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Anopheline Mosquitoes of Myanmar. I.

Anopheles (cellia) dirus Peyton and Harrison, 1979

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Results of the study involving distribution, breeding habitats, biology under laboratory conditions, adult bionomics and its relationship to malaria transmission under different ecological conditions have been studied and presented. Cytogenetics and iso-enzyme studies to differentiate its sibling species were also presented. Of the sibling species of An.dirus, species D was found to predominate in Myanmar, whereas species A was recorded from Central Burma. Susceptibility of An. dirus to DDT and Bacillus sphaericus was also reported.

INTRODUCTION

Malaria workers in Burma previously referred to the leucosphyrus group of species as An. leucosphyrus. Tin (1) and Rao (2). Christophers (3) included Upper and Lower Burma in the distribution fo An. leucosphyrus. Khin-Maung-Kyi(4) examined specimens of An. leucosphyrus from the collections at the Malaria Institute of Myanmar and identified them as An. balabacensis balabacensis Baisas, which he incriminated as an important vector of malaria in Myanmar. When the Department of Medical Research (DMR) initiated studies on malaria vectors in 1983, the prevalence of Anopheles dirus Peyton & Harrison was recognized for the first time in Burma. Subsequent surveys by DMR teams in various parts of the country did not yield any An. balabacensis- balabacensis. In view of the fact that An. dirus is a primary vector of malaria in Burma and that the last detailed study of this species was carried out about 19 years ago, during which period many ecological changes have occurred, DMR endeavoured to investigate into its bionomics and relationship to malaria transmission under varied ecological conditions. In addition to detailed morphological studies, cytogenetics & isoenzyme studies were undertaken to differentiate sibling species. Breeding habitats were surveyed with special ecological studies being carried out in domestic wells of Mudon Town. Adult bionomics with special reference to biting and resting behaviours were critically studied. Other relevant data pertaining to its role as a major vector were also collected.

Identification

Identification of An. dirus was done according to Peyton & Harrison(5). The main morphological characters used for identification being:

(a) Adult wing : Basal Extension fo dark mark on Vein R extending to the level of humeral dark area on Costa.

(b) Pupal chaetotaxy : Length of Seta 9, IV approximately 0.50 of Seta 9,V

Distribution in Burma

Khin Maung Kyi (4) described the distribution of An. balabacensis balabacensis in Burma and stated that this species is mostly associated with forested foot-hills and deep forests (Fig 1).

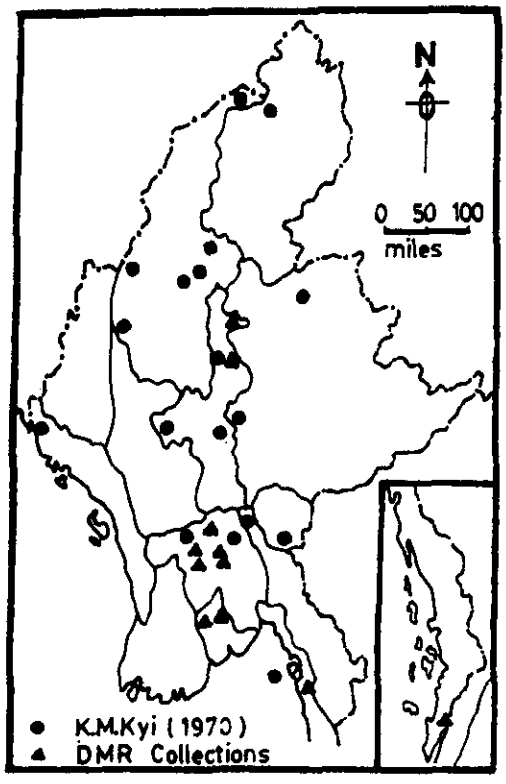

Fig.(1).Map of Burma showing distribution of An. dirus

DMR entomological survey teams collected An. dirus from the following locations Table 1.

