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Anopheline fauna and incriminatory malaria vectors in malaria endemic areas on Lombok Island, Indonesia

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Abstract: A systematic mosquito collection was carried out for 3 years from November 2001 to September 2004 in western Lombok Island, Indonesia to clarify *Anopheles* fauna, and to confirm vector species in malaria endemic areas. Adult mosquitoes were collected at 14 sites in the study area by using double-walled mesh nets with human or cow bait. A total of 11 species were encountered. *Anopheles vagus* was the most predominant. The second most abundant species differed among the sub-study areas; *An. sundaicus* was abundant in the coastal plain area, and *An. balabacensis* in the mountainous area. *Anopheles balabacensis* showed high anthropophily and exophagy and *An. sundaicus* moderate anthropophily and exophagy. Malaria parasite detection from the collected mosquitoes was also carried out through the detection of circumsporozoite protein by the VecTest™. Fourteen and 4 samples, which were positive for *Plasmodium falciparum* and *P. vivax* antigen respectively, were found from *An. subpictus*, *An. sundaicus* and *An. balabacensis*. We conclude that malaria in the coastal plain area is transmitted by *An. sundaicus* and *An. subpictus*, whereas *An. balabacensis* is the primary vector in the mountainous area of Lombok Island.

Key words: *Anopheles* fauna, malaria vector, *An. balabacensis*, *An. sundaicus*, biting behavior, Lombok Island

INTRODUCTION

Malaria is still a major public health problem in Indonesia with 6 million clinical cases and 700 deaths each year (Laihad, 2000). There are 80 *Anopheles* mosquito species, and 24 species are potential malaria vectors in Indonesia (Sallum et al., 2005). Seven *Anopheles* species; *An. sundaicus*, *An. subpictus*, *An. aconitus*, *An. balabacensis*, *An. barbirostris*, *An. punctulatus* and *An. minimus*, are listed as important malaria vectors (Kirnowardoyo, 1985). The risk of malaria transmission exists throughout the year in the whole country except in Jakarta municipality, big cities, and areas of tourist resorts such as Bali and Java (WHO, 2005). In the outer islands, a higher incidence of malaria is reported (Dachlan et al., 2005). However mosquitoes of these islands including Lombok Island have not been well documented (Lee et al., 1984).

Five *Anopheles* species; *An. subpictus*, *An. maculatus*, *An. barbirostris*, *An. sundaicus* and *An. aconitus*, are suspected as malaria vectors in Lombok Island, and *An.*

sundaicus and *An. subpictus* found in lagoons and fish ponds along the coastal areas are suggested as the most important vectors (Yotopranoto et al., 1996; Gebrak Malaria Team in West Lombok, 2000). Forest malaria vectors in Southeast Asia, such as *An. maculatus*, *An. minimus* and *An. leucosphyrus* group, are also potential malaria vectors (Miyagi et al., 1994). However, because of short study period, details about the breeding patterns, feeding preference and the local distribution of *Anopheles* mosquitoes on Lombok Island are still unclear. The role of these potential vectors in local malaria transmission is also unknown. Therefore, we conducted systematic mosquito collections for 3 years from November 2001 to September 2004 on western Lombok Island to clarify *Anopheles* fauna and confirm vector species in the malaria endemic areas.

Materials and Methods

Study area

Adult mosquito collections were conducted in the Batulayar subdistrict, Meninting County, Lombok Island, Indonesia (Fig. 1). The entire study area covered approximately 10 km south-north and 8 km east-west, with the western edge facing Lombok strait. Two seasons, the dry season from June to October and rainy season from November to March, occur in the study area. Hills and dense forests characterize the northern and eastern part of the study area, while rice fields, schools, residential areas and markets are located in the southern part. Based on the number of dry months and rainfall, 5 vegetation types are distinguished for Nusa Tenggara (Monk et al., 1997). The study area was divided into 4 sub-study areas based on vegetation type, distance from the sea coast and altitude: coastal plain area, coastal hilly area, plain area and mountainous area (Table 1). Characteristics of each sub-study area are briefly described below.

