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Tempo-spatial variation in feeding activity and density of *Aedes albopictus* (Diptera: Culicidae) at peridomestic habitat in Nagasaki, Japan

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Abstract: To clarify the tempo-spatial variation in feeding of *Aedes albopictus,* a field investigation was conducted in a field where the species heavily infested, in Nagasaki. Feeding activity was observed in the nighttime as well as at dawn and dusk. The density was high at sites with vegetation and low at sites without vegetation and indoors. At sites with vegetation, the proportion of females decreased at dawn and dusk, while it increased at sites without vegetation and indoors. Feeding activity in nighttime is possibly common in *Ae. albopictus,* and the place of feeding expands from sites with vegetation to sites without vegetation and indoors at dawn and dusk.

INTRODUCTION

Tempo-spatial analysis of feeding behavior is essential to understand the risk of a host-vector contact and the transmission dynamics of diseases. Therefore, the feeding behavior has been investigated on *Aedes albopictus,* an important vector of dengue fever and a potential vector of other arboviruses, in Japan, Southeast Asia and other countries. From these investigations the species has been considered as an outdoor feeder in crepuscular periods (Bekku, 1954; Wang, 1962; Gould *et al.,* 1968, 1970; Ho *et al.,* 1973; Hawley, 1988; Schultz, 1989; Yee and Foster, 1992; Hassan *et al.,* 1996). However, it was also suggested that the species showed variation in both time and place of feeding. With regard to the time of feeding, Bekku (1954) found that the species bit at night other than at two feeding periods, dawn and dusk, by 24-hour observation in a field although most of the studies were concentrated on daytime collection. Yee and Foster (1992) also showed agreement with the result of Bekku (1954) by a laboratory experiment. Despite these facts, the authors considered the feeding at night was exceptional, which should be noteworthy because recent findings have revealed that *Ae. albopictus* is an opportunistic feeder on a wide variety of animal hosts (Savage *et al.,* 1993; Niebylski *et al.,* 1994). The species possibly feeds on those animals at night. Therefore, 24-hour collection in various habitats should be still necessary to examine in detail the time of feeding of *Ae. albopictus.* With regard to the place of feeding, Takagi *et al.* (1995a, b) showed that the number of females varied from site to site depending on the environmental conditions of the collection sites.

To clarify the tempo-spatial variation in feeding of *Ae. albopictus* in more detail, a series of adult female collection was conducted in a field where *Ae. albopictus* heavily infested, using a dry ice-enhanced suction trap.

MATERIALS AND METHODS

The study area was located at the northern edge of a small woods on the campus of Nagasaki University School of Medicine, Nagasaki, Japan, previously described by Takagi *et al.* (1995a, b) (Fig. 1). The area was enclosed with a fence and a building of Institute of Tropical Medicine, and was seldom disturbed by human activity and artificial lighting in the nighttime. It included a stock house (3.7×5.8 m) surrounded by vegetation which was spottedly distributed. These conditions offered a typical peridomestic habitat of *Ae. albopictus* in Nagasaki.

Due to limited manpower, it was difficult to conduct simultaneous human-baited collection at multi sites. Therefore, suction traps baited with dry ice were alternatively used to evaluate the feeding activity.

Twenty-four-hour collection was repeated 5 times at 2-day intervals from 3 to 12 of September, 1998. Seven fixed sites with different conditions (1 indoors, 4 outdoor with and 2 outdoor without vegetation, respectively) were selected for the collection. One hour before start of the collection, all doors and windows of the stock house, which was used as an indoor collection site, were opened. The suction trap used in this study was a CDC miniature light trap type without light bulbs, and was continuously operated with 1 kg of dry ice for 24 hours at each site. The collection period was divided into 4 phases, dawn=4:00–8:00, daytime=8:00–17:00, dusk=17:00–21:00 and nighttime=21:00–4:00. Mosquitoes collected were removed hourly at dawn and dusk when feeding was expected to be active. At daytime and nighttime the mosquitoes were removed every 2 hours. The removed mosquitoes were identified in a laboratory.

Variations in mosquito density among collection sites and day phases were analyzed by ANOVA. All statistical calculations were performed by Systat 7.0.

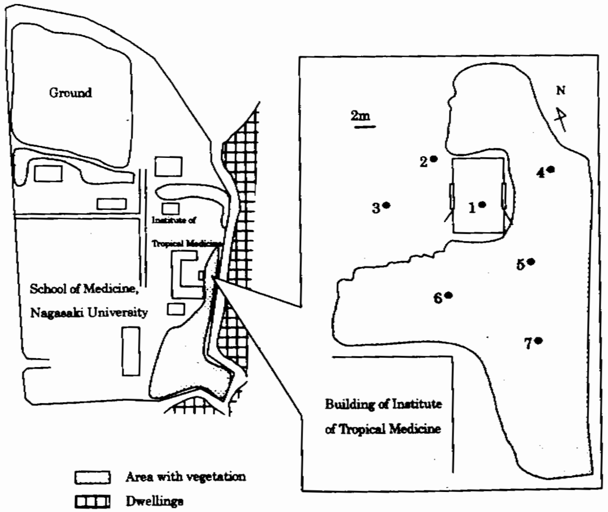

Fig. 1. A map showing location and the environmental conditions of the study area.