Breeding habitats

Khin Maung Kyi (4) quoting various reports on work carried out in Myanmar during the late 1930s and in the 1940s, stated that An. balabacensis balabacensis is essentially a jungle breeder found in rain-filled trenches, puddles and marshes in the forest and in rocky beds of ravines. Based on these observations, Khin Maung Kyi concluded that this species is capable of breeding in a great variety of stagnant waters not exposed to sunlight. Breeding of An. dirus in wells of Mudon was reported by Vector-borne Diseases Control Unit in their 1980 Departmental Annual report (6).

DMR survey teams located An. dirus breeding sites in the following locations.

(1) Mudon : Out of about 900 domestic wells in Mudon Town, An. dirus larvae could be collected from about 50% during the rainy season, especially from Kwan-ka-thaung (Myoma 4 ward). In the dry season, larval density decreases but some larvae can still be located from some wells in Kwan-ka-thaung. The domestic wells, which are in daily use, are lined by laterite rocks and are situated in shaded areas.

(ii) Phado : At Hti-phado wood cutting camp situated in a deep forest area 5 miles from the forest fringe, An. dirus larvae were found breeding in rocky pools along the banks of a stream under dense shade.

(iii) Tha-bye-wa : (a) Rock pools in a tree shaded ravine situated deep inside a teak forest reserve.
(b) Bamboo stumps in the teak forest reserve situated on a hill east of Tha-bye-wa.

Moe Moe (7) investigated into the ecological factors influencing the breeding of An. dirus in the wells. The factors that directly influence breeding were found to be:

(a) Rainfall : Rainfall was found to be an important factor. During the rainy season, the larval density in the wells of Kwan-ka-thaung (Myoma 4) ward, Mudon increased significantly. Similarly, larval density increased in wells of Myoma 3 ward. However, the remaining wards, Myoma 1 and 2 had very little An. dirus breding in the wells, even in the rainy season. In the dry season, An. dirus larvae could be collected only from Kwan-ka thaung (Myoma 4) ward.

(b) Water level of the wells : Distance of water level of the wells was found to be another important factor. Deep wells are more shaded and provide more attractive oviposition sites for An. dirus. The wells in Kwan-ka-thaung (Myoma 4) Wards which were found to be deeper

Table 1. Location where An. dirus was collected by DMR survey teams

Locality	Township	Season	Topography & Remarks on location
YANGON DIVISION			
Ye-sit-kan; Gyo-byu	Taik-kyi	Jun-Jul	Reservoir & catchment area with thick forests. Yangon Water Supply Center.
Lein-hmaw-chan	Taik-kyi	Jun-Jul	Low foot-hills with thick vegetation & forest
Ye-net-kyee, Phaung-Gyi	Hle-gu	June	Low foot-hills with thick forest. Road construction camp
BAGO DIVISION			
Hti-phado, Phado	Kyauk-ta-gar	Jun-Feb	Deep forest wood-cutting camp in Bago Yoma range
Kyar-kyaung-thaik Phado	"	Jun-Jan	Temporary settlement on edge of forested foot-hill
Ngok-to & Gwe-gon,	"	Jun-Jan	Villages within 1½ miles of forested foot-hill
Kat-se-ne Camp,	"	June	Timber extraction camp in Bago Yoma range Deep forest.
Ye-bya Camp	Ok-po	Nov	Timber extraction camp in deep forest of Bago Yoma range.
Waing & Kaing-taw-su villages	Ok-po	Nov	Villages at the foot-hills of Bago Yoma
Tha-bye-wa village	Oktwin	Jul-Jan	Village surounded by Teak forest reserve and teak plantations. Situated in a valley in Bago Yoma range at an altitude of 700' above sea level
MANDALAY DIVISION			
Se-daw-gyi	Madaya	Aug-Sept	Foot-hills & near forest in an irrigation system area
Kabaing	Mogok	Aug-Sept	Hilly area with forest at an altitude of about 4000 feet above sea level
MON STATE			
Kwan-ka-thaung Ward, Mudon Town	Mudon	Throughout the year, but very few during Feb to Jun	One of the four wards comprising Mudon urban area. Edge of town near rubber plantation
TANINTHARYI DIVISION			
Nam-tun Border Camp	Kawthaung	January	Deep forest timber extraction camp adjacent to Isthmus of Kra on Thailand. Hilly topography with many perennial streams in the area

than those in other Wards were harbouring more An. dirus larvae.

