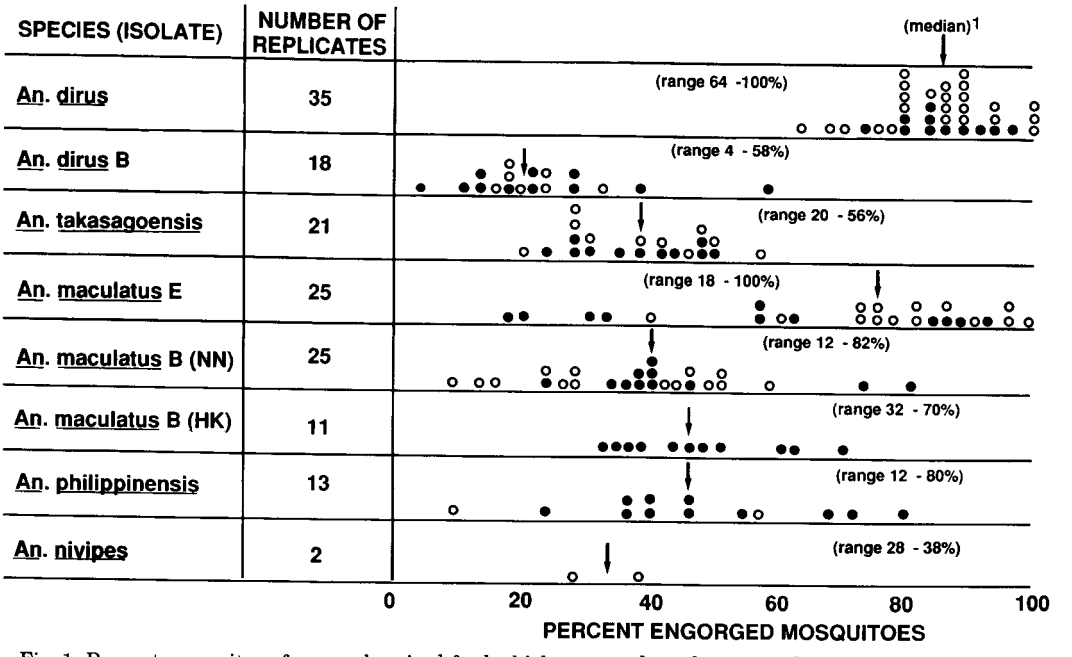

Fig. 1. Percent mosquitoes from each paired feed which engorged on rhesus monkeys infected with *Plasmodium cynomolgi* parasites. Closed circles indicate paired feeds for all anophelines. Open circles indicate paired feeds of the test anophelines with only *Anopheles dirus*, the reference species. [1]Arrow marks the median percent of mosquitoes that took a blood meal.

dirus to *P. cynomolgi* was compared with the susceptibility of each of the 7 test species/isolates.

Mosquito feeding techniques: Fifty, 3–6 day old female *An. dirus* and one or more of each of the test species were placed in separate screen-topped 215 cm^3 (pint) cartons. They were starved for 5–6 h, then allowed to feed during the same period for 30 min on infected rhesus monkeys at the second peak of parasitemia and on successive days thereafter when gametocytes were present. All mosquitoes were fed between 1300 and 1400 h to avoid variation in feeding due to periodicity. Females fed to repletion were

Table 2. *Plasmodium cynomolgi* oocyst infections of 7 *Anopheles* isolates compared with *Anopheles dirus* examined 7 days after feeding on gametocytic rhesus monkeys.