Coastal plain area and coastal hilly area:

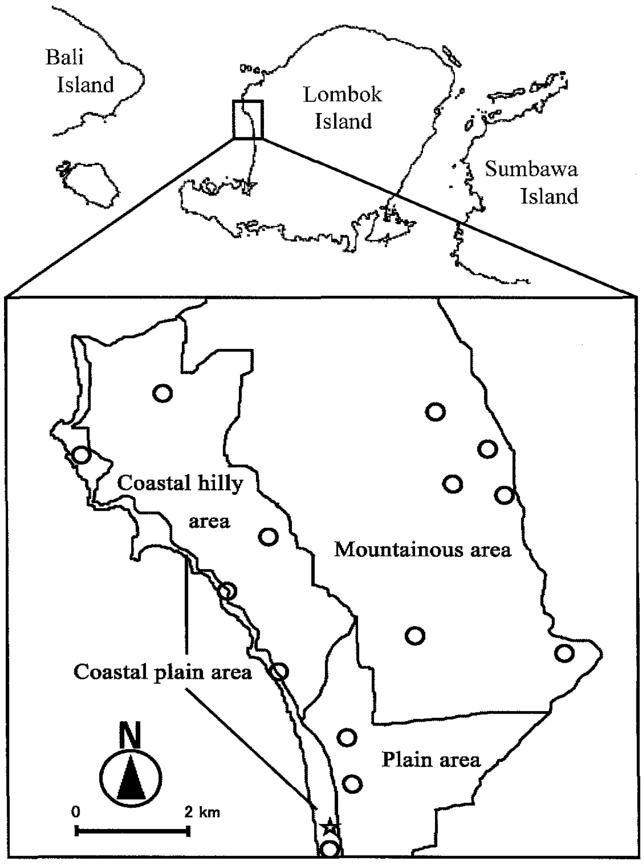

Fig. 1. Map showing the 4 sub-study areas and adult collection sites (○) in Meninting County, Lombok Island, Indonesia. ☆=S8°32′28″, E116°04′17″.

these areas are located in the western part of the county. The hilly part of this area is near to the seashore and the plain is narrow. Villages are situated within 1 km from the sea coast. Domestic animals found around the houses include cows, horses, goats, chickens, dogs and cats. Cattle are often found grazing on the plain along the coast.

Plain area: the southern area stretches over the alluvial plain. Rice fields, various shops, markets, schools and public offices are found in the populated areas. Domestic animals such as cows, horses, goats, chickens, dogs and cats are common in the villages.

Mountainous area: a densely forested area (approximately 600 m elevation). The tree canopy is thick and various fruit trees such as durian, banana, jackfruit are encountered around the area. Animals in the area include wild birds, chickens, dogs,

Table 1. Vegetation type, distance from the sea coast and altitude of the 4 sub-study areas examined.

Sub-study area	Vegetation type	Distance from sea coast	Altitude
Coastal plain area	Dry deciduous forest	0–1 km	0–50 m
Coastal hilly area	Moist deciduous forest	1–3 km	50–250 m
Plain area	Dry deciduous forest	0.5–4.5 km	10–100 m
Mountainous area	Moist deciduous forest	>2 km	100–600 m

cats as well as two species of black and gray wild monkeys but cows are rather scarce. Houses are distributed sparsely from the side to the top of the mountain. No transportation is available so the area is accessible only by foot.

Mosquito collection

Human and cow baited collections using double-walled mesh nets were carried out at 14 sites in the study area from November 2001 to September 2004. Mosquito collections were done 3 days a week every month. Except for the mountainous area, mosquitoes were collected hourly from 18 : 00 to 24 : 00. In the mountainous area, a whole night mosquito collection (from 18 : 00 to 6 : 00) was carried out.

