Ecology of Malaria Vectors

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OBJECTIVE: To investigate the bionomics and population dynamics of known and potential vectors of human malaria in Southeast Asia and their relationship to the dissemination of chloroquine resistant strains of P. falciparum.

DESCRIPTION: Specific factors being studied in the process of defining actual and potential vector species in Thailand include the following: incidence of malarial oocysts and sporozoites in wild anopheline populations, susceptibility of colonized strains of Anopheles to infection with P. falciparum, patterns of biting activity of vector species, ovipositional habits of anopheline mosquitoes and the of viability their eggs under varying environmental conditions.

PROGRESS:

1) Malaria field studies in Prachinburi province. Entomological field studies were carried out in Prachinburi province in support of a longitudinal study of the epidemiology of malaria in the area. The study site was located in a forested valley in the mountains about 100 Km south of Korat and, malaria is endemic in the human population of the area. Periodic collections of adult and larval anophelines were made in and near the villages of Ban Bu Phram and Ban Thap Lan. Initially, a comparison was made of the number of anopheline adults attracted to CDC light–traps and lard–can traps, both baited with a CO_2 attractant (dry ice), and those obtained from concurrent human–bait collections. Use of the two types of traps was discontinued because they yielded few mosquitoes, and collection of anophelines resting inside and outside houses and biting human subjects was relied upon thereafter. A total of 35 night time collections, made between dusk and dawn, were carried out at weekly intervals in the two villages from June 1971 through March 1972. Weekly collections were terminated at the end of the rainy season in November, and begun again in February 1972 during the peak of the dry season. During the latter period collections were also made at a logging camp in the forest 2 miles NW of Ban Bu Phram. A total of 571 A. balabacensis females were collected over the whole period (Table 1). Smaller number of A. minimus, A. aconitus and A. maculatus females were also collected. The largest number of balabacensis were collected during June and July: both villages were sprayed with DDT at the end of July and comparatively few balabacensis adults were collected thereafter (Table 2). A. balabacensis was the only anopheline found infected during this period, and the overall infection rate for the 571 A. balabacensis collected during the study period was 1.7%. Two infected mosquitoes were collected in each of the following situations: biting indoors, biting outdoors, and resting outdoors. Biting activity by balabacensis began outdoors as early as 1900 hours and indoors by 2000 hrs. Biting activity reached a peak between 2300–2400 hrs. however the differences in hourly collections between 2100 and 0400 outdoors and between 2200 and 0500 indoors were small. A. balabacensis were collected resting outdoors from 1900 to 0500 and indoors from 1900 to 0600. Peak numbers were found resting outdoors between 2200–2300 hours and between 2000 and 2300 hours indoors. Efforts were made during this period to locate the daytime resting places of adult A. balabacensis, but only three males and two females were collected during daylight hours resting in an adandoned charcoal pit near Ban Bu Phram. No A. balabacensis adults were collected resting in vegetation during daylight hours, even though collections were begun at daybreak in areas where A. balabacensis had been collected the previous night or when these collections were conducted near known ovipositional sites.

Larval habitats of A. balabacensis in the vicinity of Ban Bu Phram and Ban Thap Lan were located and described. During this period balabacensis larvae were found in 43 ground-pools in 18 locations. Once discovered these pools were visited weekly and sampled for larvae. The temperatures and pH of water in these pools were measured weekly. The majority of these pools were formed by elephant footprints, however larvae were also collected from water which collected in buffalo hoofprints, wheel ruts, natural depressions and in fallen tree trunks. All these sites were shaded from the sun during part of the day. The daytime water temperatures ranged from 26 to 31°C and the pH values ranged from 6.0 to 7.0 throughout the study period. Larvae were present in one or more of these sites from June until November. Because of irregular rainfall, the majority of the larval pools were intermittently dry, and none had balabacensis larvae continuously during the period of observation. Larvae of at least 8 other anopheline species were collected in association with A. balabacensis, including A. barbirostis, members of the A. byrcanus complex, A. barbumbrosus, A. bodgkini, A. insulaeflorum, A. kochi, A. tesselatus and A. vagus. Throughout the rainy season samples of moist soil were taken from edges and bottoms of dry pools from which balabacensis larvae had been previously collected. These samples were returned to the laboratory and immersed in pans of water. One hundred twenty such samples were obtained and first stage balabacensis larvae were recovered from 10. Presumably these larvae hatched from eggs present in the soil samples, although it is also possible they represented larvae trapped in the mud at the bottoms of the drying pools. However, the former assumption is supported by the observation that gravid balabacensis females from the SMRL colony, given the opportunity to oviposit in pans containing pools of water in a sand substrate, deposited their eggs only on the sand at the margins of the water rather than on the surface of the water. In addition, eggs of colonized A. balabacensis have remained viable for up to 56 days when kept on moist filter paper in SMRL laboratory.

