BREEDING AND DAY RESTING HABITATS OF *ANOPHELES DIRUS* IN ASSAM, INDIA

Anil Prakash, DR Bhattacharyya, PK Mohapatra and J Mahanta

Regional Medical Research Centre (ICMR), NE Region, Post Box No. 105, Dibrugarh - 786 001, Assam, India

Abstract. Longitudinal surveys at monthly frequency were carried out during 1995-1996 in a forest fringed village of district Dibrugarh, Assam to decipher the breeding and day resting habitats of *Anopheles dirus*. It regularly bred in small, shallow, rain filled, transient, shady or partly shady puddles/ground pools in the rainy months and in the perinneal streams in the adjoining forest of the village during dry months. In pools, the degree of interspecific association (0.238 ± 0.174) and index of association (0.428) of *An. dirus* breeding was highest with *Aedes caecus*. *An. dirus* was completely exophilic and rested during day time in the forest mostly on tree trunks upto the height of 4-5 feet from the ground in dark moist niches avoiding direct sun light.

INTRODUCTION

Anopheles dirus is a major vector of malaria in northeast India (Sen *et al*, 1973; Das and Baruah, 1985; Dutta *et al*, 1989a). However, most of the available work on *An. dirus* in India deals with either its incrimination (Das and Baruah, 1985; Dutta *et al*, 1989a, b) or feeding habits (Sen *et al*, 1973; Dutta *et al*, 1996) and a majority of them lack information on other aspects of the bionomics. Moreover, in the present global scenario of rapidly changing ecological and environmental conditions there is a need to monitor the bionomics of disease vectors on a regular basis. In view of this a longitudinal systematic study was conducted during 1995-1996 in a forest clearing village of district Dibrugarh, Assam to generate the updated information on various facets of the bioecology of *An. dirus*. This report contains our obsevations on breeding and day resting habitats of this vector.

MATERIALS AND METHODS

The year long study was carried out between August 1995 and July 1996 in a malaria endemic village, *viz* Soraipung under the direction of the primary health center Tengakhat, Dibrugarh District, Assam. The study village, situated at the fringes of a tropical rain forest with disturbed ecology, its topography, climate and people have been described elsewhere (Anil Prakash *et al*, 1997). Surveys of mosquito breeding and day resting habitats within the village and the adjoining forest areas were conducted in the third week of every month throughout the study. Searches in the forest were restricted to about 500 meters from the fringes due to operational and security reasons.

Breeding habitat surveys

All possible permanent and temporary mosquito breeding sites in and around the village and forest area were sampled with the help of either 300 ml capacity dipper or laddle or Pasteur pipette, depending upon the size of the habitat. From the positive habitats mosquito immatures were collected in separate wide mouthed plastic jars, brought to the laboratory and reared till emergence. Mortalily of immatures, if any, during rearing was ignored. Species emerging from each sample from different habitats were identified and recorded seperately.

The degree of interspecific association (C_{AB}) was calculated following Cole (1949). Value of C_{AB} is based on the joint occurrence of 2 species in samples of immatures and varied from +1 (for complete association) to -1 (for complete dissociation). Further, the index of association (I) was calculated by the method of Whittaker and Fairbanks (1958) as modified by Southwood (1966). This index also has a normal range of +1 to -1 and takes into consideration the number of individuals of 2 species occurring together.

Day resting collections

Both indoor and outdoor day resting mosquitos were collected between 6.00 and 9.00 hours by 3-4 experienced collectors. Indoor mosquitos from 8 randomly selected human dwellings and 2 cattle sheds in the village were collected with the help of suction tube and flashlights and their per man hour densities calculated. Outdoor resting mosquitos were collected from (i) ground level vegetation in the village as well as forest area with the help of a dropnet and (ii) various possible resting biotopes in the forest such as tree trunks, shrubs, fallen logs, tree holes, crab holes along the stream bank, etc by suction tube method. Mosquitos, thus collected, were identified, their abdominal condition recorded and *An. dirus*, if present, were dissected for parity and gland infection.

RESULTS

Breeding habitats

The study village was topographically situated in a plain area in a broken forest. Among the permanent breeding habitats there were a couple of small perennial streams passing through the village after emerging from the adjoining forest. In addition, most of the houses had a small pond for fish rearing and a kuccha shallow well for drinking water. Paddy fields, ground pools and small ditches were semipermanant/temporary, peridomestic breeding habitats available in and around the village. In none of these sites was the breeding of *An. dirus* detected in the village. Immatures of 9 anopheline and 11 culicine species which bred in the village included *Anopheles aconitus, An. barbirostris, An. hyrcanus* gp, *An. kochi. An. nivipes, An. philip-pinensis, An. splendidus, An. vagus, An. varuna, Culex bailyi, Cx. bitaeniorhynchus, Cx. fusco-cephalc, Cx. halifaxi, Cx. mimeticus. Cx. pseu-dovishnui. Cx. tritaeniorhynchus, Cx. vishnui, Orthopodomyia anopheloides, Aedes caecus,* and *Aediomyia* spp.