For human baited indoor and outdoor collections a small double-walled mesh net was used. Two villagers sat inside the inner net (1.2 by 1.2 by 2 m height), and mosquitoes trapped between walls of the inner and outer nets (3.2 by 3.2 by 2 m height) were collected with an aspirator for 45 minutes hourly. For the cow baited collections, a double-walled mesh net of larger size (inner net=4 by 4 by 2 m height, outer net=6 by 6 by 2 m height) was used. A cow was tethered at the center of the inner net from 17 : 00 to midnight or 6 : 00 the next morning. Mosquitoes found between the two walls and on the outside wall of the large net were collected hourly for 15 min each time by 3 collectors. Collected mosquitoes were kept in plastic cups and labeled with the date, hour, site and bait, and then carried to the laboratory for identification and dissection.

Mosquito identification and detection of malaria antigen from the mosquitoes

All anopheline mosquitoes were identified to species using identification keys by Reid (1968) and Peyton and Scanlon (1966). Based on the literature the following 5 anopheline species, *An. balabacensis*, *An. sundaicus*, *An. subpictus*, *An. flavirostris* and *An. maculatus* s. l. were selected as suspected vectors. For the suspected vectors a part of the collected females (48–72%) were used for detection of specific peptide epitopes (Pf, Pv 210 and Pv 247) of circumsporozoite proteins of 3 types of *Plasmodium* sporozoites by the VecTest (Medical Analysis Systems, Inc.) from June 2002 to September 2004. Heads and thoraxes of female *Anopheles* mosquito samples of the same species (individuals or pooled less than 20 individuals) were used in the examination. The suspected vector species showed a higher biting density in the dry season, so 5 other anopheline species common in the rainy season were additionally selected, namely *An. annularis*, *An. tessellatus*, *An. indefinitus*, *An. barbirostris* and *An. vagus*, and a total of 50 pools (204 females) were checked for malaria antigens by the VecTest during the rainy season of 2003.

Data analysis

Density of *Anopheles* mosquitoes, represented as the number of mosquitoes/cow/half night or the number of mosquitoes/2 men/half night, was averaged and compared among sub-study areas by ANOVA or t-test with log transformations, log (number of mosquitoes+1). Means and standard deviations were back transformed and are shown in the tables. A pair wise comparison of means

Table 2. Total number (upper) and average number of each *Anopheles* per collection (lower) at sub-study areas from November 2001 to September 2004.

Host animal	Site	Area	Species											Total
			vag	*sund*	*ind*	*tesse*	*sub*	*bal*	*bar*	*ann*	*flav*	*koc*	*mac*	
Cow (/cow/half night)	outside	*cp*	57,698	2,574	2,560	1,311	1,018	0	680	367	449	217	158	67,032
		m	5,159	2	135	134	13	160	56	19	83	149	179	6,089
		p	5,325	0	252	185	33	0	11	234	0	29	0	6,069
		ch	358	1	6	3	1	0	0	1	23	0	29	422
Human (/2men/half night)	outdoor	*cp*	216	450	38	13	39	0	14	10	3	0	2	785
		m	129	2	4	3	2	893	4	3	35	2	18	1,095
		p	7	0	0	0	0	0	0	0	0	0	0	7
		ch	2	0	0	0	1	0	0	0	2	0	0	5
	indoor	*cp*	45	115	7	3	14	0	1	1	0	0	0	186
		m	13	0	0	0	0	3	0	0	4	0	0	20
		p	1	0	0	1	0	0	1	0	0	0	0	3
		ch	1	0	0	0	0	0	0	0	0	0	0	1
		Total	68,954	3,144	3,002	1,653	1,121	1,056	767	635	599	397	386	81,714
Cow (/cow/half night)	outside	*cp*	363±537a	16.1±46.2a	16.1±30.6a	8.3±19.3ab	6.4±24.3a	0	4.3±10.6a	2.3±7.9a	2.8±10.2a	1.4±3.7a	1±2.9a	
		m	32.9±128b	0.01±0.2b	0.9±6.8b	0.9±4.6b	0.1±0.6b	1±1.7	0.4±0.9b	0.1±0.7a	0.5±2.2b	1±5.2a	1.1±3.2a	
		p	592±1011a	0	28.0±42.8a	20.6±36.1a	3.7±7.3ab	0	1.2±1.4ab	26±51.2b	0	3.2±6.8	0	
		ch	22.4±30.6b	0.1±0.3ab	0.4±0.7ab	0.2±0.5b	0.1±0.3ab	0	0	0.1±0.3a	1.4±2ab	0	1.8±4a	
Human (/2men/half night)	outdoor	*cp*	1.9±7.9a	3.9±15.2a	0.3±1.3a	0.1±0.6a	0.4±1.5a	0	0.13±0.5a	0.1±0.8a	0.03±0.2a	0	0.02±0.1a	
		m	0.4±1.8b	0.01±0.1b	0.01±0.2b	0.01±0.1b	0.01±0.1b	2.8±4	0.01±0.1b	0.01±0.1a	0.1±0.5a	0.01±0.1	0.1±0.4a	
		p	0.8±1.6ab	0	0	0	0	0	0	0	0	0	0	
		ch	0.1±0.3ab	0	0	0	0.1±0.3ab	0	0	0	0.1±0.3a	0	0	
	indoor	*cp*	0.4±1.2a	1.1±4.4	0.1±0.3	0.03±0.2a	0.1±0.5	0	0.01±0.1a	0.01±0.1	0	0	0	
		m	0.2±0.7a	0	0	0	0	0.1±0.2	0	0	0.08±0.3	0	0	
		p	0.1±0.3a	0	0	0.11±0.3a	0	0	0.1±0.3b	0	0	0	0	
		ch	0.1±0.2a	0	0	0	0	0	0	0	0	0	0	