RESULTS

The average number of female *Ae. albopictus* collected by suction traps from all collection sites is shown in Fig. 2. Feeding activity of females evaluated by the average density of females (the number of females per site per hour) was the highest at dusk. Then the activity gradually decreased during nighttime. Feeding activity in nighttime was higher than that in daytime. Activity of males was higher at dawn and dusk, and a small peak was observed at nighttime.

The average densities of females at each site are shown in Table 1. The result of 2-way ANOVA showed that density of *Ae. albopictus* was significantly different among both the collection sites and the day phases (collection site: $F=41.502$, df=2, $P<0.001$; day phases: $F=29.716$, df=3, $P<0.001$). The density at sites with vegetation was higher than at sites without vegetation and indoors, irrespective of phases.

The proportion of females at each site on each phase was calculated from the average number per site and is depicted in Fig. 3. Day phase-related-change in the proportion of females among the sites was observed although it was not statistically significant ($\chi^2=5.372$, df=6, $P>0.05$). At dawn and dusk, when the feeding activity was high, the proportion of females decreased at sites with vegetation, and increased at sites without vegetation. On the other hand, at day and night, the proportion increased at sites with vegetation but decreased at those without vegetation and indoors.

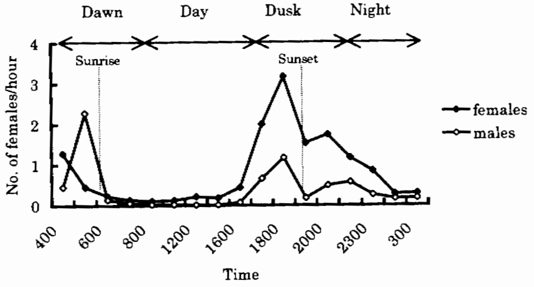

Fig. 2. Feeding activity of females and activity of males of *Ae. albopictus* observed from 3 to 12 September 1998 in Nagasaki, Japan.

Table 1. The average number of female *Ae. albopictus* (no. of females/hour) collected at different sites during 3 to 12 September 1998 in Nagasaki, Japan.

Phase	Collection sites			ANOVA
	Without vegetation	Indoors	With vegetation	
Dawn	0.23 ± 0.47^{b}	0.15 ± 0.36^{ab}	0.78 ± 1.44^{a}	$F=4.404$, $P<0.05$
Day	0.06 ± 0.24^{b}	0.16 ± 0.37^{b}	0.69 ± 1.06^{a}	$F=11.820$, $P<0.001$
Dusk	0.95 ± 1.07^{b}	1.00 ± 1.21^{b}	3.11 ± 3.53^{a}	$F=9.501$, $P<0.001$
Night	0.22 ± 0.42^{b}	0.17 ± 0.37^{b}	1.49 ± 2.24^{a}	$F=16.503$, $P<0.001$
Overall average	0.34 ± 0.70^{b}	0.35 ± 0.76^{b}	1.45 ± 2.40^{a}	$F=24.898$, $P<0.001$

Averages in the same row followed by the same letter were not significantly different (Tukey's HSD test, $P>0.05$).

have been many analytical studies on the relationship between each environmental factor and mosquito density (Clements, 1999). However, environmental factors do not vary independently but interrelate to each other. Mosquitoes may respond not to each environmental factor separately, but to a set of factors as a whole. Therefore, for understanding of the exophagy of *Ae. albopictus* and effective environmental factors on their feeding behavior, we should compare the combination of environmental factors among different types of collection sites and analyze the association with mosquito density.

To evaluate the environmental factors influencing the exophagy, we conducted a field study in Nagasaki, Japan, providing 4 different environments with a combination of structural factors (inside a normal-walled building, net-walled buildings, outdoor site with vegetation and outdoor site without vegetation) and microclimatic parameters (temperature, relative humidity, light intensity and wind velocity), and examined female density at these environments (Experiment 1). Since physical blocking by walls was considered to be an effective factor in house-entering behavior as suggested on African mosquitoes (Snow, 1987; Gillies, 1988; Lindsay and Snow, 1988), it was also evaluated separately in Thailand by using a bamboo hut (Experiment 2).

High density was observed at outdoor sites with vegetation and inside net-walled buildings. The effect of physical blocking by building walls on densities also was confirmed. Based on the results, factors affecting the exophagy of *Ae. albopictus* are discussed.