Stream and marshy areas were permanent, while puddles, ground pools, elephant footprints and treeholes were temporary breeding habitats in the forest area. Breeding of *An. dirus* was never detected in marshes or tree holes but was found in all other habitats at some point or the other during the study. Rain water filled transient, shady or partly shady ground pools/puddles, usually found in footprints of elephants or natural depressions in the ground, were the preferred breeding sites of *An. dirus* in most parts of the year with maximum species diversity (Table 1). Most of these pools with clear to muddy water were shallow (about 15-20 cm deep), frequently had rotton leaves at the bed and usually became dried up during a dry spell of 6-8 days indicating their transient nature. During cool months (November-December), however, such ground pools were not available and the breeding of *An. dirus* was detected in pools connected with the stream at its margins. The nearest breeding site of *An. dirus* was detected at a distance of about 200 meters from the village in a pool. The breeding of *An. dirus* was found to occur in association with 11 species. The degree (C_{AB}) and index of association (I) between *An. dirus* and other cohabiting species were calculated for jungle pools and elephant footprints (considered together); the values are given in Table 1. Of all species breeding in association with *An. dirus, Ae. caecus* had the highest degree ($+0.238 \pm 0.174$) and index of association ($+0.428$), while species like *An. vagus* and *Cx. bailyi* were negatively associated. The index of association or intensity of breeding of all species other than *Ae. caecus* recorded negative values with *An. dirus*.

Day resting habitats

In 27 man hours of indoor collection from human dwellings and 9 hours from cattle sheds during the study period, not a single resting *An. dirus* adult could be collected. However, on one occasion 6 freshly fed *An. dirus* females were found trapped inside a torn bednet from a house. Day resting mosquitos in human dwellings comprised mainly *An. vagus* (63.3%) and *Cx. quinquefasciatus* (34.3%) with PMHD of *An. vagus* varying from 34.5 (September) to 0.6 (December) and that of *Cx. quinquefasciatus* from 22.4 (October) to 2.0 (January).

Drop net collections, both in forest and village, failed to capture any resting *An. dirus* adult from the ground level vegetation and yielded mainly culicines, *An. barbirostris* and *An. hyrcanus* gp mosquitos.

Extensive scanning of various resting biotopes in the forest yielded 22 (8 males, 14 females) resting adults of *An. dirus* (Table 2). The majority of them (20 out of 22) were collected from the moist,

Table 1

Species and habitat association of immatures of *An. dirus* along with Degree and Index of interspecific association between *An. dirus* and other cohabiting species in jungle pools.

Species	Breeding habitats in forest				In forest pools	
	1	2	3	4	Degree of interspecific association (C_{AB})	Index of association (I)
Ae. caecus	X	X			+ 0.238 ± 0.174	+ 0.428
An. aconitus	X				+ 0.048 ± 0.038	- 0.422
An. hyrcanus group	X		X		+ 0.048 ± 0.038	- 0.826
An. kochi	X		X		+ 0.002 ± 0.091	- 0.700
An. vagus		X			- 0.032 ± 0.682	- 0.760
Cx. bailyi		X		X	- 0.025 ± 0.381	- 0.668
Cx. fuscocephala	X				- 0.032 ± 0.682	- 0.820
Cx. pseudovishnui	X				+ 0.048 ± 0.038	- 0.860
Cx. tritaeniorhynchus	X				+ 0.048 ± 0.038	- 0.648
Or. anopheloides	X				+ 0.048 ± 0.038	- 0.424
Uranotaenia spp	X				+ 0.048 ± 0.038	- 0.424

1 = Shady, transient puddle/ground pools
2 = Elephant footprints
3 = Drying pools in seasonal streams
4 = Pools connected with margins of slow moving streams

Table 2

Outdoor day resting habitats of *An. dirus*.

Resting niches in forest	Man hours searched	No. *An. dirus* collected					Total
		Males	Females				
			UF	FF	SG	G	
Tree trunks	45.0	8	8	2	2	0	20
Shrubs	6.0	-	-	-	-	-	-
Crab holes along stream bank	5.0	-	-	-	-	-	-
Creepers	7.0	-	-	2	-	-	2
Tree holes number	202	-	-	-	-	-	-
Felled logs	6.0	-	-	-	-	-	-

dark corners of the large tree trunks, avoiding direct sunlight, upto the height of 1.5 meters from the ground level. Only 2 females were found resting on a bunch of intermingled creepers at a height of 1.8 meters. The nearest resting *An. dirus* was found at a distance of about 150 meters from the village. Of the 14 day resting females, 8 were unfed, 4 full fed and 2 semigravid with 57.1% parity and none was sporozoite positive.