vag=*Anopheles vagus*; *sund*=*An. sundaicus*; *ind*=*An. indefinitus*; *tesse*=*An. tessellatus*; *sub*=*An. subpictus*; *bal*=*An. balabacensis*; *bar*=*An. barbirostris*; *ann*=*An. annularis*; *flav*=*An. flavirostris*; *koc*=*An. kochi*; *mac*=*An. maculatus* s.l.

Means from the same collection method in the same colimn followed by the same letter were not significantly different ($p<0.05$, Tukey's HSD test).

Area=sub-study area; *cp*=coastal plain area; *m*=mountainous area; *p*=plain area; *ch*=coastal hilly area.

was conducted using Tukey's HSD test. All statistical analyses were performed using Systat statistical software.

To evaluate the degree of anthropophily and exophagy of anopheline mosquitoes, ratio of cow to outdoor human mean biting density and ratio of outdoor to indoor human mean biting density was calculated, respectively.

Results

Comparisons of species composition among sub-study areas

During the study period, a total of 81,714 *Anopheles* mosquitoes consisting of 11 species were collected from outside cow baited, and outdoor and indoor human baited collections. The average densities of these species were calculated for 4 sub-study areas (Table 2). The species composition and abundance differed among the sub-study areas. Among 11 species, *An. vagus* was the most abundant in cow baited collections in all sub-study areas composing >85% of the total. The second most abundant species differed among the sub-study areas; *An. sundaicus* in the coastal plain area, *An. balabacensis* in the mountainous area, *An. indefinitus* and *An. annularis* in the plain area, and *An. flavirostris* and *An. maculatus* s.l. in the coastal hilly area. The following 3 species, *An. balabacensis*, *An. subpictus* and *An. sundaicus* showed a concentrated distribution. *Anopheles balabacensis* was distributed only in the mountainous areas, whereas >95% of *An. subpictus* and *An. sundaicus* were collected from the coastal plain area.

Anthropophily and exophagy

Ratio of mean biting density of cow baited collections to outdoor human baited collections (C/OH), and the ratio of outdoor to indoor human baited collections (OH/IH) were calculated for the 8 major species in Table 3. A small C/OH value indicates high anthropophily of the species. The ratio was the smallest for *An. balabacensis* and the largest for *An. vagus*. *Anopheles sundaicus* and *An. subpictus* showed moderate anthropophily. *Anopheles balabacensis* showed the highest OH/IH value indicating highly exophagous, whereas *An. sundaicus* and *An. subpictus* were slightly exophagous.