MATERIAL AND METHODS

Experiment 1

Experimental field and mosquito collection

The same experimental field as that of Higa et al. (2000), located in the northeastern part of the campus of Nagasaki University, School of Medicine, Japan, was used in the present study. Total of 9 sites with 4 different environmental conditions (①: inside one normal-walled and ②: inside 2 net-walled buildings. All of these were surrounded by vegetation (ID1-3). ③: 3 outdoor sites located in vegetation (VG1-3) and ④: 3 outdoor sites located outside of the vegetation (BR1-3)) were set up for mosquito collection (Fig. 1). Among the 9 sites ID1-3 were used as indoor collection sites. A building used for ID1 was sized 3.7×5.8×2.0 m (Fig. 2). ID2 and ID3, located about 50 m from ID 1, were surrounded by similar vegetation to ID 1, which was composed of herbaceous plants approximately 1 m height, shrubs and trees. ID2 and ID3, the net-walled buildings, which comprised 2 m cube frames covered with a green, nylon mesh sheet, were constructed 1.5 m apart (Fig. 3). The mesh size of the sheet was 0.8 mm×0.8 mm which was small enough to prevent mosquitoes from entering through the mesh. The ground was cleared before the construction of the net-walled buildings. ID2 had a mesh sheet roof, while ID3 had a roof made of plywood (2×2 m) on the top. The bottom edges of one side of the net-walled buildings were pulled up and fixed at 50 cm above the ground to allow entrance and exit of mosquitoes. VG1-3 were defined as outdoor sites with vegetation, and BR1-3 were those without vegetation.

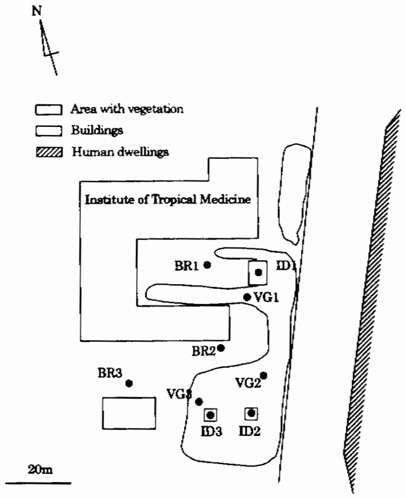

Fig. 1. A map showing location and the environmental conditions of the study area in Nagasaki, Japan.
ID1, normal-walled building; ID2, net-walled building; ID3, net-walled building with roof; VG1-3, outdoor sites with vegetation; BR1-3, outdoor sites without vegetation.

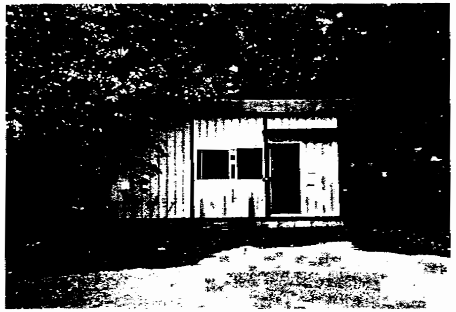

Fig. 2. The normal-walled building in Experiment 1 in Nagasaki, Japan.

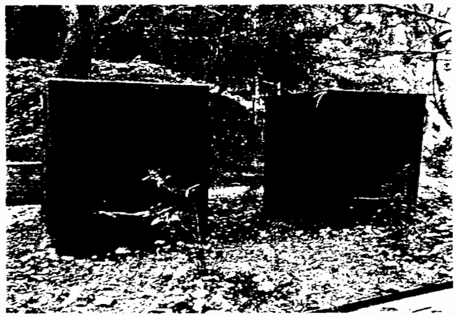

Fig. 3. The net-walled buildings in Experiment 1 in Nagasaki, Japan.

Twenty-four-hour collection was repeated 4 days in July 1999. One hour before start of the collection, all the doors and windows of ID1 facing both outdoor sites with and without vegetation were opened. Suction traps used in this study were CDC miniature light trap type without light bulbs, and were continuously operated with 1 kg of dry ice for 24 hours at each site. Female mosquitoes collected by the traps were regarded as feeding/host-seeking females as described by Gillies (1980). The collection period was divided into 4 phases, dawn = 4 : 00–8 : 00, daytime = 8 : 00–17 : 00, dusk = 17 : 00–21 : 00 and nighttime = 21 : 00–4 : 00. Mosquitoes collected were removed hourly at dawn and dusk. At daytime and nighttime they were removed every 2 hours. Mosquitoes removed were identified into species and counted in the laboratory.

Collections of environmental data

Other than the 4 pre-set environmental factors, four microclimate parameters (temperature, relative humidity, light intensity and wind velocity) were measured at each collection site. Temperature (℃) and relative humidity (%) were recorded hourly by automatic recorder (HOBO 8, Onset Computer Corporation, Bourne, USA). Light intensity (lx) and wind velocity (m/s) were measured hourly or every 2 hours by light intensity meter (KENIS Kagaku Kyoeisha Ltd., Tokyo) and wind velocity meter (Sato Keiryoki Mfg. Co., Ltd., Tokyo).