(c) Shade , grass and shrubs on inner walls and debris on water surface : Shade and debris in the wells were also found to be yet another important factor influencing breeding of An. dirus. 70% of the An. dirus positive wells had shade & shrubs on sides and debris on water surface.

(d) Salinity of the water : It was observed that Sodium chloride (NaCl) concentration in the well water had an inverse correlation with the mean density of An. dirus larvae & pupae per dip. In Mudon wells it was observed that at NaCl concentrations above 200 ppm, the larvae and pupae collected per dip decreased to a very low level.

The factors influencing the breeding of An. dirus in the deep forest breeding sources such as rock pools in ravines or stream beds are :

(a) Situated under heavy shade in deep forest. No direct sunlight falling on the water surface.

(b) Breeding water was found to be clear and clean but the pools contain large amounts of decayed leaves at the bottom.

(c) Many species of Culex were found to be co-breeding in these pools.

A few bamboo stumps in the deep forest were found to harbour An. dirus breeding. Water in these bamboo stumps were also found to contain decayed leaves. Co-breeders identified were Aedes (Finlaya) albolateralis (Theobald) and Orthopodomyia albipes Leicester, the latter incidentally being recorded for the the first time in Myanmar. : in

Adult Bionomics

Seasonal prevalence : Khin Maung Kyi (4) basing on surveys carried out by his staff from 1959 onwards, stated that An. balabacensis balabacensis (= dirus) is prevalent from June to October, with its peak in August and September. DMR survey teams at Phado, during two years of longitudinal surveys, observed that An. dirus was collected regularly fron June till February from foot-hill villages of Bago Yoma. This species was more prevalent in the moonsoon months and the peak density was obtained during September. In the deep forest wood-cutting camp of Hti-phado, 5 miles inside Bago Yoma, high density of An. dirus was still collected in February.

These An. dirus mosquitoes were also presumbaly having a good survival rate at Phado as observed from the Table (2) presenting man-biting rates and vectorial capacities. In comparison to the foot-hill village man-biting rates (mbr), the rate in Hti-phado Camp was found to be about 30 per man night, an excessively high rate.

Table 2. An. dirus vectorial capacities & man-biting rate at Phado (1984-1986)

MONTH	An. dirus				Raintall(mm)	
	1984/1985		1985/1986		1984/85	1985/86
	ma	v.c	ma	v.c		
Sep/Oct	1.14	2.63			346	176
Oct/Nov	1.8	5.68			112	60
Nov/Dec	0.44	0.13	1.27	2.1	-	76
Dec/Jan	0.95	0.60				
Jan/Feb	0.25	0.79				
Feb/Mar	0	0	0.09	0.16		
Mar/Apr	0	0				
Apr/May	0	0			14	33.5
May/Jun	0.45	1.71	0.82	0.11	392	55.5
Jun/Jul	0.91	3.41			871	388.6
Jul/Aug	0.73	2.77			637	642.2
Aug/Sep	1.36	0.21			1039	656.0
Sep/Oct	0.64	0.14	0.55	1.19	176	289.1
Total	0.77		0.68			

ma= man biting rate v.c= vectorial capacity

At Tha-bye-wa village, Oktwin Township, a man-biting rate of 4.6 is obtained during July 1987, Myo Paing et al (8). In January 1988, only 3 An. dirus were caught during the whole trip whereas in February, none could be collected.

In the deep forest timber extractio camps in Kawthaung Township, the man-biting rate of An. dirus recorded during January 1989, was 4.0 per man night.

From the Department of Medical Research (DMR) survey results it can be concluded that An. dirus was prevalent from June to February in the deep forest and foot-hill areas with a peak in the late moonsoon month of September.