Malaria antigen detection from collected mosquitoes

Among 661 pools composed of 3,553 females of 10 anopheline species, 14 and 4 were positive for *P. falciparum* and *P. vivax* antigen, respectively (Table 4). Positive samples were found only in the mountainous and coastal plain areas. No

Table 3. Ratio of mean biting density of cow baited collections to outdoor human baited collections and indoor to outdoor human baited collections for 8 species in the coastal plain and mountainous areas from November 2001 to September 2004.

Ratio Ratio	Species							
	vag	*sund*	*ind*	*sub*	*bal*	*bar*	*flav*	*mac*
Cow/HB out	255.1	8.2	97.3	33.6	0.2	54.6	18.7	22.5
HB out/in	2.1	1.3	2.0	1.1	108.9	6.7	3.5	—

vag=*Anopheles vagus*; *sund*=*An. sundaicus*; *ind*=*An. indefinitus*; *sub*=*An. subpictus*; *bal*=*An. balabacensis*; *bar*=*An. barbirostris*; *flav*=*An. flavirostris*; *mac*=*An. maculatus* s.l.
Cow/HB out=average density of cow baited collection/average density of outdoor human baited collection.
HB out/in=average density of outdoor human baited collection/average density of indoor human baited collection.
average density of cow baited collection: (no./cow/half night).
average density of outdoor human baited collection: (no./2men/half night).

Table 4. Malaria sporozoite detection by VecTest® from 10 anopheline species.

Species	No. mosquitoes	No. pools	No. of positive pools: Pf	Pv	Total (%)	Source of the samples: Bait	Indoor/ outdoor	Substudy area
	126	59	3	1	4 (6.8)	Cow	Outside	Mountainous
bal	632	179	9	2	11 (6.1)	Human	Outdoor	Mountainous
	3	3	0	0	0		Others	
	246	69	1	0	1 (1.4)	Human	Outdoor	Coastal plain
sund	48	20	1	0	1 (5.0)	Human	Indoor	Coastal plain
	1,201	119	0	0	0		Others	
sub	480	72	0	1	1 (1.4)	Cow	Outside	Coastal plain
	21	11	0	0	0		Others	
flav	379	34	0	0	0		All	
mac	213	45	0	0	0		All	
ann	81	19	0	0	0		All	
tesse	60	11	0	0	0		All	
ind	40	14	0	0	0		All	
bar	20	3	0	0	0		All	
vag	3	3	0	0	0		All	
Total	3,553	661	14	4	18 (2.7)			

bal=*Anopheles balabacensis*; *sund*=*An. sundaicus*; *sub*=*An. subpictus*; *flav*=*An. flavirostris*; *mac*=*An. maculatus* s.l.; *ann*=*An. annularis*; *tesse*=*An. tessellatus*; *ind*=*An. indefinitus*; *bar*=*An. barbirostriis*; *vag*=*An. vagus.*

positive sample was detected in the plain and coastal hilly areas. All 18 positive samples were derived from only 3 mosquito species, 15 from *An. balabacensis,* 2 from *An. sundaicus* and 1 from *An. subpictus*, and none from the other 7 anopheline species. Only 1 positive sample was collected from indoor human baited collections in the coastal plain area, and all positive samples in the mountainous area were collected from outdoor human or cow baited collections. The percentage of positive samples for *An. balabacensis* was 6.8% and 6.1% for samples collected from outdoor cow and human baited collections in the mountainous area, respectively. The percentage of positive samples for *An. sundaicus* and *An. subpictus* ranged from 1.4% to 5.0%.

Table 5 shows the time course of mosquito collections for the 18 positive samples. In *An. balabacensis*, 10 out of 15 samples were collected before midnight suggesting an early-night transmission of malaria in the mountainous areas. Positive samples were also collected from *An. subpictus* and *An. sundaicus* before midnight. Mosquito were only collected before midnight, so the positivity of these 2 species for the VecTest™ during the latter half of the night is unknown.