Experiment 2

Experimental field and mosquito collection

Because of experimental design and operational limits, Experiment 2, an examination to evaluate the physical blocking effect of walls on invading to feed, was conducted in the yard at the northeastern part of a building complex of Office of Vector Borne Disease Control No. 2 in Chiangmai, Thailand. The yard was grassy and partly shaded by trees, and *Ae. albopictus* and *Ae. aegypti* inhabited there (Suwonkerd et al., 1996). A bamboo hut (2.0 × 3.0 × 2.0 m), which comprised iron frames with bamboo walls and roof, was constructed under the trees in the yard. The walls of the hut were detachable. Three varieties of walls with approximately 25%, 50% and 75% openings to the total area of the walls were prepared (Fig.

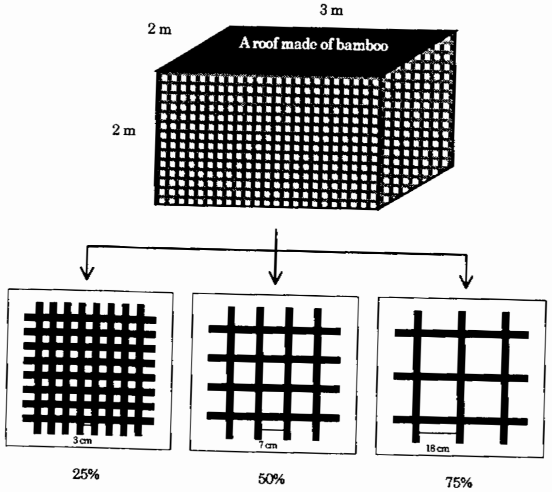

Fig. 4. The bamboo hut with different percentage of opening area (25, 50 and 75%) in walls in Experiment 2 in Chiangmai, Northern Thailand. (Width of bamboo stick is approximately 3 cm.)

4). Each variety of walls was attached to the frames in order, and indoor- and outdoor-densities of females were compared under the different wall conditions. Human-bait collections were conducted at both inside and outside the hut (2 m distant from the hut) for 10 min. from 17:00 to 18:00 in June–August 1999. The collection was repeated 5, 7 and 7 times under the wall conditions of 25, 50 and 75% openings, respectively.

Collections of environmental data

Temperature (℃), relative humidity (%) and light intensity (lumens) at the indoor and outdoor sites were recorded by the automatic recorder used in the first experiment.

Statistical analysis

In Experiment 1, data obtained in the daytime (4:00–20:00 including dawn and dusk) and in the nighttime (20:00–4:00) were separately analyzed since the microclimate conditions, especially light intensity, markedly changed from daytime to nighttime. For the analysis of density variations among different environments, the environments were ranked within each data set depending on the number of *Ae. albopictus* at each sampling time, and nonparametric analysis of variance (Friedman's test) was applied because *Ae. albopictus* had a clear diel feeding activity (Higa et al., 2000) and the underlying assumption of the usual ANOVA was not valid. For pair-wise comparisons among different environments, multiple comparisons for nonparametric randomized block analysis of variance (Zar, 1984) were conducted. For easy understanding of variation among the environments, the average number of females/hour/site also was calculated and shown in the Tables. Since the degree of many or a few of *Ae. albopictus* shown by Friedman's analysis was based on rank, the density referred in the following text was regarded as relative density. Since microclimate parameters showed periodic changes within a day, the level and variability of each parameter were used for the comparison of environmental conditions among the collection sites. The significance of difference in the level and the variability of the microclimate was tested by Friedman's test and the F-test, respectively. Light intensity was transformed in logarithm for the analyses and then backtransformed for showing the average in the Tables.

In Experiment 2, the numbers of *Ae. albopictus* collected were compared between inside and outside the bamboo hut by the chi-square goodness of fit test. The variation of the microclimatic parameters between the collection sites was analyzed by Friedman's test. All statistical calculations were performed by statistical software Systat.

RESULTS

Experiment 1

Difference in the density of Ae. albopictus *at outdoor collection sites surrounding the normal-walled and the net-walled buildings*

