SOME OBSERVATIONS ON SYMPATRIC POPULATIONS OF THE MALARIA VECTORS *ANOPHELES LEUCOSPHYRUS* AND *ANOPHELES BALABACENSIS* IN A VILLAGE-FOREST SETTING IN SOUTH KALIMANTAN

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INTRODUCTION

The *Anopheles leucosphyrus* group of mosquitoes includes several serious vectors of human malaria in the forested areas of Southeast Asia. *Anopheles dirus* Peyton and Harrison, itself a complex of at least four distinct biological species (Baimai *et al.* 1984), is a primary vector on the Southeast Asian mainland. *Anopheles leucosphyrus* Dönitz and *An. balabacensis* Baisas are principal vector species in parts of Indonesia and Malaysia. According to Colless (1956b, 1957) and Reid (1968), *leucosphyrus* occurs in Sumatra, the Malay Peninsula, Sarawak and Kalimantan. Ramalingam (1974) reported the occurrence of this species in Sabah. *Anopheles balabacensis* is known from Palawan and Balabac Islands (Philippines), Sabah, Brunei, the extreme north of Sarawak and western Java. Populations of the latter species are also known to occur in East and South Kalimantan (Kirnowardoyo, 1985). The exact distributions of these species in Kalimantan are unknown.

Anopheles leucosphyrus is a vector of human malarial parasites in Sumatra (Doorenbos, 1931) and Sarawak (de Zulueta, 1956; de Zulueta and Lachance, 1956). *Anopheles balabacensis* has been incriminated in Sabah (McArthur, 1947, 1950; Colless, 1952; Hii, 1985b). Stoker (1934) and Goelarso (1934) found naturally infected specimens in East Kalimantan which could have been either *leucosphyrus* or *balabacensis*. Our present knowledge of the biology and importance of these species in relation to malaria transmission is very limited. This knowledge is based on studies of *leucosphyrus* in Sarawak (Colless, 1956a; de Zulueta, 1956; de Zulueta and Lachance, 1956) and *balabacensis* in Brunei and Sabah (McArthur, 1947; Colless, 1950, 1952, 1953; de Zulueta, 1956; Hii, 1985b). There has been no published report about these species in areas where they occur together. In September 1986, we had the opportunity to make some observations on sympatric populations while collecting specimens in a remote area of South Kalimantan for cytogenetic studies of *leucosphyrus* in Southeast Asia and the development and testing of DNA probes for the identification of members of the *leucosphyrus* group in Thailand (Panyim *et al.*, 1985). Additionally, we were able to test many of the specimens for the presence of sporozoites using enzyme-linked immunosorbent assays (ELISA) for *Plasmodium falciparum* and *P. vivax*. Our observations are reported herein as they may be of interest to workers engaged in studies on the ecology and control of malaria vectors in Kalimantan.

The views of the authors do not purport to reflect the positions of the supporting agencies.

MATERIALS AND METHODS

Collections were made at Salaman, an isolated community of 564 persons located about 45 km east of Peleihari and approximately 15 km northwest of Kintap in Tanah Laut Regency, South Kalimantan, Indonesia. The general area is hilly and densely forested. Much of the village area is overgrown with secondary flora interspersed by small cultivated areas and wooded stretches along gullies and streams. A few areas are used for grazing small numbers of cattle and water buffalo. The vegetation changes abruptly from secondary growth to tall trees at the edge of the village clearing. Villagers live in wooden huts and houses mainly scattered along the road and major streams some 200-500 m from the forest.

Human bait collections were made on five consecutive nights between 19 and 24 September 1986 by two teams each consisting of a supervisor and four collectors. Collectors worked in pairs outside houses located at the south end of the village. On two nights, one team was taken into the forest near the north end of the village where three men collected on the forest floor while one man collected on a platform built in a tree about 18m above the ground. Collections were made from 2000 to 0600 hours. This period was chosen because the feeding activity of *leucosphyrus* and *balabacensis* usually commences between 2000 and 2100 hours and continues until dawn (Colless, 1956b).