Rainfall ceased in Ban Bu Phram valley on 4 November, and all larval pools under observation on the valley floor were dry by 15 November. During the remainder of November and in December efforts were made to locate balabacensis breeding sites at the margins of the forested hills surrounding the valley where water was still present in stream beds and seepage areas. Larvae of A. balabacensis were found in such sites during November and December, but no larvae were collected between January and March 1972, although suitable larval habitats were still present. Larvae of A. balabacensis were obtained from soil collected from two former breeding sites during December, but samples collected between January and March were negative.

2) Susceptibility of A. balabacensis and A. minimus to infection with P. falciparum. A study was undertaken to determine if there is a difference in the susceptibility of colonized strains of A. balabacensis and A. minimus to infection with naturally occurring P. falciparum in an area where chloroquine resistant falciparum is endemic. This study was conducted in the town of Phra Phutthabat, Saraburi province approximately 130 km north of Bangkok. Laboratory reared A. balabacensis and A. minimus from SMRL colonies were fed simultaneously on 36 persons with P. falciparum infections. These subjects came from a wide area of central Thailand for treatment of acute malaria at either the local hospital or Malaria Eradication Center in Phra Phutthabat. Only those with single infections of P. falciparum and gametocyte densities of at least 100 per cmm were selected for study. Mosquitoes which engorged on these subjects were dissected eight to ten days later, and the oocysts present on their guts counted. The proportions of mosquitoes which had oocysts (per cent positive) and the mean number of oocysts per infected female (oocyst index) were used to compare the susceptibility of the two mosquito species. The results of the 36 attempts to infect the two mosquito species are summarized in Table 3. More A. balabacensis fed and were dissected than A. minimus. Sixteen of the 36 subjects were infectius for A. minimus, while A. balabacensis were infected by these and an additional eight subjects. Twelve of the subjects were non-infectious for both mosquito species. The results of the 16 paired feedings which resulted in infection of both mosquito species are summarized in Table 4. The medians of per cent positive and oocyst numbers were 2.5. and 4.5 greater, respectively, for A. balabacensis than for A. minimus. The results of the feedings on the eight subjects which infected only A. balabacensis are presented in Table 5. There were no noticeable differences in size or morphology of the oocyst which developed in the two species.

The A. balabacensis and A. minimus oocyst indices from 13 subjects are shown in Figure 1. The results were highly correlated ($r = .80$, $p < .01$). In Figure 2 are presented the per cent positive results for these subjects: association was less marked ($r = .39$, $p < .15$). The data from the additional three subjects which infected both species are not included in Figures 1 and 2 because in each case less than ten A. minimus were dissected and, of these, only one was infected.

The percents positive for both mosquito species were also compared with the gametocyte densities in the subjects. When mosquitoes were infected, larger proportions of A. balabacensis were infected at each gametocyte density. However, the association between gametocytemia and proportion infected for the above mentioned 13 subjects was not appreciable for either A. balabacensis ($r = 0.36$, $p < .15$) or A. minimus ($r = .15$, $p < .50$). Similar results were obtained for both mosquitoes when oocyst indices were compared with levels of gametocytemia. The gametocyte densities and percentages of mosquitoes infected for all 36 subjects are shown in Figure 3.

Table 1.

Numbers of A. balabacensis Collected in Ban Bu Phram Valley.

Location	Biting		Resting		Total
	Outdoors	Indoors	Outdoors	Indoors	
Village	161	193	111	45	510
Forest	11	1	44	5	61
Totals	172	194	155	50	571

Table 2.

Summary of the A. balabacensis collected at Prachinburi study site. (June 71 – March 72)

Location	Month	Biting				Resting	
		Total Number Collected		Number Collected /man/night		Total Numbers Collected	
		Outdoors	Indoors	Outdoors	Indoors	Outdoors	Indoors
Village	June	69	90	4.3	6.4	60	4
"	July	53	72	2.8	2.7	37	40
"	Aug	1	21	0.2	3.5	2	1
"	Sept	20	9	0.8	0.3	8	0
"	Oct	16	1	0.5	0.04	1	NC*
"	Nov	2	0	0.7	0.0	0	NC
"	Feb	0	0	0	0.0	3	0
Forest	Feb	11	11	1.8	0.5	43	5
"	Mar	0	0	0	0	1	NC
Total	8	172	194	–	–	155	50

* NC: No. collection made

Table 3.