The number of *Ae. albopictus* collected at 3 outdoor sites with vegetation (VG1-3) located near the 3 buildings was analyzed by Friedman's test to examine the differences in mosquito density around the 3 buildings since the two net-walled buildings (ID2 and ID3) were constructed about 50 m from the normal-walled building (ID 1). There was no significant difference in the density of females among the 3 outdoor sites with vegetation throughout the experimental period (1st collection day: $\chi_r^2=0.81$, $P>0.05$ in the daytime and $\chi_r^2=0.50$, $P>0.05$ in the nighttime; 2nd day: $\chi_r^2=2.42$, $P>0.05$ and $\chi_r^2=3.38$, $P>0.05$; 3rd day: $\chi_r^2=3.29$, $P>0.05$ and $\chi_r^2=2.38$, $P>0.05$; 4th day: $\chi_r^2=1.57$, $P>0.05$ and $\chi_r^2=0.38$, $P>0.05$). No significant differences in outdoor density were also observed among the 3 outdoor sites without vegetation (BR1-3) (1st collection day: $\chi_r^2=3.50$, $P>0.05$ in the daytime and $\chi_r^2=0.38$, $P>0.05$ in the nighttime; 2nd day: $\chi_r^2=0.12$, $P>0.05$ and $\chi_r^2=0.38$, $P>0.05$; 3rd day: $\chi_r^2=0.38$, $P>0.05$ and $\chi_r^2=0.00$, $P>0.05$; 4th day: $\chi_r^2=1.50$, $P>0.05$ and $\chi_r^2=3.38$, $P>0.05$). These results clearly showed that the differences in mosquito density around the 3 experimental buildings were negligible. Thus, sampling data from the 3 outdoor sites with (VG1-3) or without vegetation (BR1-3) were pooled, and the relative density or the average was used to represent density of outdoor sites in the following analyses.

Difference in the density among 4 varieties of environmental conditions

Difference in adult density of *Ae. albopictus* among 4 different environments was significant both in the daytime ($\chi_r^2=28.5$, $P<0.001$) and the nighttime ($\chi_r^2=23.6$, $P<0.001$). Statistical analysis based on rank sums among the varieties of the environments revealed the following.

In the daytime, the inside of the net-

Table 1. The results of Friedman's test and the average number of *Ae. albopictus* females per hour (±SD) at the collection sites with different environmental conditions.

Phase		Indoors			Outdoors		Friedman's test statistics
		Normal-walled	Net-walled		With vegetation	Without vegetation	
			With roof	Without roof			
Daytime[1]	Rank sum[2]	117.0^{bc}	157.5^{ab}	158.5^{a}	159.0^{a}	98.0^{c}	$\chi_r^2=28.5$, $P<0.001$
	Average	0.4±0.8	1.8±4.2	1.2±2.0	1.0±1.8	0.2±0.6	
Nighttime	Rank sum	30.5^{b}	52.5^{ab}	62.5^{a}	61.5^{a}	33.0^{b}	$\chi_r^2=23.6$, $P<0.001$
	Average	0.1±0.3	0.9±1.5	1.4±2.5	0.5±1.1	0.1±0.4	

1) Daytime inclueded dawn and dusk phases.

2) The values in the same row followed by the same letter were not significantly different ($P>0.05$) by Friedman's test.

Table 2. The results of Friedman's test, the averages of temperature, relative humidity, light intensity and wind velocity and variance at the collection sites with different environmental conditions.

Parameters		Indoors			Outdoors		Friedman's test statistics
		Normal-walled	Net-walled				
			With roof	Without roof	With vegetation	Without vegetation	
Daytime[1]							
Temperature (℃)	Rank sum[2]	120.0^{b}	149.0^{b}	127.0^{b}	137.0^{b}	247.0^{a}	$\chi_r^2=83.3$, $P<0.001$
	Average	25.6	26.8	26.7	26.6	28.6	
	Variance[3]	3.6^{c}	9.6^{b}	8.4^{b}	7.3^{b}	23.0^{a}	
Relative humidity (%)	Rank sum	192.5^{a}	179.0^{ab}	204.5^{a}	137.0^{b}	67.0^{c}	$\chi_r^2=96.1$, $P<0.001$
	Average	84.0	78.5	79.4	78.1	70.8	
	Variance	75.7^{c}	185.0^{b}	193.2^{b}	176.9^{b}	364.8^{a}	
Light intensity (lx)	Rank sum	63.0^{d}	116.5^{c}	135.0^{c}	197.0^{b}	253.5^{a}	$\chi_r^2=170.9$, $P<0.001$
	Average	14	260	491	1202	7085	
	Variance	1.7^{c}	7.3^{b}	8.4^{b}	10.9^{b}	15.2^{a}	
Wind velocity (m/s)	Rank sum	111.0^{b}	109.5^{b}	111.0^{b}	198.0^{a}	235.5^{a}	$\chi_r^2=111.8$, $P<0.001$
	Average	<0.01	<0.01	<0.01	0.20	0.43	
	Variance	—	—	—	0.09^{b}	0.36^{a}	
Nighttime							
Temperature (℃)	Rank sum	52.5^{ab}	41.5^{b}	30.5^{b}	40.5^{b}	75.0^{a}	$\chi_r^2=28.9$, $P<0.001$
	Average	25.0	24.8	24.6	24.8	25.3	
	Variance	2.9^{a}	4.0^{a}	4.0^{a}	4.0^{a}	4.0^{a}	
Relative humidity (%)	Rank sum	46.0^{ab}	64.5^{a}	70.0^{a}	38.5^{bc}	21.0^{c}	$\chi_r^2=39.5$, $P<0.001$
	Average	84.9	86.0	86.6	84.5	83.1	
	Variance	20.3^{a}	32.5^{a}	36.0^{a}	30.3^{a}	34.8^{a}	
Light intensity (lx)	Rank sum	35.0^{b}	30.0^{b}	33.0^{b}	62.0^{a}	80.0^{a}	$\chi_r^2=48.5$, $P<0.001$
	Average	0.1	0.1	0.1	0.2	0.8	
	Variance	0.04^{c}	0.04^{c}	0.00^{d}	0.08^{b}	0.32^{a}	
Wind velocity (m/s)	Rank sum	39.5^{b}	36.0^{b}	36.6^{b}	60.0^{ab}	68.5^{a}	$\chi_r^2=23.1$, $P<0.001$
	Average	0.01	<0.01	<0.01	0.10	0.13	
	Variance	0.00^{b}	—	—	0.04^{a}	0.04^{a}	