DISCUSSION

Colless (1956) described the typical breeding place of *An. dirus* as a small pool or group of pools under some degree of shade containing clear, still or with a barely perceptible flow water. Breeding of this species was reported from small depressions such as foot prints of man, elephant, buffalo and other animals in jungles of India (Clark and Choudhury, 1941; Dutta *et al*, 1989a) and well adapted to sustain life in temporary pools in the forest (Rosenberg, 1982). Present observations agree with these reports but differ with the reports of its breeding in containers (Ismail *et al*, 1974) or domestic wells (Tun-Lin *et al*, 1987). Breeding of *An. dirus* was detected in only a fraction of breeding habitats, suggesting that even under similar environmental conditions some habitats were more preferable than others, perhaps due to the ovipositional preferences of females as well as the capability of immature stages to withstand the environmental conditions in those habitats. A change in breeding sites of *An. dirus* with the season was noticed - while pools and puddles were the major breeding habitats during rainy months, its breeding shifted to pools connected with the slow moving stream at the margins in cool dry months. This situation was also reported from Bangladesh (Rosenberg, 1982) and Thailand (Wilkinson *et al*, 1978).

Ae. caecus was the most associated species, in terms of breeding frequency and intensity, with *An. dirus* indicating their common breeding preference for transient puddles/forest pools. Negative values of index of association (Table 1) pointed towards relatively low immatures densities of cohabiting species with *An. dirus*. This could possibly be due to predation by *An. dirus* on the associated species in temporary breeding habitats. Rosenberg (1982) demonstrated the predation of I and II instar larvae of other species by IV instar larvae of *An. dirus* in the laboratory, and attributed this behavior to nutrient scarcity in small transitory bodies of water, and thought it to be an adaptation of *An. dirus* to temporary breeding places. Interspecific association for vectors like *An. fluviatilis* (Gunasekaran *et al*, 1993) and *An. culicifacies* (Bhatt *et al*, 1990) have been recorded in the past. However, ours is perhaps the first report regarding interspecific association of *An. dirus* in India. This observation will be further elucidated in future.

An. dirus is an exophilic mosquito (Rosenberg, 1982), however, its day resting habitats are not properly elucidated. Day time search of resting mosquitos in the forest is labor intensive, tedious and poses several operational problems. There is only one report (Sen *et al*, 1973) of collection of 1 specimen of *An. balabacensis* from a stream bank after 216 hours of outdoor searching in Tirap district of Arunachal Pradesh. Considering this, the present work provides a satisfactory account of day time resting habitats of *An. dirus*, perhaps for the first time in India. *An. dirus*, in conformity with other reports (Colless, 1956; Ismail *et al*, 1973; Sen *et al*, 1973; Dutta *et al*, 1989a) was exophilic, and we found them taking shelters during day time on the tree trunks up to the height of 1.5 meters from the ground in the forest not very far from the village. Collection of good numbers of females of *An. dirus* in unfed, fullfed and semigravid stages on tree trunks in the forest indicates its preferred natural day resting shelter in Assam in contrast to deserted anthills in West Malaysia (Sloff and Verdrager, 1972), gem mining pits in Thailand (Scanlon and Sandhinand, 1965), shaded mud cavities or holes along canal banks in Bangladesh (Rahman *et al*, 1977). These observed differences in natural day resting shelters may be attributed to the most suitable resting niches available in an area or perhaps a different sibling species. Of course this needs further investigations.

Control of *An. dirus* poses a serious challenge due to its behavior, adaptability and operational difficulties. The vastness and scattered nature of its breeding habitats, largely in inaccesible areas, makes the prospects of larval control, either chemically or by source reduction, unattractive and uneconomic. Similarly its exophilic nature help avoiding the fatal contact with the sprayed walls, thus rendering residual insecticides ineffective. This situation demands specific and effective ways of reducing man-mosquito contact by personal protection methods. The bednet usage in Northeastern states of India is fairly high (RMRC, unpublished data) and insecticide impregnated bednets may be useful in controlling *An. dirus* transmitted malaria. The limited flight range ($\leq$ 500 m) of this mosquito (Colless, 1956; Kalra, 1989) may also be exploited for its control by clearing the forest greater than its flight range around the villages as suggested by Kalra (1991).