The median and range for the number of engorged and dissected mosquitoes (groups of 50) for 36 attempted infections.

		A. balabacensis		*A. minimus*	
		Infected	Not Infected	Infected	Not Infected
No. groups		24	12	16	20
No. fed	Median	41.5	38.0	25.5	22.5
	Range	31 – 49	18 – 48	7 – 42	7 – 44
No. dissected (survived)	Median	37.5	32	20.0	17.5
	Range	30 – 47	14 – 47	6 – 38	6 – 33
Percent fed dissected		90.3	84.2	78.4	77.7

Table 4.

Results of dissections of 16 groups of A. balabacensis and A. minimus when both groups were oocyst positive.

		A. balabacensis	A. minimus
Percent positive	Median	83.3	33.2
	Range	6.5 — 100	3.9 — 69
Oocyst index	Median	13.4	3
	Range	1.4 — 248	2 — 42

Table 5.

Results of dissections of eight groups of A. balabacensis when the corresponding A. minimus group negative

Percent positive	Median — 36 Range — 2.3 — 100
Oocyst index	Median — 1.5 Range — 1 — 34.8

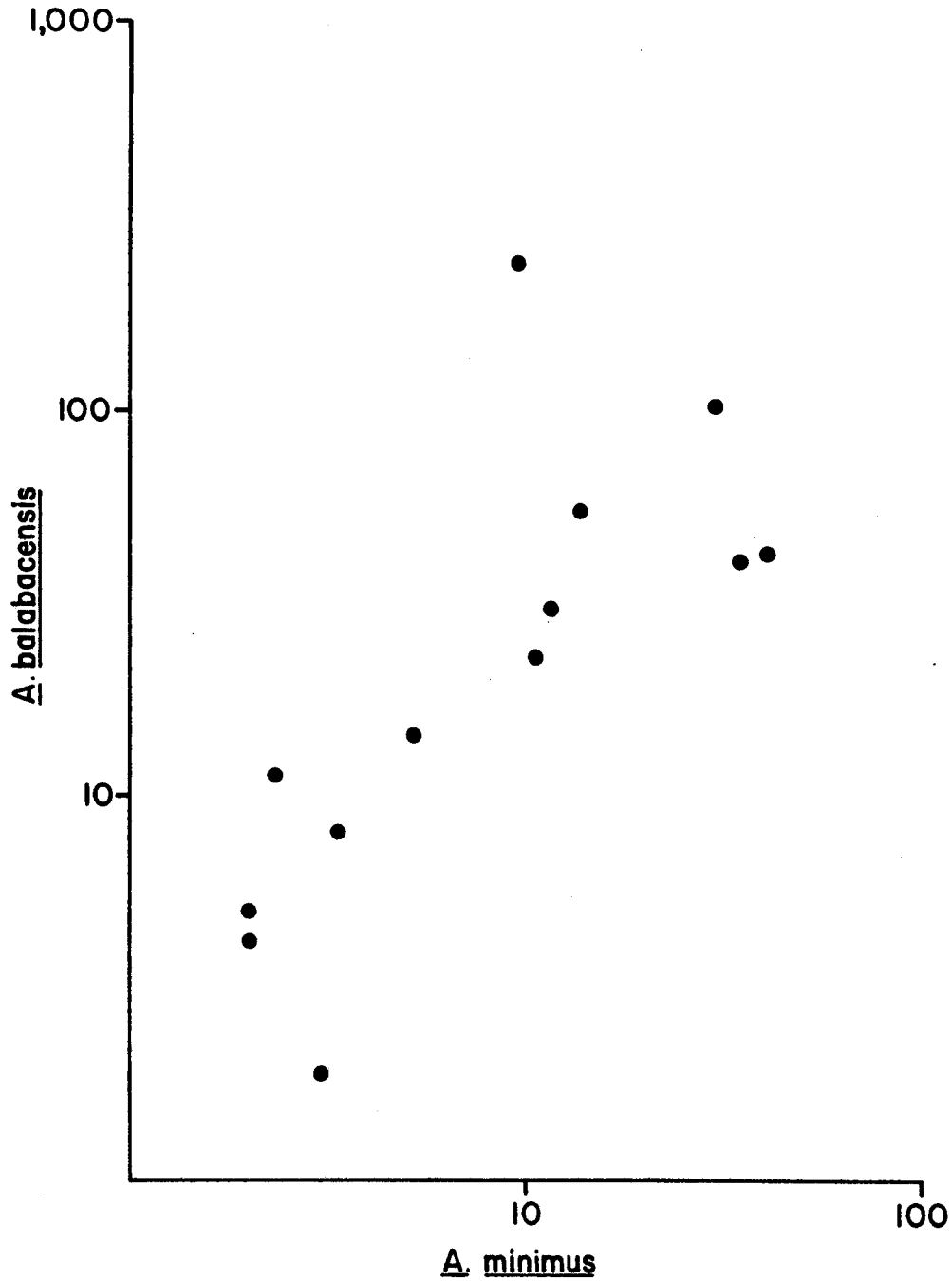

Fig. 1. Oocyst index for A. balabacensis and A. minimus by subject.