Our primary aim at Salaman was to collect blooded females, maintain them alive and take them to Bangkok for egg laying and colonization. All mosquitoes were allowed to engorge before being removed in glass vials. Supervisors gathered the vials hourly and marked each with the hour of collection. On the following morning, the specimens were identified, transferred to paper cups with screen tops, provided with a wad of cotton moistened with a sucrose-vitamin solution and placed in a cool, humid environment. The cups were checked periodically and any dead mosquitoes were removed, examined to confirm the initial species determination and placed on filter paper in small petri dishes to dry. Specimens that reached Bangkok alive were placed in oviposition vials upon arrival. When these specimens died, they were reidentified and allowed to dry. Dried specimens were analysed for sporozoite infections of *P. falciparum* and *P. vivax* using the ELISA developed by Burkot *et al.*, (1984) and Wirtz *et al.*, (1985). Specimens were assayed individually. Mosquito extracts which gave mean optical density readings at least twice as high as the lowest mean positive reference control that exceeded the highest mean negative control were defined as positive. Some of the dead specimens were mounted on pins and retained as voucher specimens.

South Kalimantan has two seasons, a rainy season which lasts from November to May and a period of little rainfall from June to October. Salaman was entirely dry when we arrived. Larval surveys were made on the first three days, but few larval habitats were found because of the dry conditions. Surveys were limited mainly to the village and forest fringe, but three short excursions were made into the forest along streams. Unexpected torrential rains and flooding on the fourth and fifth days prevented additional surveys.

RESULTS

A total of 217 anopheline females belonging to six species were captured outside houses at Salaman during 32 man-nights of collecting (Table 1). *Anopheles leucosphyrus* and *An. balabacensis* were by far the dominant species, comprising 51.6 and 46.1 % of the total catch, respectively. Specimens of *An. minimus* Theobald, *An. kochi* Dönitz, *An. peditaeniatus* (Leicester) and *An. vanus* Walker were col-

lected in much smaller numbers and accounted for only 2.3% of the catch. Each collector received an average of 3.5 bites per night from *leucosphyrus* and 3.1 from *balabacensis*.

Table 1

Numbers of *Anopheles* females caught biting humans outside houses during five nights at Salaman (19-24 September 1986).

Species	Catch	Bites/ man-night
An. leucosphyrus	112	3.5
An. balabacensis	100	3.1
An. kochi	1	0.03
An. minimus	1	0.03
An. vanus	2	0.06
An. peditaeniatus	1	0.03

The overall biting activity of the *leucosphyrus* and *balabacensis* captured outside houses is compared in Fig. 1. The limited data indicate that the biting patterns of the two species are similar. Both species commenced to bite shortly before 2100 hours and continued to bite thoughout the night. The biting activity of *leucosphyrus* was greatest between 2000 and 0300 hours while the maximum attack rates for *balabacensis* occurred between 2100 and 0400 hours. The biting activity of both species fell to a low level before dawn. *Anopheles balabacensis* appeared to have a higher peak of activity between 2100 and 2300 hours.

Small numbers of anophelines other than *leucosphyrus* and *balabacensis* were caught during the human bait collections. To see if other species were feeding on non-human hosts, resting collections using a water buffalo as bait were made on two occasions. One collection was made in a grazing area with considerable scrubby secondary vegetation. The second collection was made in a clump of trees beside an abandoned house. Only four engorged *An. vanus* were captured during two hours of collecting between the