Anopheles species (isolate)	No. of trials	No. of mosquitoes dissected	Oocyst infection rate[1]	Mean no. of oocysts[2,3] (range)	Paired *t*-test *P* value[4]
dirus	18	274	88	>68 (<1–144)	
dirus B		83	83	>31 (<1–106)	<0.001
dirus	21	389	89	>63 (15–145)	
takasagoensis		160	86	>48 (7–100)	NS
dirus	25	520	93	>71 (32–145)	
maculatus E		399	77	>25 (3–72)	<0.001
dirus	25	520	93	>71 (32–145)	
maculatus B (NN)		226	70	>25 (1–67)	<0.001
dirus	11	223	92	>68 (32–145)	
maculatus B (HK)		93	57	10 (2–30)	<0.001
dirus	13	263	94	>87 (32–195)	
philippinensis		140	55	>14 (<1–62)	<0.001
dirus	2	44	95	>96 (4–100)	ND
nivipes		17	6	1 (0–1)	

[1] Number of mosquitoes with oocysts/number mosquitoes dissected.

[2] Number of oocysts counted/number of mosquitoes dissected.

[3] Individual oocyst estimates of 101–200 and >200 were recorded as 100 or 200, respectively, for mean oocyst calculations. Therefore, actual mean oocyst counts are greater than the calculated values. Individual oocyst counts did not exceed 100 oocysts for *An. maculatus* B (HK) and *An. nivipes*.

[4] NS = not significant, ND = not done.

placed in screened cardboard stool specimen cups and maintained on a 5% sucrose solution for the duration of the study. Partially engorged and unfed mosquitoes were discarded. Mosquitoes from each paired feeding were kept under identical conditions in the insectary at AFRIMS.

Mosquito examinations: The oocyst positive rate, the mean number of oocysts and oocyst development in mosquitoes on day 7 after infection, were determined by microscopic examination. Midguts from 50% of the surviving mosquitoes from each group were dissected, placed in a drop of saline and examined under a compound microscope (400×) for oocysts. Estimates of 101–200 and >200 oocysts were recorded as 100 or 200, respectively, for mean oocyst calculations. The diameter of ≤10 oocysts from each mosquito were measured with an ocular micrometer.

The salivary gland infection rate and the proportion of each species with oocysts that also produced sporozoites in the salivary glands were determined. The number of sporozoites in the salivary glands was counted (≤100) or estimated (>100). Salivary glands were removed from all surviving mosquitoes on day 14 after infection, transferred to a drop of Hayes saline, gently crushed under a coverslip to release the sporozoites and examined at 400×. Numbers of sporozoites were counted or estimated by counting a sample of sporozoites in the occular grids when numbers exceeded 100. Midguts were examined as described above for mature, ruptured and melanized oocysts.

RESULTS

Feeding: Anopheles dirus was compared with *An. dirus* B, *An. takasagoensis* Morishita, *An. maculatus* E, *An. maculatus* B, NN and HK isolates, *An. philippinensis* and *An. nivipes* Theobald. The number of paired feeds for each of the test *Anopheles* species/isolates ranged from 11 to 25, except for *An. nivipes*, in which only 2 paired feeds were done (Fig. 1). The blood-feeding response of *An. dirus* ranged from 64 to 100% ($\bar{x}$ = 85%) for different replicates and was significantly greater than the test anophelines ($P < 0.01$, paired t-test) (Fig. 1). Because six t-tests were conducted, P values were adjusted using Bonferroni's procedure to control the per experiment error rate (O'Brien and Shampo 1988). T-tests were performed, as usual, but then the P value was multiplied by the number of comparisons undertaken to obtain adjusted P values. The largest P value obtained after adjustment was 0.011. The descending order of feeding response follows: *An. dirus* > *An. maculatus* E > *An. maculatus* B (HK), *An. philippinensis* > *An. maculatus* B (NN) > *An. takasagoensis* > *An. nivipes* > *An. dirus* B.

Significance in the susceptibility results depended on the number of mosquitoes fed to repletion that were available for dissection on days 7 and 14 after infection. A large number of

female *An. dirus* ($\bar{x}$ = 19.7 mosquitoes) were dissected on day 7 after infection. However, the low blood-feeding response of *An. dirus* B resulted in only a few females available for dissection on day 7 ($\bar{x}$ = 4.6), which increased experimental error.

