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Vector Mosquitoes of Japanese Encephalitis (Diptera: Culicidae) in Northern Thailand: Seasonal Changes in Larval Community Structure¹

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Larval mosquito communities in rice fields were examined monthly for 2 years at 3 study areas (15 subareas) near Chiangmai, Thailand. The temporal and spatial variations of community structure were analyzed based on the similarity of species composition among mosquito communities. Subareas were grouped depending on the similarity of the species composition. A large cluster which was composed of several small groups of subareas supporting mosquito communities of a similar species composition was observed in the rainy season. In the subareas of the large cluster, 2 Japanese encephalitis (JE) vector mosquitoes, *Culex vishnui* THEOBALD and *C. tritaeniorhynchus* GILES, occupied more than 60% of the mosquito communities, and a large number of JE vectors appeared from the rice fields. In the dry season, the large cluster of subareas disappeared and the species composition of the mosquito community varied greatly among the subareas. The successional change of mosquito colonization and an asynchronous cultivation of rice plants were suggested as important ecological factors related to the development of rice field mosquito communities.

Key words: Japanese encephalitis, mosquito community, Thailand

INTRODUCTION

Rice fields are the main breeding sites of Japanese encephalitis (JE) vector mosquitoes, and various kinds of organisms appear in an aquatic community of rice fields (SIRIVANAKARN, 1976; HECKMAN, 1979; LACEY and LACEY, 1990). There are clear successional changes in the structure of rice field communities. Successional changes in the colonizing of mosquito species have been observed during the course of rice cultivation (REUBEN, 1971; CHANDLER and HIGHTON, 1975; SNOW, 1983; MOGI and MIYAGI, 1990).

Although the temperature conditions in tropical countries allow the continuous rice cultivation, the availability of rice fields for mosquito breeding depends largely on seasonal changes in rain fall. Artificial control of the water supply to rice fields by irrigation is another important ecological factor determining the community structure of rice field mosquitoes (AMERASINGHE and ARIYASENA, 1990). To understand the development of mosquito communities in rice fields, both the successional changes of the rice field ecosystem and the availability of rice fields for mosquito breeding are important ecological factors.

In Thailand there are 7 potential vectors of JE, and more than one species transmit JE

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in a given area (REUBEN et al., 1994). Ecological and epidemiological studies on JE vectors have been made in northern and central regions of Thailand, and basic ecological characters of JE vectors, such as seasonal prevalence, host preference and flight ability, have been examined (GOULD et al., 1974; BAILEY and GOULD, 1975; SOMBOON et al. 1989; GINGRICH et al., 1992). No comparative studies on community structure of rice field mosquitoes have been made in Thailand. We examined the larval population of JE vectors in 3 areas with different environmental conditions in northern Thailand, and analyzed the variation of larval abundance among the areas in relation to agricultural practices (TAKAGI et al., 1997). In this paper, the seasonal change in larval community structure of rice field mosquitoes was analyzed and the ecological factors related to the development of the mosquito community in rice fields are discussed.

MATERIALS AND METHODS

Study area. Three areas around Chiangmai city were selected for the larval survey. The first study area, Mae Taeng (coded MT), was 50 km north of Chiangmai city. It was situated in a shallow valley and several clumps of dwellings were scattered within 4 km along an unpaved road. The flat areas were used as terraced rice fields or for dwellings. The slopes behind the flat areas were occupied by orchards and secondary forest. The second area, Mae Joh (coded MJ), was in a developing, moderately populated area, 15 km north of Chiangmai city. A wide flat area was used for rice cultivation but the area had been reduced by road and house construction. Beside the newly constructed road, unmanaged rice fields and ditches were found in large numbers and small patches with trees and shrubs were distributed among the rice fields. The third study area, Nong Tong (coded NT), was in a typical rice cultivation area, 20 km south of Chiangmai city. A modern irrigation system had been developed and rice was produced in a wide flat area.