Relationship of density to distance from forest : DMR survey team results show that there was definite relationship between An. dirus man-biting rate (mbr) and the distance of the catching station from the forest. Highest (mbr) was observed at Hti-phado wood cutting camp. Next highest rate was at Kyar-Kyaung-thaike (KKT) temporary settlement at the edge of forested foot-hills. At Ngok-to (NGT) village about 1/3 mile from the forest edge, An. dirus was collected at a lower density, while at Gwe-gone village (GG), situated about 1½ miles away from the forest the density of An. dirus recorded was very low. Similar situation exists for An. minimus in the same Phado area, Myo Paing et al (9).

Biting habits

(a) Host preference : Anopheles dirus was collected by DMR survey teams from both human and animal baited bed-nets and from human baits by direct hand catches. However, no An. dirus has ever been collected by CDC light traps by our teams. Host preference of An. dirus was studied at Phado by placing two big bed nets (11' X 11' X 6') about 150 feet apart with a house in between and placing two adult humans in one net and a cow in the other. The nets were operated from dusk till just before dawn. Results obtained showed that of the total An. dirus collected in both the nets, human-baited nets yielded 75-90%. This showed that in Phado, An. dirus was highly anthropophillic. At Tha-bye-wa village the ratio of An. dirus collected by animal baited net to human baited net was 4:46. However, a similar study conducted by one of the authors (W.T.L) at Mudon, showed An.dirus in that area to be more zoophillic although the breeding sites were situated very close to human dwellings.

(b) Biting rhythm : Anopheles dirus biting peak occurred in the 2nd and 3rd quarters of the night(2100 hours to 0300 hours). However, in the cool season its biting activity started earlier. In the early monsoon month of June 1984, An.dirus was caught biting man outdoors from about 0700 hours at Kat-se-ne Forest Extraction camp, Kyauk-ta-gar Township by one of the authors (AAS).

Place of biting

DMR survey teams observed An. dirus to be highly exophilic although not as much as An. minimus. In Phado, the indoor and outdoor biting activities were found to be almost equal during the cold months of November to February. In other areas, An. dirus was observed to be more exophilic in its biting behaviour.

Resting habits

Daytime indoor resting catches carried out longitudinally at Phado, did not yield any An dirus. DMR survey teams collected An. dirus adults resting on under-surfaces of banana leaves at Ye-sit-kan, Taik-kyi Township and in small crevices on the laterite walls of domestic wells in Mudon Town.

Relationship to malaria transmission

Anopheles dirus is regarded as a primary vector of malaria in Myanmar from dissection results and also from epidemio-logical evidences. During the longitudinal

surveys at Phado, out of a total of 147 An. dirus dissected, 2.7% (4/147) was found to be positive for sporozoites.

At Tha-bye-wa, An. dirus collected were not dissected as they were all brought back alive to DMR laboratory for cyto-genetic studies. The total number of An. dirus collected was 46 and the man biting rate was 4.6. At the time of this collection in July 1987, other malaria vectors like An. minimus and An. maculatus were caught in very small numbers. However, blood examination of villagers showed that the malaria incidence was very high, the slide positivity rate in children under 10 years of age being 66.7% and infant parasite rate 50%, Myo Paing et al (8). From this evidence, An. dirus could presumed to be an important vector of malaria in the area. Similar evidences also point out that An. dirus is an important vector in the timber extraction camps of Kawthaung Township.

Colonization of An. dirus in the laboratory

Anopheles dirus has been colonized in DMR laboratory since 1982 using the induced mating technique. The larval food used for the early instars were oatmeal, dry yeast. wheat gum and corn starch or Farex, and for the late instars, a mixed larval food containing Nestum or Lactogen, corn starch of Farex, ox liver powder and Vitamin B complex.

Laboratory biology of An. dirus

Nwe Nwe Yin (10) studied the biology of An. dirus under laboratory condition at the DMR.