Oocysts: While the oocyst rate and numbers of oocysts were greater for *An. dirus* than *An. takasagoensis* infected with *P. cynomolgi*, differences were not significant. *Anopheles dirus* was significantly more susceptible to midgut infections of *P. cynomolgi* than the other test mosquitoes ($P < 0.01$, paired t-test) (Table 2). The mean oocyst diameters were estimated from a sample of 10 or fewer oocysts from each infected mosquito of the test species/isolates and were similar in size to *An. dirus* ($\bar{x}$ = 32.4 μ). The mean oocyst diameters from the test mosquitoes dissected 8 days after infection ranged from 39.5 to 55.0 μ.

T-tests were also computed for paired feeds of *An. maculatus* E and *An. maculatus* B (HK) and for *An. maculatus* E and *An. maculatus* B (NN). The mean oocyst counts of *An. maculatus* E (25.3) and *An. maculatus* B (NN) (25.1) were not significantly different. For paired feeds of *An. maculatus* E, the mean oocyst counts (14.6) were always higher than *An. maculatus* B (HK) (9.7) but were not significantly different ($P < 0.05$).

The distribution of oocyst counts of individual mosquitoes for paired replicates of *An. dirus* and the test anophelines is shown in Fig. 2. Most of the infected *An. dirus* had oocyst counts ranging from 100 to 200 while only a few had >200 oocysts. The distribution of oocyst counts for *An. dirus*, *An. takasagoensis* and *An. dirus* B were similar (Fig. 2). More *An. maculatus* E and B and *An. philippinensis* replicates were negative for oocysts, or had fewer oocysts than *An. dirus*. None of these species/isolates had more than 200 oocysts (Fig. 2).

Sporozoites: The frequency of the test anophelines with infected salivary glands was highest for *An. dirus* B (85%) and *An. takasagoensis* (76%) and similar to *An. dirus* (Table 3). *Anopheles maculatus* E and B (NN) had more than 6 times as many uninfected mosquitoes as *An. dirus*, whereas *An. maculatus* B (HK), *An. philippinensis* and *An. nivipes* had more than 9 times as many uninfected mosquitoes. The percentages of *An. dirus*, *An. dirus* B and *An. takasagoensis* with >1,000 sporozoites in the salivary glands were also much higher than those of the other test species/isolates.

DISCUSSION

Rhesus monkeys infected with *Plasmodium cynomolgi* B strain by the mosquito-sporozoite-monkey-inoculation technique were used as a model for human malaria to determine the susceptibility of *Anopheles* mosquitoes to infection with *Plasmodium* parasites (Collins et al. 1965, 1972). This model has been used in *Plasmodium* parasite susceptibility studies for 3 reasons (Warren et al. 1963; Bennett et al. 1966a, 1966b; Rutledge et al. 1970): 1) the susceptibility of *Anopheles* species to *P. cynomolgi* roughly parallels their susceptibility to human malaria parasites (Hodgkin 1956), 2) *Plasmodium cynomolgi* shows a close morphological and cyclical relationship with *P. vivax* (Grassi and Feletti) (Coatney 1968), and 3) *Plasmodium cynomolgi* has been transmitted to man under both artificial and natural conditions (Coatney et al. 1961, Schmidt et al. 1961, Cheong and Coombs 1970).

Anopheles dirus is anthropophilic but will feed readily on rhesus monkeys (Fig. 1). Feeding

Table 3. Numbers of *Plasmodium cynomolgi* sporozoites in the salivary glands of *Anopheles dirus* compared with 7 other *Anopheles* species dissected 14 days after feeding on gametocytic rhesus monkeys.