Sampling of larvae. In each study area, five subareas were selected for larval survey. Mosquito larvae were sampled every month by a dipper (13 cm in diameter and 6.5 cm in depth) from September 1991 to December 1993. Forty samples, each of which consisted of 10 dips made at the same rice field, were collected in one subarea. The collected larvae were kept in a small glass vial with 15 ml of 70% alcohol for later identification in the laboratory. For each sample, the height of rice plants was measured and the appearance of flower or seed was recorded.

Data analysis. Only the data of culicine mosquitoes were analyzed in this paper. The total number of larvae collected was calculated and the species composition was compared among the 3 study areas. The seasonal changes in species composition were also analyzed. A test of independence using a *G*-test (SOKAL and ROHLF, 1981) was used for the analysis of variation in species composition. To measure the species diversity, a Shannon-Weaver index was calculated by the equation, $H = -P_i \log_e P_i$, where P_i is the proportion of the species (i) in the total sample of larvae.

Based on the seasonal variation of rainfall, 4 seasons were distinguished; November to January (early dry season), February to April (late dry season), May to July (early rainy season) and August to October (late rainy season). Seasonal variations in the proportion of the 2 dominant species (*Culex vishnui* THEOBALD and *C. tritaeniorhynchus* GILES) were analyzed by ANOVA using the arcsine transformation, and multiple comparisons of the averages were made by the TUKEY and KRAMER method.

To show the seasonal development of mosquito community, variations of community

structure among subareas were analyzed in each season based on the similarity of the species composition. Species composition (%) was calculated for each subarea using the average larval density in each season. The percentage similarity (WHITTAKER, 1952) was used to calculate the similarity of species composition between subareas and a similarity matrix was derived for each season. For each season the similarity of species composition among subareas is shown in a figure where each subarea was plotted using the observed proportion of the 2 most abundant species in the season: *C. bitaeniorhynchus* and *C. tritaeniorhynchus* in the dry season of 1991–1992, and *C. vishnui* and *C. tritaeniorhynchus* in the other seasons. The subareas having more than 70% similarity were grouped by a box in the figure.

As an environmental factor related to colonization of mosquito species in a rice field, the height of rice plants was analyzed using data from the late rainy seasons in 1992 and 1993. The height of rice plants was broken down by 10 cm intervals into 10 classes (from 10 cm to >100 cm). Average densities of *C. vishnui*, *C. tritaeniorhynchus*, and *C. gelidus* were calculated for each class. The variations in height of rice plants among the study areas were analyzed by ANOVA. All statistical analyses were performed using a Systat statistical software package.

RESULTS

Culex vishnui and *C. tritaeniorhynchus* were the most abundant in the present study followed by *C. gelidus* THEOBALD and *C. bitaeniorhynchus* GILES (Table 1). The proportion of these 4 species to the total number was about 92%. Although *C. fuscocephala* THEOBALD was abundant in previous studies in northern Thailand, the density in this study was very low, accounting for only 0.63% of the total number. The species compositions differed significantly among the study areas ($G_{adj} = 2607.44$, $p < 0.01$). The mosquito community in MJ showed the lowest species diversity and the total number of larvae collected was the largest.

Seasonal variation in species composition was significant ($G_{adj} = 3873.8$, $p < 0.01$), and species diversity was higher in the dry season than in the rainy season (Table 2). The average proportion of *C. vishnui* in the late rainy season was significantly higher than in the other seasons, whereas no significant differences were found among seasons in the average

Table 1. A list of culicine mosquitoes, the number of larvae collected and species diversity in the 3 study areas

Species	Study area			Total (%)
	MT	MJ	NT	
<i>Culex vishnui</i> THEOBALD	526	3,441	1,188	5,155 (33.57)
<i>C. tritaeniorhynchus</i> GILES	853	2,250	1,295	4,398 (28.64)
<i>C. gelidus</i> THEOBALD	97	1,801	1,036	2,934 (19.10)
<i>C. bitaeniorhynchus</i> GILES	418	348	900	1,666 (10.85)
<i>C. quinquefasciatus</i> SAY	81	49	411	541 (3.52)
<i>C. pseudovishnui</i> COLLESS	266	175	90	531 (3.46)
<i>C. fuscocephala</i> THEOBALD	27	37	32	96 (0.63)
<i>C. whitmorei</i> (GILES)	29	5	1	35 (0.23)
Total	2,297	8,106	4,953	15,356 (100)
Species diversity	1.624	1.331	1.644	1.561