1) Daytime inclueded dawn and dusk phases.
2) The values in the same row followed by the same letter were not significantly different ($P>0.05$: Friedman's test).
3) The values in the same row followed by the same letter were not significantly different ($P>0.05$: F-test).

walled buildings showed higher density. The average number of females/trap/hour was 1.8 and 1.2, for the net-walled buildings with and without a roof, respectively, and both of the densities and that of the outdoor sites with vegetation (average = 1.0) were higher than those in the normal-walled building (average = 0.4) and outdoor sites without vegetation (average = 0.2) (Table 1). The variation of the density in the nighttime also showed a similar tendency to that in the daytime. There was no significant difference in the densities between the presence and absence of a roof on the net-walled buildings.

Difference in microclimate conditions among 4 varieties of environmental conditions

The microclimate conditions in the outdoor sites without vegetation were largely different from those with vegetation in the daytime, although differences in the microclimate conditions were obscure in the nighttime (Table 2). The difference in temperature between the inside of net-walled building and the outdoor sites with vegetation were not significant both in the daytime and the nighttime, while differences in relative humidity in the nighttime, light intensity and wind velocity both in the daytime and the nighttime were significant. Between the roofed and unroofed net-walled buildings, no significant differences were observed in the microclimate conditions. The microclimate with dark, less windy and humid conditions in the normal walled building was significantly different from those at outdoor sites with vegetation, except for temperature in the daytime, while in the nighttime, difference in microclimate conditions between the inside of the normal-walled building and outdoor sites with vegetation was obscure, and was observed in only light intensity. Except for light intensity in the daytime, there were no significant differences in the microclimate conditions between the normal-walled and the net-walled buildings.

Table 3. The total number and the average number (No./10 min) of females collected at inside and outside of the bamboo hut with different percentage of opening area in walls.

Opening area	No. collections	Total no. collected (No./10 min)		χ^2 test
		Inside	Outside	
25%	5	2 (0.4)	12 (2.4)	$\chi^2=7.143$, $P<0.01$
50%	7	9 (1.3)	6 (0.9)	$\chi^2=0.600$, $P>0.05$
75%	7	6 (0.9)	6 (0.9)	$\chi^2=0.000$, $P>0.05$

The variability of the microclimate conditions within a day was compared based on the variance of each parameter. The microclimate at outdoor sites without vegetation during the daytime was more variable than at outdoor sites with vegetation. In the daytime, differences between outdoor sites with and without vegetation were significant in all microclimate parameters except for wind velocity. Between the net-walled buildings and outdoor sites with vegetation, there was no significant difference in the variability of all microclimate conditions except for light intensity in the nighttime and wind velocity both in the daytime and the nighttime. The microclimate in the normal-walled building during the daytime was more stable than at outdoor sites with vegetation. There was a significant difference in the variability between the normal-walled building and the net-walled buildings although the levels of most parameters showed no significant difference.

Experiment 2

Difference in the average number of Ae. albopictus *females between inside and outside of a bamboo hut*

When the opening of bamboo walls was the smallest (25%), the difference in the average number of *Ae. albopictus* between inside (0.4 females/10 min) and outside (2.4) of the hut was significant (Table 3). However, when the opening was larger (50 and 75%), no significant difference in the average number was found between inside and outside of the bamboo hut (1.3 and 0.9 in 50%, and 0.9 and 0.9 in 75%, respectively).

Microclimate conditions at collection sites

No significant effects of the bamboo walls were observed on temperature, relative humidity and light intensity at inside of the bamboo hut under the wall conditions of 50 and 75% openings (Table 4). Temperature at outside (average = 27.7℃) was significantly higher than inside (average = 27.0℃) under the wall condition of 25% opening, while relative humidity and light intensity were not significantly different between inside and outside of the bamboo hut.

Table 4. The results of Friedman's test and the averages of microclimate conditions (±SD) at inside and outside of the bamboo hut with different percentage of opening area in walls.