ACKNOWLEDGEMENTS

We sincerely acknowledge the technical help rendered by Mr PK Doloi, AC Rabha and Raju Sonowal during the study.

REFERENCES

Anil Prakash, Bhattacharyya DR, Mohapatra PK, Mahanta J. Seasonal prevalence of *Anopheles dirus* and malaria transmission in a forest fringed village of Assam, India. *Indian J Malariol* 1997 (In press).

Bhatt RM, Sharma RC, Kohli VK. Interspecific associations among anophelines in different breeding habitats of Kheda district, Gujrat Part I : Canal irrigated area. *Indian J Malariol* 1990; 27 : 167-72.

Clark RHP, Choudhury MA. Observations on *Anopheles leucosphyrus* in the Digboi area, Upper Assam. *J Mal Inst India* 1941; 4 : 103-7.

Cole LC. Measurement of interspecific association. *Ecology* 1949; 30 : 411-24.

Colless DH. The *Anopheles leucosphyrus* group. *Trans R Entomol Soc Lond* 1956; 108 : 37-116.

Das SC, Baruah I. Incrimination of *Anopheles minimus* Theobald and *Anopheles balabacensis* Baisas (*An. dirus*) as malaria vectors in Mizoram. *Indian J Maluriol* 1985; 22 : 53-5.

Dutta P, Bhattacharyya DR, Sharmah CK, Dutta LP. The importance of *Anopheles dirus* (*A. balabacensis*) as a vector of malaria in Northeast India. *Indian J Malariol* 1989a; 26 : 95-101.

Dutta P, Bhattacharyya DR, Dutta LP. Incrimination of *Anopheles dirus* as a vector of malaria in Dibrugarh district, Assam. *Indian J Malariol* 1989b; 26 : 149-52.

Dutta P, Bhattacharyya DR, Khan SA, Sharmah CK, Mahanta J. Feeding pattern of *Anopheles dirus*, the major vector of forest malaria in Northeast India. *Southeast Asian J Trop Med Public Health* 1996; 27 : 378-81.

Gunasekaran K, Jambulingam P, Das PK. Interspecific association and Index of association of *Anopheles fluviatilis* James in the breeding habitats in hill tract of Koraput district, Orissa. *J Com Dis* 1993; 27 : 156-63.

Ismail IAH, Notananda V, Schepens J. Studies on malaria and responses of *Anopheles balabacensis* and *Anopheles minimus* to DDT residual spraying in Thailand. Part I. Pre-spraying observations. *Acta Trop* 1974; 31 : 129-64.

Kalra NL. Forest malaria vectors in India : Ecological characteristics and epidemiological implications. In: Sharma VP, Kondrashin AV, eds. Forest malaria in Southeast Asia. Proceedings of an informal consultative meeting. WHO/MRC, New Delhi, 1991: 93-114.

Rahman M, Elias MD, Ameen MU. Bionomics of *Anopheles balabacensis balabacensis* Baisas (Diptera: Culicidae) in Bangladesh and its relation to malaria. *Bangladesh J Zool* 1977; 5 : 1-23.

Rosenberg R. Forest malaria in Bangladesh III. Breeding habits of *Anopheles dirus*. *Am J Trop Med Hyg* 1982; 31 : 192-201.

Scanlon JE, Sandhinand V. The distribution and biology of *Anopheles balabacensis balabacensis* in Thailand. *J Med Entomol* 1965; 2 : 61-9.

Sen AK, John VM, Krishnan KS, Rajagopal R. Studies on malaria transmission in Tirap district, Arunachal Pradesh (NEFA). *J Com Dis* 1973; 5 : 98-110.

Sloff R, Verdrager J. *Anopheles balabacensis balabacensis* Baisas 1936 and malaria transmission in south eastern areas of Asia. WHO/MAL/72. 765. (Mimeographed document) 1972 : 1-26.

Southwood TRE. Ecological methods, with particular reference to study of insect population. London Methuen : 1966; 1-391.

Tun-Lin W, Htay-Aung, Moe-Moe, Sebastian A, Myopaing, Myat-Myat-Thu. Some environmental factors influencing the breeding of *Anopheles balabacensis* complex *(dirus)* in domestic wells in Burma. *J Com Dis* 1987; 19 : 291-9.

Whittaker RH, Fairbanks CW. A study of plankton copepod communities in the Columbian basin, south eastern Washington. *Ecology* 1958; 30 : 46-65.

Wilkinson RN, Gould DJ, Boonyakanist P, Segal HE. Observations on *Anopheles balabacensis* (Diptera: Culicidae) in Thailand. *J Med Entomol* 1978; 14 : 666-71.