Table 2. Seasonal variation of species composition and species diversity

Species	Dry season		Rainy season	
	Early	Late	Early	Late
<i>Culex vishnui</i>	344	867	650	3,294
<i>C. tritaeniorhynchus</i>	1,061	880	1,334	1,123
<i>C. gelidus</i>	391	254	1,313	976
<i>C. bitaeniorhynchus</i>	298	581	117	670
<i>C. quinquefasciatus</i>	190	267	84	0
<i>C. pseudovishnui</i>	55	220	147	109
<i>C. fuscocephala</i>	26	4	32	34
<i>C. whitmorei</i>	10	1	2	22
Total	2,375	3,074	3,679	6,228
Species diversity	1.558	1.648	1.411	1.295

Table 3. Average proportion of *C. tritaeniorhynchus* and *C. vishnui* in each season

Season	<i>C. tritaeniorhynchus</i>	<i>C. vishnui</i>
Early dry	39.8 a	21.4 a
Late dry	31.6 a	22.6 a
Early rainy	40.5 a	20.6 a
Late rainy	30.9 a	44.6 b

Averages in the same column followed by the same letter are not significantly different (TUKEY and KRAMER method, $p > 0.05$).

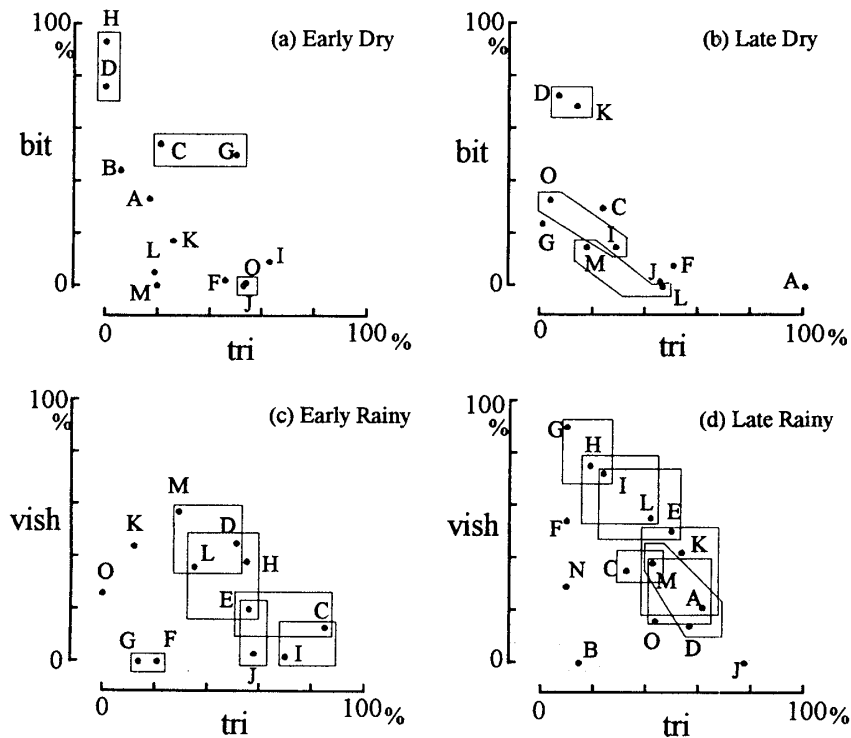


Fig. 1. Similarity of species composition among subareas in four seasons of 1991–1992. Letters A–E, F–J, and K–O show subareas of MT, MJ, and NT, respectively. The axes bit, vish, and tri are the proportion of *C. bitaeniorhynchus*, *C. vishnui*, and *C. tritaeniorhynchus*, respectively. The subareas in the same box showed more than 70% similarity of species composition.

proportion of *C. tritaeniorhynchus* (Table 3).