Seasonal prevalence of detected vectors observed in study periods

Total number of mosquitoes collected by outdoor and indoor human baited collections and cow baited collections of the same night was calculated, and monthly averages for the total are depicted for *An. balabacensis*, *An. sundaicus* and *An. subpictus* in Fig. 2. Densities of the 3 species were abundant in the dry season, and the peak densities were observed in August in *An. sundaicus*. The peak density of *An. subpictus* was observed in August 2002 and 2003, and June 2004. The seasonal variation of the density was the smallest in *An. subpictus* in 2003. The density level of *An. balabacensis* was the highest in the dry season and the peak density was ob-

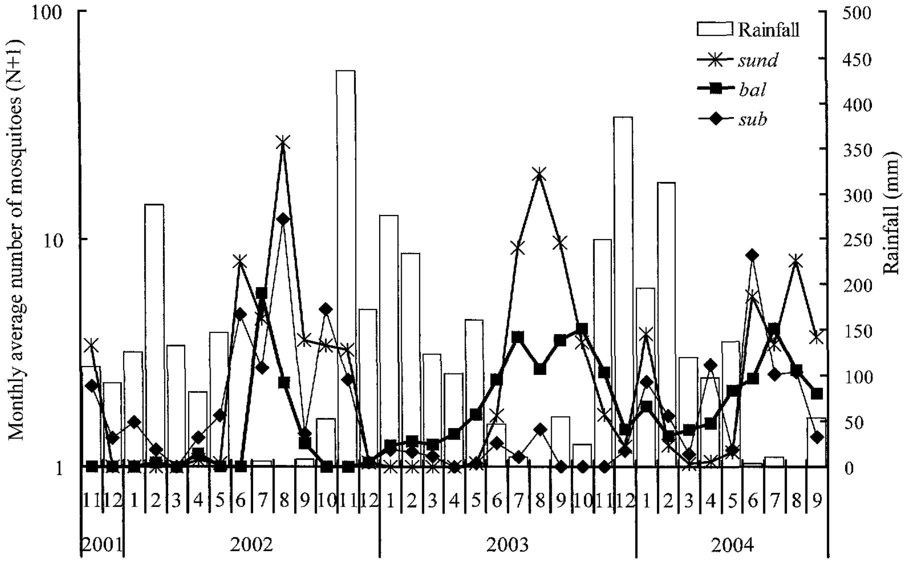

Fig. 2. Seasonal prevalence of suspected malaria vectors in the mountainous and coastal plain areas observed in this study. *sund*=*Anopheles sundaicus*, *bal*=*An. balabacensis*, *sub*=*An. subpictus*.

served in July or October. The population growth rate in the early dry season was the highest in *An. sundaicus*.

Biting activity of detected vectors

Based on the results of outdoor human baited collections in the mountainous area from July 2002 to September 2004, the frequency of collected mosquitoes at the different collection times were calculated for *An. balabacensis* and is shown in Fig. 3 (a). The difference in biting activity between the dry season and the rainy season was not observed. The biting activity of *An. balabacensis* was the highest from 19 : 00 to 21 : 00 and gradually decreased toward morning. Although biting activity of *An. sundaicus* and *An. subpictus* was examined only from 18 : 00 to 24 : 00, the frequency distributions of these species are shown in Fig. 3(b) based on the results of outdoor human baited collections in the coastal plain area from November 2001 to September 2004. The biting tendency was clearly higher toward midnight in *An. sundaicus* during the dry season.

Discussion

These results clearly indicate that *An. balabacensis* is a vector of malaria in the mountainous areas, and *An. sundaicus* and *An. subpictus* are vectors in the coastal plain areas of western Lombok Island, Indonesia. Malaria endemic areas in Lombok Island are confined to the coastal area, and three species *An. subpictus*, *An. maculatus* s.l. and *An. barbirostris* are the primary malaria vectors (Gebrak Malaria Team in West Lombok, 2000; WHO, 2001; Dachlan et al., 2005). However, intensive spleen examination of school children in our study area in 2002 suggested that malaria endemic areas exist not only in the coastal areas but also the mountainous areas (Yoshinaga et al., 2008). Furthermore, the slide positive rate examined by a malaria survey in 2002 and 2003 revealed that the rate was constantly positive in subvillages of the mountainous area, while unstable and low in subvillages along the coast. The confined distribution, higher density throughout the year, and higher percentage of malaria positive samples in *An. balabacensis* clearly explains the higher malaria endemicity in the mountainous area. This is the first report to confirm the role of *An. balabacensis* in malaria transmission in the mountainous areas of Lombok Island, Indonesia.