Anopheles species/(isolates)	No. trials	No. dissected	% mosquitoes by sporozoite counts				
			0	1–10	11–100	101–1,000	>1,000
dirus	17	302	11	0	1	2	86
dirus B		66	15	0	3	9	73
dirus	21	333	9	0	<1	3	87
takasagoensis		136	23	0	2	10	65
dirus	23	410	5	0	<1	2	92
maculatus E		354	33	3	10	24	30
dirus	20	346	5	0	<1	2	92
maculatus B (NN)		178	39	1	10	22	28
dirus	10	163	8	0	<1	2	89
maculatus B (HK)		81	67	6	11	9	7
dirus	13	218	7	0	1	2	90
philippinensis		128	63	6	9	14	8
dirus	2	36	0	0	0	0	100
nivipes		14	57	0	0	14	29

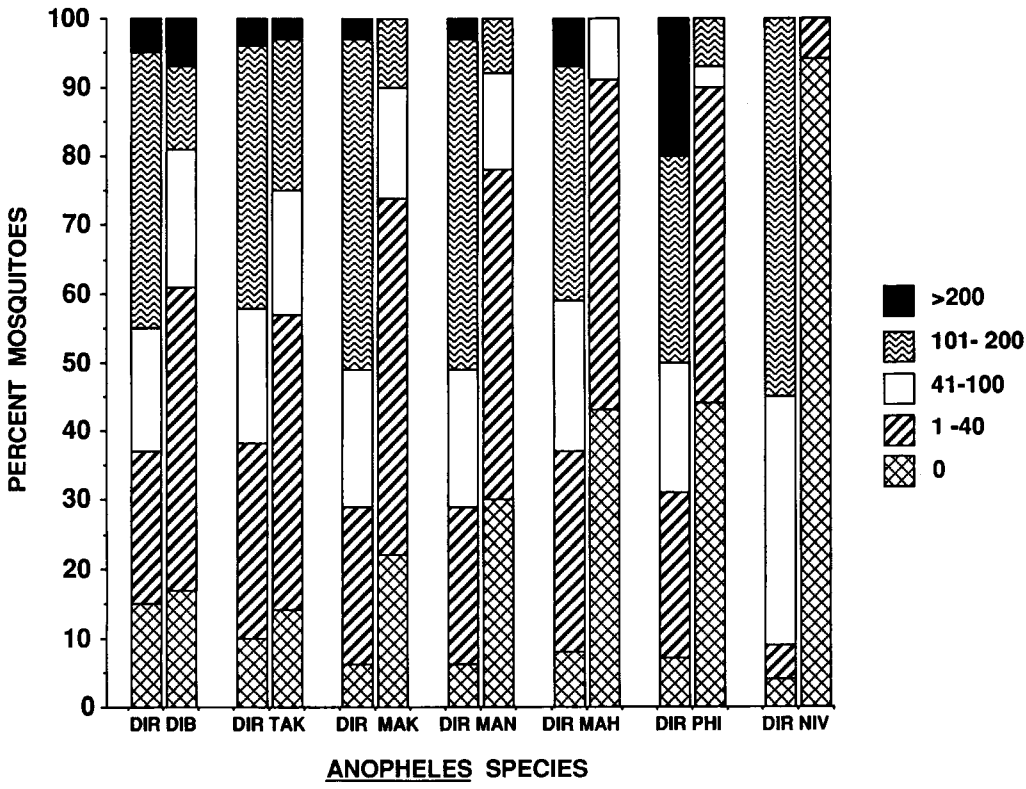

Fig. 2. Distribution of mosquitoes negative for oocysts (0) and mean oocyst counts of 1–40, 41–100, 101–200 and >200 for *Anopheles dirus* and 7 test anopheline species/isolates determined from paired feeds on rhesus monkeys infected with *P. cynomolgi*. The mean oocyst counts for *An. dirus* are shown for the paired feeds illustrated in each figure. *Anopheles dirus* = DIR; *An. dirus* B = DIB; *An. takasagoensis* = TAK; *An. maculatus* (KL) = MAK; *An. maculatus* (NN) = MAN; *An. maculatus* (HK) = MAH; *An. philippinensis* = PHI; *An. nivipes* = NIV.