Only 3 groups of subareas showed more than 70% similarity in the early and late dry season of 1991–1992, and each of these groups did not overlapped (Figs. 1a and b). However, in the early rainy season of 1992, there were 6 groups of subareas having more than 70% similarity (Fig. 1c). The subareas of the 5 groups overlap each other and thus, the 5

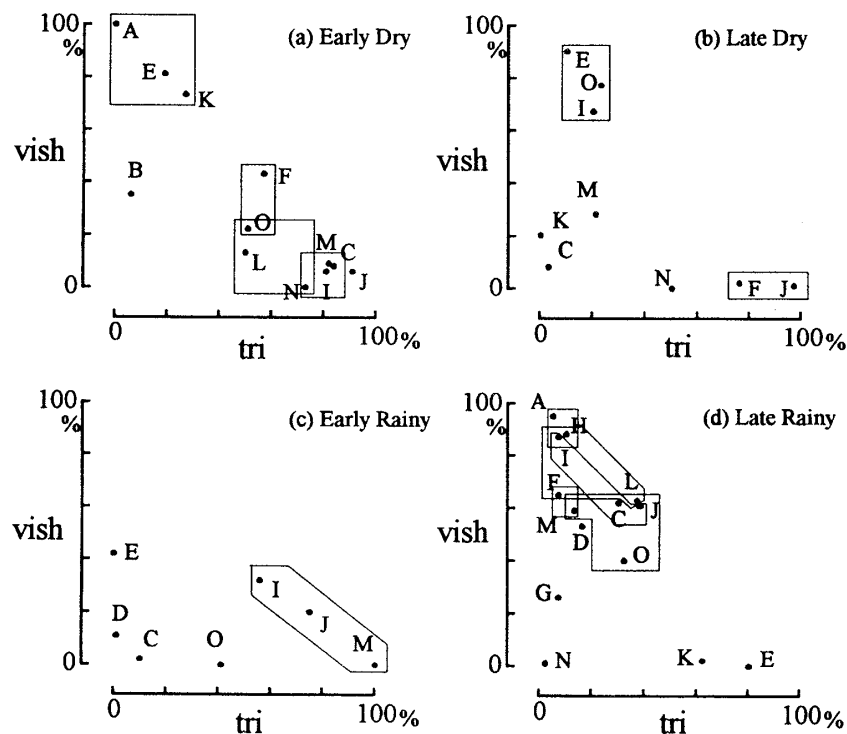


Fig. 2. Similarity of species composition in four seasons of 1992–1993. Letters A–E, F–J, and K–O show subareas of MT, MJ, and NT, respectively. The horizontal and vertical axes are the proportion of *C. tritaeniorhynchus* and *C. vishnui*, respectively. The subareas in the same box showed more than 70% similarity of species composition.

groups made up a large cluster of subareas as a whole. One large cluster of subareas, which consisted of 7 small groups, appeared in the late rainy season of 1992 (Fig. 1d). Eleven out of 15 subareas were included in the large cluster.

The large cluster of subareas broke down and only 4 groups of subareas were observed in the early rainy season of 1992–1993 (Fig. 2a). The groups of subareas continued to break down until the early rainy season in 1993, where only one group of subareas showed more than 70% similarity (Figs. 2b and c). Thereafter, reconstruction of a large cluster of subareas was observed in the late rainy season of 1993 (Fig. 2d). Nine out of 14 subareas were included in a large cluster. In the large clusters, 2 dominant species, *C. vishnui* and *C. tritaeniorhynchus*, occupied >60 and >69% of the mosquito communities in 1992 and 1993, respectively.