Anopheles balabacensis is a principal

Table 5. Hourly collection number of mosquitoes collected with a positive pool by the VecTest®.

Year	Month	Collectiion	*Plasmodium* sp. detected	Hour 18	19	20	21	22	23	0	1	2	3	4	5	Total/pool
Mountainous area (*Anopheles balabacensis*)																
2002	July	Outdoor Human	Pf	1			1	1	1					1	1	6
2003	May	Outdoor Human	Pf							1						1
	June	Outdoor Human	Pf							1		1	1			3
		Outdoor Human	Pf					2								2
		Outside Cow	Pf	1				2								3
	September	Outdoor Human	Pf		1		2	1								4
		Outdoor Human	Pf	1	3	1	3	4	1							13
		Outdoor Human	Pf	2	4		1	2	1							10
		Outside Cow	Pf							1			1			2
	November	Outdoor Human	Pf							1	1					2
		Outside Cow	Pv		1	1			1							3
	December	Outdoor Human	Pf		2		1					1				4
		Outside Cow	Pf			1	1			1						3
2004	July	Outdoor Human	Pv	4	4	1	2	1								12
	August	Outdoor Human	Pv			2	1		1							4
Coastal plain area (*An. sundaicus* and *An. subpictus*)																
2002	October	Outside Cow	Pv					1			no collection (0 : 00 to 6 : 00 hr)					1
	November	Indoor Human	Pf					1			no collection (0 : 00 to 6 : 00 hr)					1
2003	August	Outdoor Human	Pf			2		1	3		no collection (0 : 00 to 6 : 00 hr)					6