periodicity could have been a factor in the low feeding response of *An. dirus* B and *An. takasagoensis* since blood meals were provided only from 1300 to 1400 h. However, these 2 species fed readily on humans in the laboratory and were regularly provided blood meals on hamsters during this same time for colony maintenance. A low blood-feeding propensity on monkeys for *An. dirus* B also has been reported for a colony at the London School of Hygiene and Tropical Medicine (J. Hii, unpublished data), and may be due to colony selection. Cheong et al. (1965) incriminated *An. balabacensis* (probably *An. dirus* B) from Perlis State, Malaysia, as a vector of *P. cynomolgi* parasites and confirmed the vector status of this species when they infected monkeys by inoculating sporozoites from wild caught mosquitoes into uninfected monkeys.

Anopheles maculatus sensu lato (*s.l.*), *An. philippinensis* and *An. nivipes* are zoophilic in Thailand. They feed primarily on water buffalo and domestic cattle but are also commonly collected in human biting collections. In areas of Thailand where these species occur in large numbers, *An. maculatus s.l.* and *An. philippinensis* have been considered either secondary or suspected vectors of *P. falciparum* and *P. vivax* parasites (Harinasuta et al. 1976).

The vector status of *An. nivipes* is unknown because the adults are not readily distinguished from *An. philippinensis*. *Anopheles nivipes* was first recognized in Thailand by Reid (1967), and examination of reared specimens and specimens from human and cow biting collections throughout Thailand indicate that *An. nivipes* is the more common of the 2 species. Green et al. (1985a) demonstrated that *An. nivipes* in Thailand actually represented 2 cytogenetic species. The Korat strain used here has not been cytotyped.

There are 3 levels of susceptibility among the test anophelines, high (*An. dirus*, *An. dirus* B and *An. takasagoensis*), moderate [*An. maculatus* E and B (NN)] and low [*An. maculatus* B

(HK), *An. philippinensis* and *An. nivipes*]. The high percentage of blood fed mosquitoes in combination with the high oocyst frequency observed in the laboratory colony of *An. dirus* indicated that it is highly susceptible to infection by *P. cynomolgi* B strain. These results are consistent with the high oocyst rates in *An. dirus* (as *balabacensis*) that Rutledge et al. (1970) and Wilkinson et al. (1972) observed. All 3 species, *An. dirus*, *An. dirus* B and *An. takasagoensis*, are members of the Leucosphyrus Group, and their high susceptibility to malaria parasites is similar to other members of the Leucosphyrus Group (Warren et al. 1963, Warren and Wharton 1963).

The oocyst and sporozoite infection rates of *An. maculatus* E and B (NN) were lower than the test Leucosphyrus Group members but were sufficiently high to consider these 2 isolates as relatively good vectors of *P. cynomolgi* parasites. Warren et al. (1963) and Bennett et al. (1966a) also showed that *An. maculatus* E (as *An. maculatus*) was susceptible to different strains of *P. cynomolgi* parasites. Differences in susceptibility between *An. maculatus* B (NN and HK) isolates were observed, but these isolates are not separable by present cytogenetic techniques. The recent elucidation of the Maculatus Complex by Rattanarithikul and Green (1987) indicates that further *Plasmodium* susceptibility studies in this complex are needed.

Anopheles philippinensis was not highly susceptible to *P. cynomolgi* B strain. These results contrast with findings of Warren et al. (1963) in Malaysia, but Bennett et al. (1966a) demonstrated that *An. philippinensis* was not susceptible to all strains of *P. cynomolgi*. Reid (1967) indicated that both *An. philippinensis* and *An. nivipes*, which are morphologically similar in the adult stage, are sympatric in Malaysia where Bennett et al. (1966a, 1966b) and Warren et al. (1963) conducted their experiments. The studies conducted in Malaysia may have been made with *An. nivipes* or a combination of *An. nivipes* and *An. philippinensis*. Relative susceptibility of *An. nivipes* could not be determined with certainty on the basis of only 2 replicates.