In the late rainy season, the variations among areas in height of the rice plants were significant both in 1992 ($F=3.63$, $p<0.001$) and 1993 ($F=41.16$, $p<0.001$). In 1992, the average heights of rice plants in most subareas ranged from 50 to 80 cm, whereas in 1993, ranged from 40 to 60 cm, suggesting a delay in rice cultivation that year (Fig. 3). Among the 3 study areas, larger variation in the height of rice plants at MT was outstanding.

Negative correlations between the height of rice plants and larval density were observed for *C. vishnui* in 1992 and 1993 (Fig. 4). No clear relationships were found between height of the rice plants and larval density of *C. tritaeniorhynchus*. Larval densities of *C. gelidus* were high in rice fields with plants of <30 cm or >90 cm in 1992, whereas they were high at the intermediate height of rice plants in 1993.

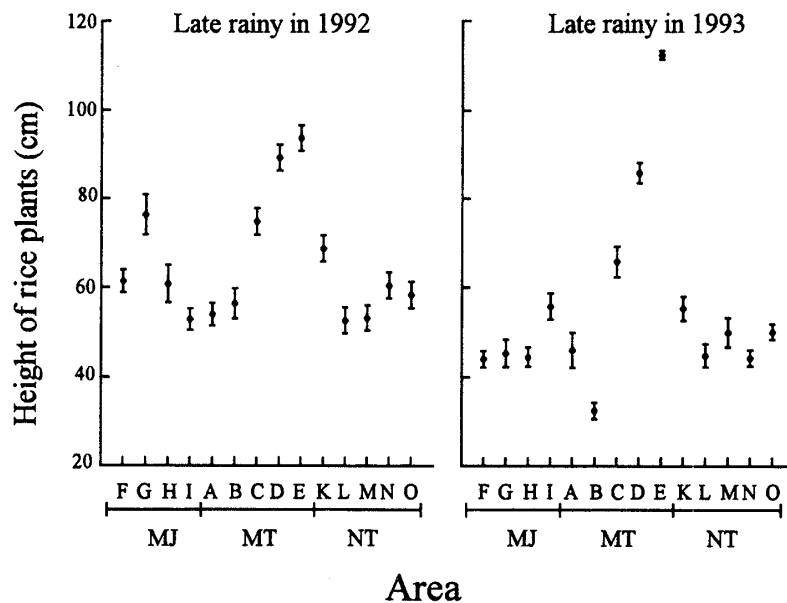


Fig. 3. Average height of rice plants (\pm S.E.) in each subarea in the late rainy seasons in 1992 and 1993.

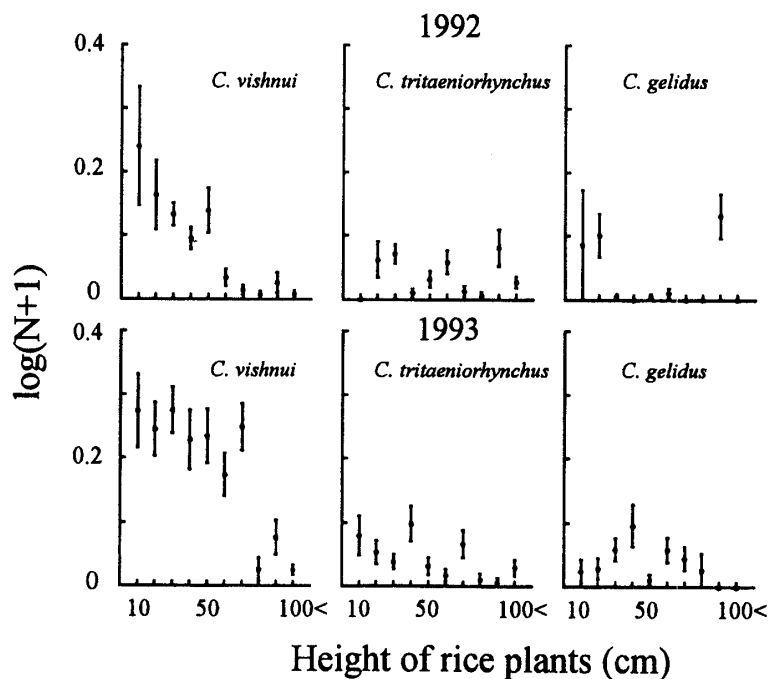


Fig. 4. Relationship between height of rice plants and larval density (\pm S.E.) of 3 *Culex* mosquitoes in the late rainy seasons of 1992 and 1993.