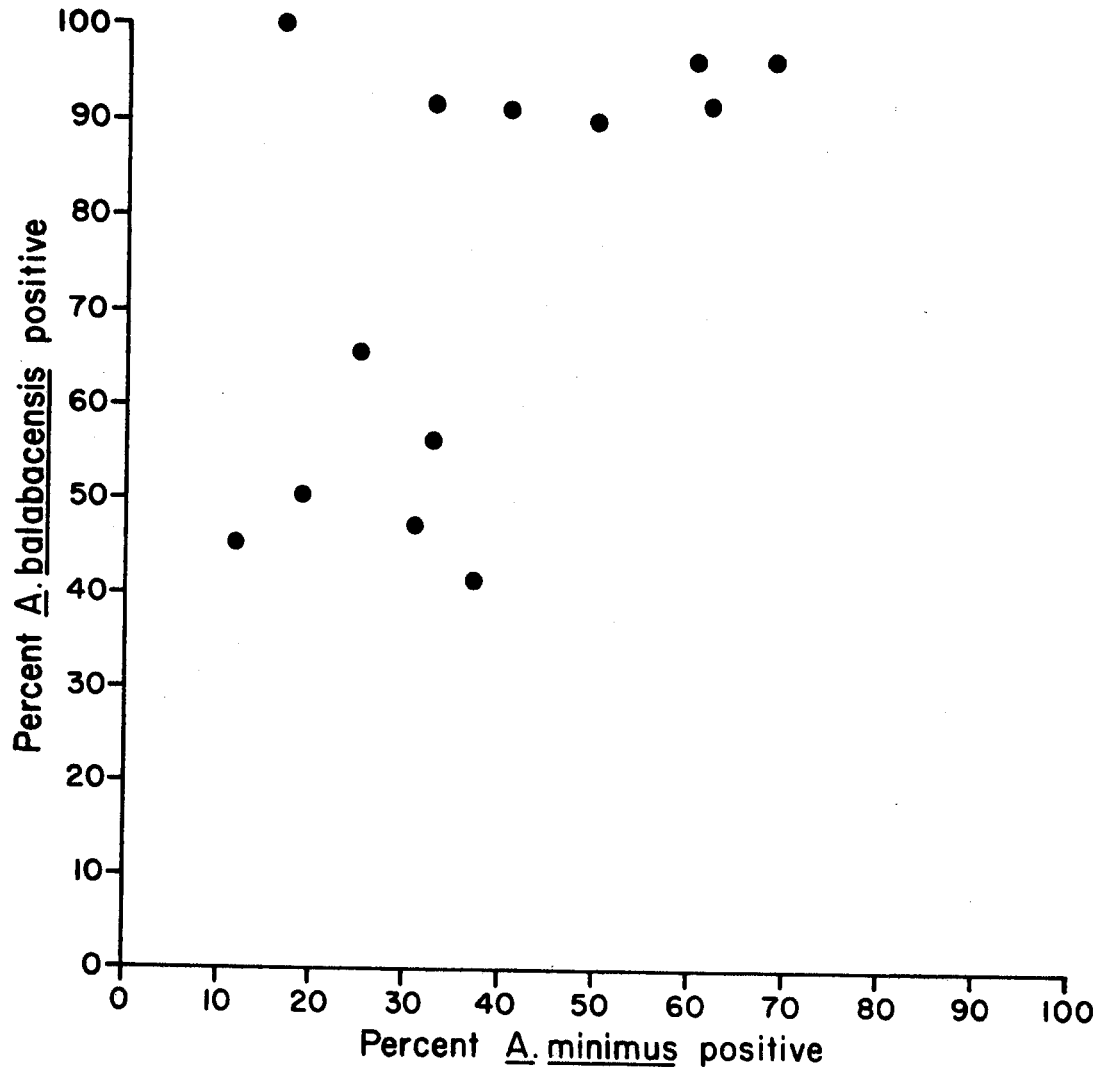

Fig. 2. Percent positive A. balabacensis and A. minimus by subject

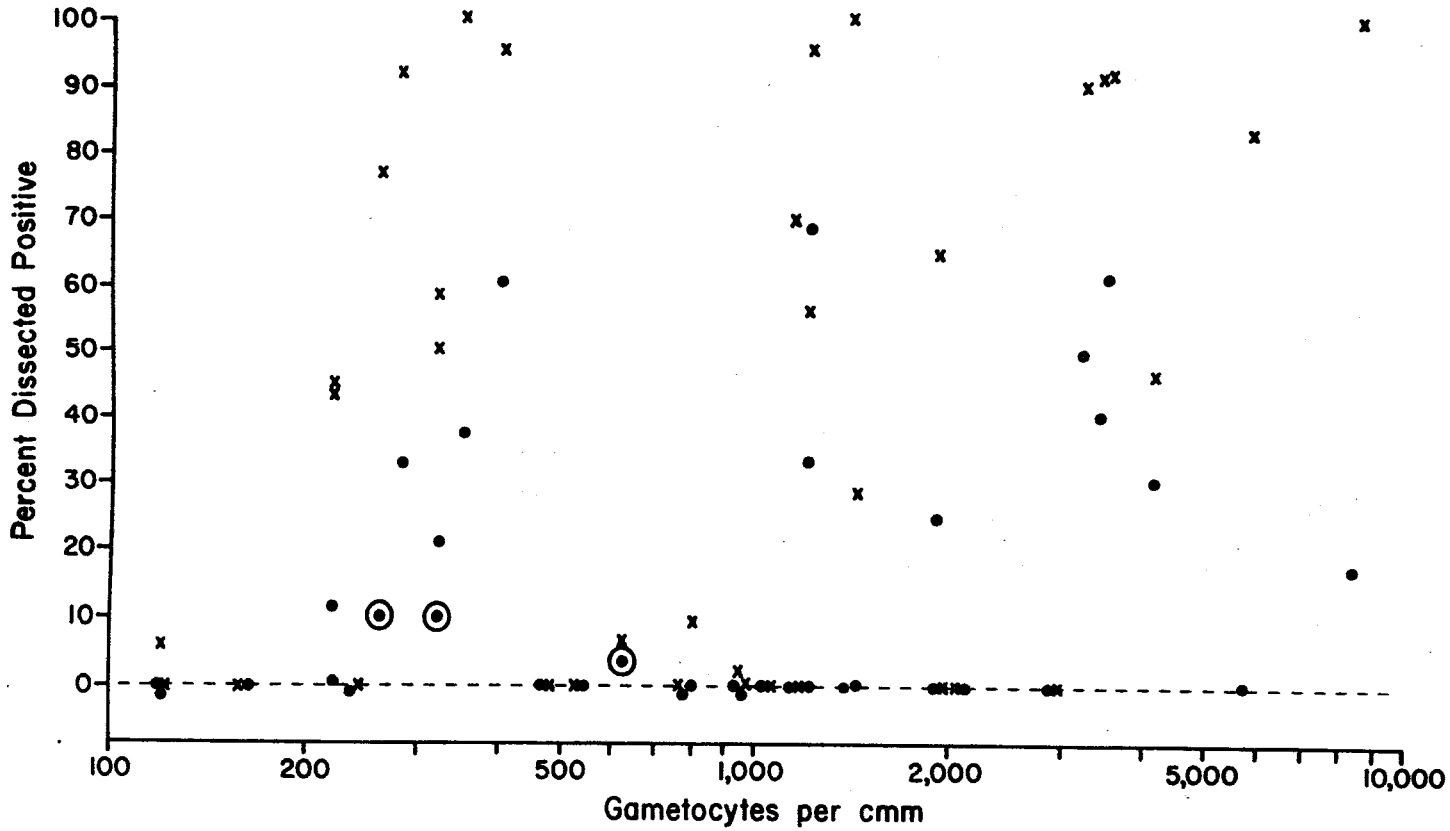

Fig. 3. Percent of A. balabacensis and A. minimus infected by feeding simultaneously on 36 subjects with P. falciparum, as related to gametocyte density. (× = A. balabacensis, • = A. minimus, ⊙ = A. minimus, less than 10 dissected.)