A number of factors, i.e., prevalence, host preference, longevity, etc., must be considered in the evaluation of mosquito species/isolates as potential vectors of malaria parasites. The degree of susceptibility to malaria parasites and ultimately its transmission by the host mosquito are but 2 aspects. The evaluation of these species in relation to susceptibility to simian *Plasmodium* species may assist in the evaluation of susceptibility to human *Plasmodium* species, especially where studies on human malarias are not possible.

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REFERENCES CITED

Anonymous. 1980. Malaria Division, Dept. Com. Dis. Control., Min. Public Health, Bangkok. (January) p. 19–21.

Baimai, V., R. E. Harbach and U. Kijchalao. 1988. Cytogenetic evidence for a fifth species within the taxon *Anopheles dirus* in Thailand. J. Am. Mosq. Control Assoc. 4:333–338.

Bennett, G. F., M. Warren and W. H. Cheong. 1966a. Biology of the simian malarias of Southeast Asia. II. The susceptibility of some Malaysian mosquitoes to infection with five strains of *Plasmodium cynomolgi*. J. Parasitol. 52:625–631.

Bennett, G. F., M. Warren and W. H. Cheong. 1966b. Biology of the simian malarias of Southeast Asia. III. Sporogony of the Cambodian strain of *Plasmodium cynomolgi*. J. Parasitol. 52:632–638.

Cheong, W. H. and G. L. Coombs. 1970. Transmission of *Plasmodium cynomolgi* (Perlis strain) to man. Southeast Asian J. Trop. Med. Public Health 1:302.

Cheong, W. H., M. Warren, A. H. Omar and S. Mahadevan. 1965. *Anopheles balabacensis balabacensis* identified as vector of simian malaria in Malaysia. Science 150:1314–1315.

Coatney, G. R. 1968. Simian malarias in man: facts, implications, and predictions. Am. J. Trop. Med. Hyg. 17:147–155.

Coatney, G. R., H. A. Elder, P. G. Contacos, M. E. Getz, R. Greenland, R. N. Rossan and L. H. Schmidt. 1961. Transmission of the M strain of *Plasmodium cynomolgi* to man. Am. J. Trop. Med. Hyg. 10:673–678.

Collins, W. E., F. E. Jones and C. G. Dobrovolny. 1965. Transmission of the RO strain of *Plasmodium cynomolgi* by *A. stephensi*, *A. quadrimaculatus*, and *A. labranchiae atroparvus*. Mosq. News 25:389–392.

Collins, W. E., P. G. Contacos, J. C. Skinner and E. G. Guinn. 1972. Studies on the transmission of simian malaria. V. Infection and transmission of the B strain of *Plasmodium cynomolgi*. J. Parasitol. 58:653–659.

Esah, S. and J. E. Scanlon. 1966. Notes on a laboratory colony of *Anopheles balabacensis* Baisas. Mosq. News 26:509–511.

Eyles, D. E. 1963. The species of simian malaria: taxonomy, morphology, life cycle, and geographical distribution of the monkey species. J. Parasitol. 49:866–887.

Gingrich, J. B., A. Weatherhead, J. Sattabongkot, C. Pilakasiri and R. A. Wirtz. 1990. Hyperendemic malaria in a Thai village: dependence of year-round transmission on focal and seasonally circumscribed mosquito (Diptera: Culicidae) habitats. J. Med. Entomol. 27:1016–1026.

Green, C. A., V. Baimai, B. A. Harrison and R. G. Andre. 1985a. Cytogenetic evidence for a complex of species within the taxon *Anopheles maculatus* (Diptera: Culicidae). Biol. J. Linnean Soc. 24:321–328.

Green, C. A., B. A. Harrison, T. Klein and V. Baimai. 1985b. Cladistic analysis of polytene chromosome rearrangements in anopheline mosquitoes, subgenus *Cellia*, series Neocellia. Can. J. Genet. Cytol. 27:123–133.