Opening area		Temperature (℃)			Relative humidity (%)			Light intensity (lumens)		
		Inside	Outside	Friedman's test statistics	Inside	Outside	Friedman's test statistics	Inside	Outside	Friedman's test statistics
25%	Rank sum	5.0	10.0	$\chi_r^2=5.0, P<0.05$	8.0	7.0	$\chi_r^2=0.2, P>0.05$	7.5	7.5	$\chi_r^2=0, P>0.05$
	Average	27.0±0.9	27.7±1.3		81.4± 6.2	79.8± 7.6		1.0±0.0	1.0±0.0	
50%	Rank sum	7.0	11.0	$\chi_r^2=2.7, P>0.05$	8.0	10.0	$\chi_r^2=0.7, P>0.05$	7.5	10.5	$\chi_r^2=1.5, P>0.05$
	Average	29.1±1.9	29.1±2.1		69.6±11.1	72.4±12.6		6.9±7.8	8.8±6.6	
75%	Rank sum	10.0	8.0	$\chi_r^2=0.7, P>0.05$	7.0	11.0	$\chi_r^2=2.7, P>0.05$	11.0	7.0	$\chi_r^2=2.7, P>0.05$
	Average	28.0±2.5	28.5±1.7		76.4±15.4	76.0±12.2		6.1±5.4	2.5±2.5	

DISCUSSION

The exophagy of *Aedes albopictus* has been well shown by previous researches. However, there were few studies to examine the factors influencing the exophagy of this species. In our study, four different environments, normal-walled building, net-walled buildings, outdoor sites with vegetation and outdoor sites without vegetation, were set up in the study area, and environmental factors affecting the exophagy of *Ae. albopictus* were evaluated through comparisons of the density of females attracted to dry ice-baited suction traps, structural factors (vegetation and buildings) and microclimate conditions among the collection sites. The results showed that the density of *Ae. albopictus* females collected in outdoor sites with vegetation and the net-walled buildings was significantly higher than that in the normal-walled building.

Since a certain site with higher aedine mosquito density is generally thought to be favorable for feeding, clarification of the environmental conditions of the site is one experimental approach to identify factors affecting the endo-/exo-phagy of the species. The present study and Higa et al. (2000) showed that the density of *Ae. albopictus* collected at outdoor sites with vegetation was higher than that at outdoor sites without vegetation, the microclimate conditions of which were different from those with vegetation and were highly variable in a day. Schultz (1989) obtained similar results; biting density of the species was high in a cemetery with much vegetation comparing with a cemetery with less vegetation. A mark-release-recapture experiment of *Ae. albopictus* at a scrap tire yard in USA revealed that approximately 85% of released females were recaptured at a forest edge ecotone with dense understory and few were collected at an open tire yard with minimal vegetation (Niebylski and Craig, 1994). These studies have clearly shown that an environment with vegetation is favorable for *Ae. albopictus*, and a habitat of the species depends on the presence of vegetation.

Our experiment in Nagasaki, Japan, has shown that even within vegetation, heterogeneity of habitat for *Ae. albopictus* was observed spatially as places where the density was high and where the density was low. The results suggested that the degree of blocking seemed to be related to the density variation in the habitat, and difference in environmental conditions of inside of buildings from the surrounding vegetation due to blocking was one influencing factor on the exophagy. In the present study, an equivalent number of females to that in outdoor sites with vegetation was collected even inside the net-walled buildings, which were less blocked than the normal-walled building. Similar microclimate conditions in levels to the normal-walled building and in variability to outdoor sites with vegetation suggested that environmental conditions in the net-walled buildings were intermediate between the normal-walled building and outdoor sites with vegetation. The high density indicated that the difference in the level of microclimate conditions between the inside of net-walled buildings and outdoor sites with vegetation was negligible for *Ae. albopictus*, and resulted in the same response of *Ae. albopictus* between the sites. Thus, equally active feeding was encountered even at inside of net-walled buildings. The same explanation may be applied to the results of Pant et al. (1973) showing that *Ae. albopictus* was collected even indoors at a village located inside a densely planted fruit garden in which the conditions at outdoors and indoors seemed to be similar.

On the other hand, the significantly lower density of females in the normal-walled building than at outdoor sites with vegetation showed that the environmental conditions in the normal-walled building were unfavorable for *Ae. albopictus*. The walls of the building, even though it was surrounded by vegetation, highly separated the inside of the building from outdoor sites with vegetation as suggested by the significant differences in microclimate conditions. The large environmental difference between the normal-walled building and the surrounding sites with vegetation might be attributed to changes of *Ae. albopictus* in the exploring behavior for blood feeding. The importance of difference in environmental conditions between indoors and outdoors for house-entering behavior has been reported in some mosquito species. A certain karyotype of *Anopheles arabiensis* which preferred drier environment was dominant at inside of houses, resulting in a differing indoor-to-outdoor proportion of the karyotype (Coluzzi et al., 1979; Gillies, 1988). Hausermann and Trpis (1977) suggested that different response to light conditions of inside and outside of houses in 2 subspecies of Ae. aegypti was attributed to different degrees of house-entering behavior.