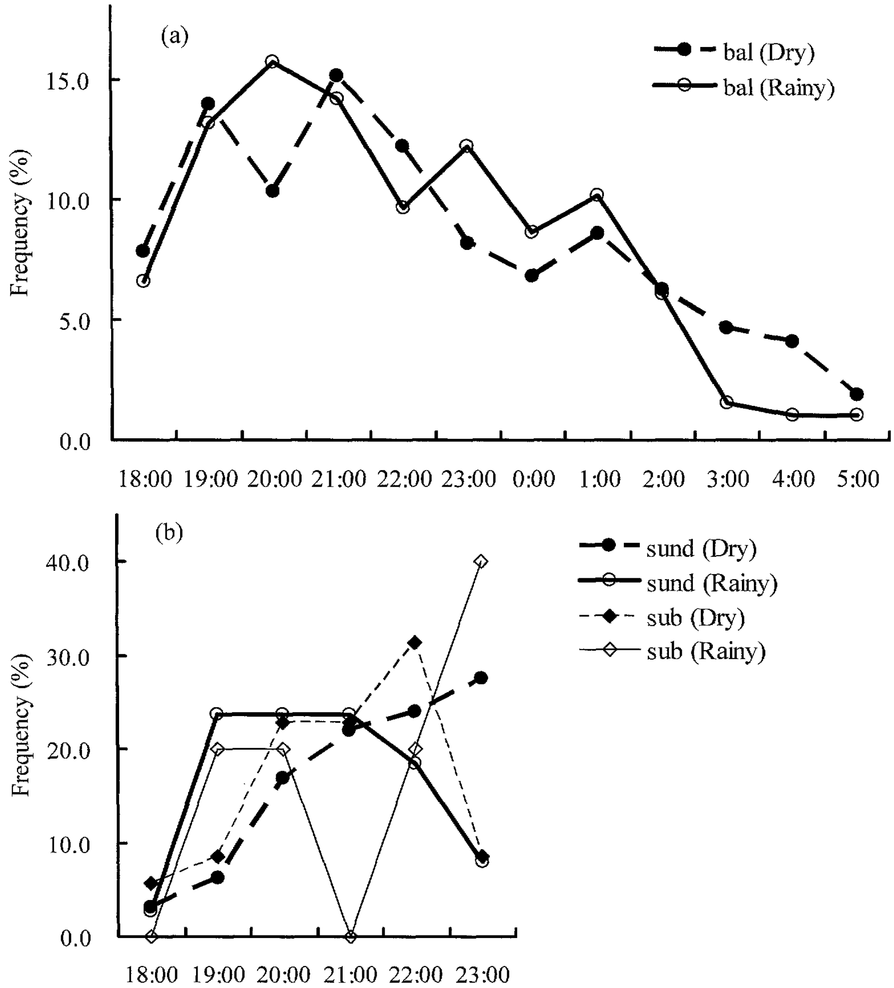

Fig. 3. Biting activity of *Anopheles balabacensis* in the mountainous area from July 2002 to September 2004 (a), and *An. sundaicus* and *An. subpictus* in the coastal plain area from November 2001 to September 2004 (b). *bal*=*An. balabacensis*, *sund*=*An. sundaicus*, *sub*=*An. subpictus*.

vector of malaria in the forested hills of South and East Kalimantan (Kirnowardoyo, 1985; Harbach et al., 1987), Central Java (Barcus et al., 2002), East Malaysia (Rohani et al., 1999) and the Philippines (Schultz, 1992), and adult densities in these areas are higher in the rainy season. However, in our study area adult densities of the species increased during the dry season. Different seasonal prevalence of *An. balabacensis* in our study area can be explained by seasonal changes in the availability and stability of larval habitats. The main larval habitats of *An. balabacensis* found in the mountainous area are small ground pools along the streams, and larvae are easily flushed out by heavy rain during the rainy season (Maekawa unpublished). However, during the dry season these ground pools provide stable larval habitats for this species.

The biting behavior of mosquitoes is an important factor to determine their vector status. Based on our results the biting behavior of *An. balabacensis* in Lombok Island is summarized as follows. *Anopheles balabacensis* was the most anthropohilic of the 8 anopheline species encountered in this study. This same behavior has been reported in the Philippines (Schultz, 1992) and Malaysia (Hii, 1985). The vector status of *An. balabacensis* in Lombok Island appears to be equivalent to *An. dirus* A in Vietnam, Laos and Cambodia (Trung et al., 2005). Strong exophagy of *An. balabacensis* was observed in Lombok Island, while house entering females of *An. balabacensis* were observed in Thailand, Malaysia and Hainan Island, China (Rao, 1984; Yang, 1983). Schultz (1992) observed a higher human biting density of indoors than outdoors in Palawan, the Philippines. Endophagy/exophagy of vector mosquitoes largely depends on housing conditions (Trung et al., 2005), so more studies on biting behavior of *An. balabacensis* under different housing conditions in Lombok Island are necessary.

Out of 15 positive samples for malaria antigen, 10 were obtained from *An. balabacensis* in the dry season of the mountainous areas. Yoshinaga et al. (2008) found a higher malaria transmission in the dry season through microscopic examination of blood samples in the same area. These results strongly suggest that malaria in the mountainous areas of Lombok Island is transmitted by *An. balabacensis* during the dry season. Therefore, malaria control activities against *An. balabacensis* is better concentrated in the dry season of the mountainous areas.

The biting activity of *An. balabacensis* in our study area was high during 19 : 00 to 21 : 00 suggesting an early night-biter. The same biting activity was observed in Palawan, the Philippines (Schultz, 1992) and Kalimantan (Harbach et al., 1987). Ten out of 15 samples with malaria antigens were collected before midnight. These results indicate that the efficacy of a bed net, which protects sleepers from an infective bite, are limited in this study area because during 18 : 00 to 21 : 00 when *An. balabacensis* is most active for blood feeding, the local people spend time by resting, playing card games, and engaging in evening conversations with neighbors on the outside terrace and bergak (Kawada et al., 2004). Alternative tools to reduce or repel infective bites at outdoors in the early-night will be more effective.

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