Harinasuta, T., H. M. Giles and A. A. Sandosham (eds.). 1976. SEAMEO-TROPMED Scientific Group Meeting: malaria in Southeast Asia. Southeast Asian J. Trop. Med. Public Health 7:641–678.

Hodgkin, E. P. 1956. The transmission of malaria in Malaya. Stud. Inst. Med. Res. Malaya 27:1–98.

Ketrangsee, S. 1989. Malaria situation in Thailand, pp. 10–17. *In*: Third Conference on Malaria Research, Thailand. WHO (SERO). Chiangmai, Thailand.

Klein, T. A., B. A. Harrison, I. Inlao and P. Boonyakanist. 1982. Colonization of Thailand strains of *Anopheles nivipes* and *Anopheles philippinensis*. Mosq. News 42:374–380.

Klein, T. A., B. A. Harrison, V. Baimai and V. Phunkitchar. 1984. Hybridization evidence supporting separate species status for *Anopheles nivipes* and *Anopheles philippinensis*. Mosq. News 44:466–470.

Klein, T. A., B. A. Harrison, J. S. Grove, S. V. Dixon and R. G. Andre. 1986. Correlation of survival rates of *Anopheles dirus* A (Diptera: Culicidae) with different infection densities of *Plasmodium cynomolgi*. Bull. W.H.O. 64:901–907.

O'Brien, P. C. and M. A. Shampo. 1988. Statistical considerations for performing multiple tests in a single experiment. I. Introduction. Mayo Clin. Proc. 63:813–820.

Ow Yang, C. K., F. L. Sta Maria and R. H. Wharton. 1963. Maintenance of a laboratory colony of *Anopheles maculatus* Theobald by artificial mating. Mosq. News 23:34–35.

Rattanarithikul, R. and C. A. Green. 1987. Formal recognition of the species of the *Anopheles maculatus* group (Diptera: Culicidae) occurring in Thailand, including the descriptions of two new species and a preliminary key to females. Mosq. Syst. (1986) 18:246–278.

Reid, J. A. 1967. Two forms of *Anopheles philippinensis* in Malaya. J. Med. Entomol. 4:175–179.

Rutledge, L. C., D. E. Hayes and R. A. Ward. 1970. *Plasmodium cynomolgi*: sources of variation in susceptibility of *Anopheles quadrimaculatus*, *An. balabacensis* and *An. stephensi*. Exp. Parasitol. 27:53–59.

Schmidt, L. H., R. Greenland and C. S. Genther. 1961. The transmission of *Plasmodium cynomolgi* to man. Am. J. Trop. Med. Hyg. 10:679–688.

Schmidt, L. H., R. N. Rossan, and K. F. Fisher. 1963. The activity of a repository form of 4, 6-Diamino-1-(p-chloro-phenyl)-1, 2-Dihidro-1, 2-Dimethyl-s-Triazine against infections with *Plasmodium cynomolgi*. Am. J. Trop. Med. Hyg. 12:494–503.

Schmidt, L. H., R. Fradkin, C. S. Genther, R. N. Rossan and W. Squires. 1982. *Plasmodium cynomolgi* infections in the rhesus monkey. I. The characteristics of untreated sporozoite-induced infections. Am. J. Trop. Med. Hyg. 31:612–645.

Warren, McW. and R. H. Wharton. 1963. The vectors of simian malaria: identity, biology, and geographical distribution. J. Parasitol. 49:892–904.

Warren, McW., D. E. Eyles, R. H. Wharton and C. K. Ow Yang. 1963. The susceptibility of malayan anophelines to *Plasmodium cynomolgi bastianelli*. Indian J. Malariol. 17:85–105.

Wilkinson, R. N., D. J. Gould and A. Boonyakanist. 1972. Comparative susceptibility of *An. balabacensis* and *Anopheles minimus* to naturally occurring *Plasmodium cynomolgi* in Central Thailand. Proc. Helminthol. Soc. Wash. 39(Special issue):423–427.