The effect of blocking on the density of *Ae. albopictus* was confirmed in our experiment in Thailand. No significant difference was observed in the number of females and microclimate conditions between inside and outside of a bamboo hut when the opening of walls were large (50 and 75%), whereas a significantly higher number of females and temperature at outside of the bamboo hut was observed when the walls with the smallest openings were used in this study (25%). In 25% opening, although the effect of bamboo walls on temperature was statistically significant, no significant difference in relative humidity and light intensity between inside and outside of the hut suggested the environmental difference was too small to change the feeding behavior of *Ae. albopictus*. Therefore, it was considered that the physical blocking effect was attributed more to the density variation in the hut with 25% opening, as compared with the temperature. Physical blocking effect by walls on house-entering behavior has been reported for African mosquitoes, *Aedes* spp., *Anopheles pharoensis*, *An. squamosus*, *Culex poicilipes* and *Cx. thalassius* (Snow, 1987; Gillies, 1988; Lindsay and Snow, 1988).

In the normal-walled building in Experiment 1, not only the difference in the environmental conditions of the inside from the surrounding vegetation but also the physical blocking might have enhanced the low indoor density of the building.

The present study indicated that the exophagy of *Ae. albopictus* is influenced by at least three factors: (1) the presence of vegetation, (2) difference in environmental conditions of inside of buildings from those in vegetation due to blocking and (2) physical blocking. Biting density of *Ae. albopictus* reported so far was always higher at outdoors than indoors (Hawley, 1988); however, the difference between outdoor and indoor densities varied depending on localities; the ratio of indoor to outdoor density was 1 : 1057 in Thailand (Gould et al., 1970), 1 : 179 in Philippines (Roseboom and Cabrera, 1964), 1 : 3.44 in Seyshelles (Lambrecht, 1971), 1 : 1.42 in Singapore (Chan, 1985) and 1 : 1.28 in Japan (Higa et al., 2000). The different degrees of exophagy among localities observed in the previous studies are partly explainable by the three factors confirmed in this study. The three factors may work in different degrees among localities, and their balance determines the indoor and the outdoor density depending on the environmental conditions of the locality.

Thus, the implication of this study's findings for vector ecology was that it is necessary for the exophagy of *Ae. albopictus* to be evaluated in consideration of, first, its habitat, and then, houses with solid walls providing a clear difference from the surrounding habitat physically and visually. Otherwise, if outdoor collection sites are without vegetation or buildings are those like the net-walled buildings in the present study, it is possible to underestimate the actual features of feeding behavior of *Ae. albopictus*. From this

point of view, ecological method for quantitative study on the endo-/exophagy of *Ae. albopictus* and other vector mosquitoes should be standardized hereafter.

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Prevalence of Lyme disease *Borrelia* in ticks and rodents in northern Kyushu, Japan

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Abstract: Between March and August 1995, the prevalence of Lyme disease *Borrelia* in ticks and rodents in the Kuju Mountains and Sobo Mountains, the highest mountainous areas in the northern half of Kyushu, and also on the Tsushima Islands in the Korean Strait was surveyed. All 20 isolates obtained consisted of 2 *Borrelia garinii* (pattern C′–C on RFLP analysis) from *Ixodes persulcatus* from Kuju, 13 *B. japonica* from *I. ovatus* and rodents from Kuju and Sobo, 1 *B. turdi* from *I. turdus* and 4 *B. tanukii* from rodents from Tsushima. All these are the first official records from this region. This evidence of *Borrelia* is suggestive, considering their dispersal from the eastern China and Korean areas to southwestern Japan, based on biogeographical knowledge.

INTRODUCTION

It is known that the most important pathogens of Lyme disease, *Borrelia garinii* and *B. afzelii*, are primarily transmitted by the *Ixodes persulcatus* tick, the northern species, and maintained among rodents which are fed by the juvenile stages of the tick (Takada, 1995; Yanagihara and Masuzawa, 1997). While such transmission dynamics have been ascertained from the northern to central part of Japan mainland (Nakao et al., 1994; Takada et al., 1994; Ishiguro and Takada, 1996), we hardly isolated any spirochetes from *I. persulcatus* and its host rodents collected throughout southwestern Japan including the Kyushu Region (Takada et al., 1994). At this time, we planned to reinvestigate the distributional range of the tick species and its *Borrelia* prevalence in the northern half of Kyushu Region including the Tsushima Islands in the Korean Strait, and also to discuss the biogeographical relation between Japan and the Asian continent, base on findings of ticks and their host rodents in this region.

MATERIALS AND METHODS

Between March and August 1995, unfed ticks were collected by flagging the vegetation along many mountain paths at elevations of 1,000–1,300 m above sea level halfway up the Kuju Mountains (1,791 m, highest peak with alpine features), and at about 1,200 m in the Sobo Mountains