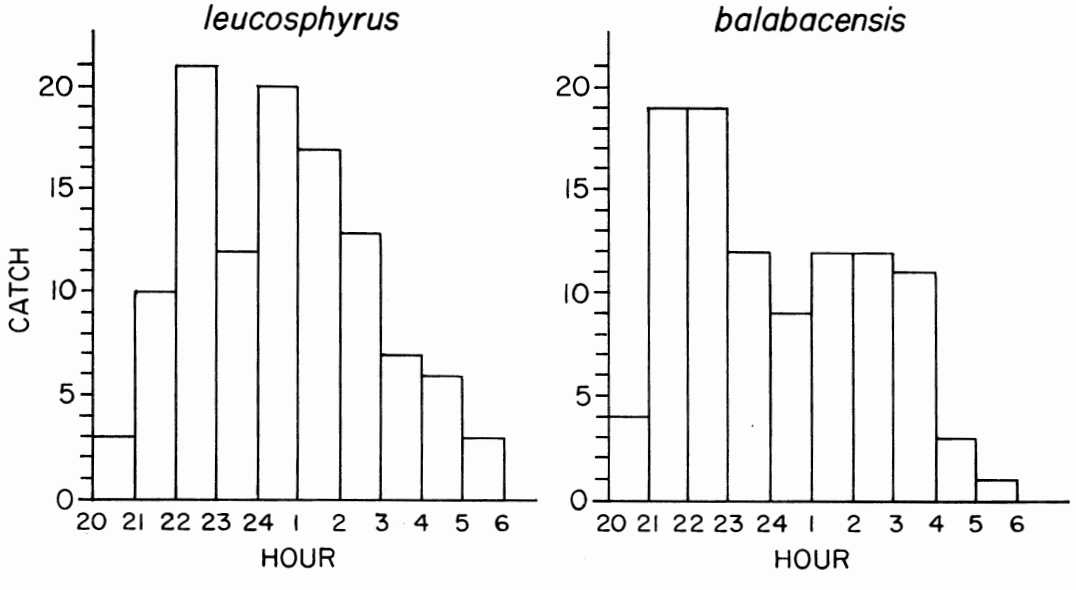

Fig. 1—Comparison of the numbers of *An. leucosphyrus* and *An. balabacensis* caught during five nights at Salaman (19-24 September 1986).

hours of 2100 and 2300 at each of the sites (total of four hours). Collections were curtailed at 2300 hours because of the paucity of anophelines encountered.

Very few specimens of *leucosphyrus* and *balabacensis* were collected by four men working in the forest on two nights (Table 2). One man collected as many specimens on the platform as the other three collected on the forest floor, suggesting that these species bite more frequently in the canopy. The two species were caught in equal numbers on the ground and the platform. Three times as many *leucosphyrus* and *balabacensis* (each species) were collected by the same number of men working outside houses in the village. A small number of specimens belonging to the genera *Mansonia*, *Coquillettidia* and *Culex* were collected in the forest, primarily in the canopy, which were not encountered in the village.

Table 2

Comparison of the numbers of mosquitoes caught during two nights in the village and forest at Salaman (21-23 September 1986).

Mosquito sp.	Village	Forest	
		Floor	Canopy
An. leucosphyrus	12(3)*	3(1)	1(1)
An. balabacensis	13(3.3)	1(0.3)	3(3)
An. vanus	2(0.5)	5(1.7)	0
An. peditaeniatus	1(0.3)	0	0
Mansonia sp.	0	1(0.3)	4(4)
Coquillettidia sp.	0	0	2(2)
Culex sp.	0	0	1(1)
Totals	28(7)	10(3.3)	11(11)

* Numbers in parentheses indicate the number of specimens caught per man.

Slightly more than 75% of the *leucosphyrus* (85 specimens) and *balabacensis* (78 specimens) captured outside houses were assayed for plasmodial infections. One specimen of each species was strongly positive for *P. falciparum*. None of the specimens were positive for *P. vivax*. The infectivity rate, based on the number of specimens assayed, was 1.0% for *leucosphyrus* and 1.3% for *balabacensis*.

Only 12 larval collections were made in and around the village. Seven of these contained anopheline larvae. *Anopheles vanus*, the predominant species, was found in four collections. *Anopheles kochi*, *An. umbrosus* (Theobald) and *An. montanus* Stanton and Hacker were found in smaller numbers in three different collections (*umbrosus* and *montanus* occurred together in one collection). *Anopheles leucosphyrus* and *An. balabacensis* were not represented in any of the collections, including two which were made in the forest, one along a stream margin and the other in a small subterranean pool. The collections contained several species of *Culex* and a species of *Uranotaenia*.