Summary of findings are : -

(a) Larval density has a direct relationship to larval mortality. The permissable density for rearing was found to be 200 larvae per tray (size 350 mm long x 240 mm wide x 60 mm deep) and with 500 ml of water.The ideal density is found to be 50 larvae per tray.

(b) At a temperature of 26-32°C pupation starts on Day 7, attains maximum pupation on Day 8 and pupation is completed on day 9, with the females measuring 5.5 mm wing-length. During the cool season when the temperature is between 21-27°C, the larvae take a day longer to pupate, reaching maximum pupation on Day 9 only. However, the adult females obtained were larger, measuring 5.7-5.8 mm wing-length.

(c) Aquatic life span was observed to be as follows:

Larval Stage	Appr: Life span (Days)	
	Monson	Cool
I	2	3
II	1	2
III	1	2
IV	2	3
Pupae	2	3
Total Duration of Aquatic stages	8	13

(d) Highest biting potential is acheived on Day 4 after which the potential falls.

(e) 4 to 5 days old male and female An. dirus give the highest insemination rate. The mating ability of males increases with age till peak is reached on Day 4-5 after which the ability declines.

(f) Ovarian development do not commence within 6 hours of a blood meal. Egg follicles reach Christopher's Stage III-IV after a lapse of 22-26 hours and full development is reached between 44-50 hours after blood ingestion.

(g) Between the temperature 27-31°C, ovipositing females deposit their eggs after a time lapse of 55-56 hours after a blood meal.

(h) Average fecundity of An. dirus was found to be 116-134. About 77% of eggs hatch on day 1 and 90% within day 3 at a temperature of 27-31℃. The average viability of eggs is found to be 91%.

Susceptibility to control agents

D D T : Laboratory-bred An. dirus adult females (unfed) were exposed to 4% DDT discriminating dose at DMR laboratory (temperature 27-31℃). All the adults tested died within the exposure period while there was no control mortality.

Bacillus sphaericus : Anopheles dirus (Mudon strain) laboratory reared 4th instar larvae were exposed to Bacillus sphaericus 2362 and 2297. The LC_{50} & LC_{90} at 24 hours were found to be 0.086 & 0.96 ppm from 2362 and 0.52 & 14.0 from 2297 respectively. Thus, An. dirus was found to be very suspectible to B. sphaericus (Tin Tin Hlaing, personal communications).

Cytogenetic studies for differentiation of sibling species

Myat Myat Thu et al (11) examined two strains of An. dirus having different behaviour & breeding sources. Forest-breeding An. dirus from Taik-kyi was compared with domestic well-breeding An. dirus from Mudon by hybridization, polytene chromosome and iso-enzyme studies. Hybridization tests showed low male fertility in F_1 hybrids which also showed inversion in arm 3R & asynaptic segments in polytene chromosome. A distinct electrophoretic banding pattern difference was also noted, indicating genetic divergence between the two strains. Myat Myat Thu et al (12) compared An. dirus of Mudon with An. dirus of Kanchanaburi, Thailand. The results of hybridization and polytene chromosome studies showed that the two strain are most diverged among the species complex.

Cytogenetic study to differentiate the sibling species of An. dirus have been in progress at DMR. Prof. Visut Baimai of Mahidol University, Bangkok was invited as Consultant and also as a collaborating scientist. Anopheles dirus specimens collected from Tha-bye-wa, Oktwin township and Kwan-ka-thaung, Mudon town were found to be species D according to Baimai et al (13). In early 1989, specimens collected from near the Thailand border in Kawthaung township were also found to be species D, whereas species A was collected from Se-daw-gyi , Madaya township, Mandalay Division, (Myat Myat Thu, personal communications).

Specimens of An. dirus preserved at DMR

The following specimens of An. dirus were preserved at DMR for reference.

(a) Adult specimens
(b) Larval and pupal skin slides
(c) Polytene chromosome slides and photographs.

Colonies of An. dirus maintained at DMR

(a) An. dirus Mudon strain
(b) An. dirus Nam-Tun, Kaw-thaung

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