DISCUSSION

A dynamic change in community structure of rice field mosquitoes was clearly shown

in the present study. Because temperature conditions in the tropics allow the continuous breeding of mosquitoes, seasonal changes in the availability of agricultural fields for mosquito breeding caused by seasonality of rain fall is one of the main factors affecting the community dynamics of rice field mosquitoes. Except for ditches and those rice fields which receive water from irrigation channels in the dry season, a large part of agricultural fields dry up, and the structure of the mosquito community changes drastically in the dry season.

A large cluster of subareas which are composed of several small groups of subareas having more than 70% similarity in species composition developed in the rainy season. In the large cluster, 2 JE vector species, *C. vishnui* and *C. tritaeniorhynchus*, occupied >60% of the mosquito communities and thus, a large part of rice fields produced JE vectors in large numbers during the rainy season. The proportion of the 2 dominant species, *C. vishnui* and *C. tritaeniorhynchus*, differed slightly among the subareas, resulting in a serial difference in the similarity of species composition between subareas. The development of a large cluster of subareas was recognized from May to July (early rainy season) in 1992, whereas in 1993 it was observed from August to October (late rainy season). The delayed development of a large cluster in 1993 was mainly ascribed to the delay of rice cultivation which was suggested in Fig. 3.

The main ecological mechanism for the development of a large cluster of subareas is the successional changes in the rice field ecosystem. During the course of rice cultivation, successional changes in the aquatic community occur (HECKMAN, 1979) and a variety of breeding sites are provided for mosquito breeding depending on the water quality and biological conditions of the field. Successional changes in colonization of mosquito species in rice fields have been examined in relation to the phase of rice cultivation (CHANDLER and HIGHTON, 1975; MOGI and MIYAGI, 1990). Between *C. vishnui* and *C. tritaeniorhynchus*, it is found that the former appeared in the earlier phase of rice cultivation, whereas the later was collected from rice fields of every phase (REUBEN, 1971; MOGI and MIYAGI, 1990). The results in Fig. 4 appear to support this finding. The seasonal variations of the proportion of *C. vishnui* and *C. tritaeniorhynchus* observed in this study may be partly explained by different responses of the two species to environmental changes.

The asynchronous cultivation of rice plants is probably another important factor related to the development of a large cluster of subareas supporting mosquito communities of a similar species composition. A wide variation in height of rice plants observed in late rainy season suggested asynchronous cultivation of rice plants in the 3 study areas. The height of rice plants has been suggested to be related to the appearance of mosquito species in rice fields (SURTEES et al., 1970; REUBEN, 1971; CHANDLER and HIGHTON, 1975; CHAMBERS et al., 1979; PRASAD et al., 1990; TAKAGI et al., 1996). From the different relationships between larval density and height of rice plants among 3 *Culex* mosquitoes in Fig. 4, it is expected that rice fields with rice plants of different heights are colonized by different mosquito species and support various mosquito communities which make up a cluster of communities as a whole in the rainy season.

In the dry season, the species composition of the mosquito community differed greatly, and only a few subareas showed more than 70% similarity. This result suggests that the quality of water and the biological conditions of agricultural fields available for mosquito breeding differ greatly from place to place, and the small number of collected larvae may reflect the small area of available agricultural fields.

There was a significant difference in species composition of mosquitoes among the 3 study areas. MT had a higher species diversity with a lower mosquito abundance, while the

opposite was observed in MJ. The variation in height of rice plants among subareas was the largest in MT, and rice fields of this area were small, separated by orchards, human dwellings, and secondary forest. These environmental characteristics in MT are quite different from NT and MJ which were characterized by more uniform and larger rice fields, and the diverse environment in MT may support a variety of mosquito species.

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