DISCUSSION

Personnel of the Indonesian Department of Health engaged in malaria detection and mosquito control recognize the presence of *balabacensis* in East and South Kalimantan (Kirnowardoyo, 1985), but do not distinguish *leucosphyrus* from this species. There is no doubt that both species occur in South Kalimantan. Our identifications were made using the wing and leg characters denoted by Colless (1956b; 1957) and verified during a current comprehensive taxonomic revision of the *leucosphyrus* group in Southeast Asia (E.L. Peyton, personal communication). To further ascertain the reliability of these characters, we examined the chromosomes of progeny raised from six *leucosphyrus* and 29 *balabacensis* (no other specimens survived long enough to lay eggs) and compared the chromosomal identifications with the original identification of each parent. There was total

agreement between the two means of identification. Chromosomal identifications were checked against the standard arrangement of the polytene chromosomes of *balabacensis* from Sabah (Hii, 1985a) and the mitotic brain chromosomes of *leucosphyrus* collected by us in central Sumatra (unpublished observations).

Colless (1956b) stated that the nocturnal biting patterns of *leucosphyrus* and *balabacensis* are essentially identical, with a peak of activity occurring after midnight. This generalization is based entirely on observations made on *leucosphyrus* in Sarawak (Colless, 1956a) and on *balabacensis* in Sabah (McArthur, 1948; Colless, 1956b). Until now, nothing has been published on the biting behavior of sympatric populations. The results of the present study indicate that there is little if any difference in the overall biting patterns of these species in areas where they occur together. Peak periods of activity for *balabacensis* are known to occur anytime between 2100 and 0500 hours on individual nights (Colless, 1956b). Variation in the time of peak biting is further illustrated by Khoon (1985) who reported that allopatric populations of these species in Sarawak (*balabacensis* occurs in the far north) are most active around midnight, while populations of *balabacensis* in Sabah exhibit peak biting between 2100 and 0100 hours. Feeding activity probably varies in response to the ecological, seasonal and meteorological conditions which exist in different geographical areas.

Under natural conditions, members of the *leucosphyrus* group of species are thought to feed primarily on primates in the forest. In Peninsular Malaysia, *leucosphyrus* prefers to feed in the forest canopy (Macdonald and Traub, 1960; Wharton *et al.*, 1964), and this is probably also true of *balabacensis* since the closely related *An. dirus* exhibits a similar preference on the Southeast Asian mainland (Eyles *et al.*, 1964; their *balabacensis*). For this reason, we expected to collect far more specimens in the forest than were actually collected there.

Both *leucosphyrus* and *balabacensis* avidly attack man at Salaman where they undoubtedly play a principal role in malaria transmission. Lower sporozoite infection rates are reported here than were reported previously for *leucosphyrus* in Sarawak (de Zulueta and Lachance, 1956; de Zulueta, 1957) and for *balabacensis* in Sabah (McArthur, 1950; Colless, 1952). We feel that a detailed entomological and parasitological investigation is needed to improve our knowledge of malaria transmission not only at Salaman, but also generally throughout Kalimantan.

SUMMARY

Human bait collections of biting anopheline mosquitoes were made on five consecutive nights during September 1986 in a remote village located in a heavily forested area of South Kalimantan, Indonesia. *Anopheles leucosphyrus* and *An. balabacensis* comprised 97.7% of the total number of specimens collected outside houses in the village. *Anopheles balabacensis* were slightly fewer in total numbers than *leucosphyrus*. Mosquitoes were collected simultaneously in the village and the forest on two nights. The numbers of *leucosphyrus* and *balabacensis* biting in the forest were small in comparison with the populations encountered in the village. Approximately 75% of the specimens were checked individually for sporozoite infections using ELISA for *P. falciparum* and *P. vivax*. Sporozoites of *P. falciparum* were detected in one specimen of *leucosphyrus* and one of *balabacensis*. The sporozoite infection rate was 1.0% for *leucosphyrus* and 1.3% for *balabacensis*.

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