

Influence of the Distribution of Host Species on Adult Abundance of Japanese Encephalitis Vectors—*Culex vishnui* Subgroup and *Culex gelidus*—in a Rice-Cultivating Village in Northern Vietnam

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Abstract. A field study was conducted in a village in northern Vietnam to investigate how host distribution influences Japanese encephalitis (JE) vector abundance. Indoor and outdoor collections were conducted from 50 compounds. We collected three JE vector species—*Culex tritaeniorhynchus* and *Culex vishnui* that comprised the *Culex vishnui* group and *Culex gelidus*. Spatial autocorrelation was not observed in the mosquito assemblies at any scale larger than the house compounds. Multivariate analyses revealed that the *Cx. gelidus* density correlated positively with both the host proximity to the breeding sites and cattle density; however, the *Cx. vishnui* subgroup density correlated positively only with cattle density. These results showed that the number of cattle in a compound influenced the JE vector abundance in that compound, and the abundance of *Cx. gelidus*, not of the *Cx. vishnui* subgroup, was affected by the host proximity to the breeding sites in the village.

INTRODUCTION

In Asian countries, *Culex tritaeniorhynchus* Giles, *Culex vishnui* s.l., *Culex fuscocephala* Theobald, and *Culex gelidus* Theobald have been implicated as vectors of Japanese encephalitis (JE).^{1–6} Although these mosquitoes feed more commonly on pig and cattle blood than on chicken and human blood,^{7–9} their feeding patterns vary depending on host availability. At any location, the feeding pattern of mosquitoes is largely governed by two parameters: 1) their innate tendency to respond to particular cues and 2) host availability. The term “host preference” can be used to describe the expression of these parameters; however, this feature is difficult to quantify because of the variability of host biomass.¹⁰ Therefore, studies on the feeding patterns of JE vectors in Asia have produced varying results, depending on the relative abundance of the host population and the sampling procedures used. In India, where the cattle population is higher than the pig population, 86–98% of all the blood meals ingested by the vectors are from cattle hosts.¹¹ On the other hand, in Okinawa, Singapore, and Taiwan, where the pig population is higher than the cattle population, up to 60% of the vector blood meals were from pig hosts.^{7,12,13} One study reported that 75–85% of the JE vectors obtained from the Chiang Mai valley, Thailand, fed on cattle blood,³ whereas another study conducted at the same location reported that 75–80% of the mosquitoes fed on pig blood.¹⁴ Furthermore, several studies have reported mixed blood feeding by *Cx. tritaeniorhynchus*.^{7,14–16}

We experimentally showed that three species of field-collected JE vectors, namely, *Cx. vishnui* s.l., *Cx. tritaeniorhynchus*, and *Cx. gelidus* from Chiang Mai, Thailand, preferred to feed on cattle blood than on pig blood.^{17,18} All three species fed on cattle in significantly higher proportions than they did on pigs when exposed to these hosts in isolation or in combination in a net. Light-trap catches obtained from the same field site revealed that < 10% of the mosquitoes had fed and that *Cx. vishnui* s.l. and *Cx. tritaeniorhynchus* had fed

more on pig blood than on cattle blood, whereas *Cx. gelidus* had mainly fed on cattle blood.¹⁸ These results indicated that the host-feeding patterns of these three JE vectors depended on host availability. Moreover, the former two species seemed to be more affected by host availability than the latter species. Thus, the extent to which host availability influences the choice of host may differ among vector species.

In this study, we focused on the manner in which mosquitoes distribute themselves on arrival in the vicinity of a host. However, it is difficult to define an appropriate scale for measuring the zone of host attraction. The host-seeking process involves several steps. First, the vector is stimulated by cues from the host, representing the initial step in a behavioral sequence that culminates in the arrival of the vector at the host site. Stimulation by appropriate wind-borne host cues activates a responsive state in mosquitoes; this elicits upwind flight that might attract the mosquito closer to a potential host; furthermore, different cues influence its final approach such that the mosquito ultimately alights on the host.¹⁰ Gilles and Wilkes¹⁹ carried out experiments that compared the range within which mosquitoes are attracted by animal baits and the carbon dioxide released by them. The number of *Cx. tritaeniorhynchus* caught decreased significantly up to a range of 36 m from both the animal baits and the carbon dioxide released by them; no further decrease was observed beyond this range. Considering this result, the zone of host attraction may possibly extend to a range of hundreds of meters.

With regard to host availability, it is necessary to determine the spatial resolution to describe host density. Therefore, by performing spatial autocorrelation analyses, we first determined the spatial scale representing the maximum heterogeneity in vector distribution. We used the best resolution to determine the mosquito density for multivariate analysis to examine the relationship between mosquito and animal host distributions.

We conducted a field study to elucidate the relationship between the host species and mosquito distributions within a rice production area in northern Vietnam. We determined the mosquito and host abundance in 50 compounds and the host abundance in an additional 29 compounds to determine the correlation between the mosquito and vertebrate host density. We carried out indoor and outdoor mosquito catches by

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using aspirators and light traps in the 50 compounds to 1) examine the distribution patterns (e.g., uniform, random, or aggregated) of the JE vectors among the compounds, 2) identify the spatial scale representing the maximum heterogeneity or similarity in vector distribution, and 3) examine whether the distribution of any domestic animal influences the mosquito distribution.

MATERIALS AND METHODS

Study area. The study was conducted in an area where JE transmission is endemic, namely, CatQue village in Hatay Province, which lies 60 km northeast of Hanoi in northern Vietnam (21°05' N, 105°68' E). Most of the village inhabitants were rice farmers; they lived in settlements that were situated within close proximity of each other and were surrounded by rice fields. For the study area, we selected a part of the village that lay adjacent to the rice fields to minimize the effect of the flight distance of the mosquitoes between the breeding sites and the hosts (Figure 1). A typical family in the village maintained livestock in their compound, including cattle, pigs, chickens, and ducks. A compound was made up of a human residence and a shed for the animals. Thus, the people lived in close proximity to their livestock, i.e., within a distance of 5–6 m. A typical house was made up of a large room (functioning as a living room, dining room, and bedroom) and, in some cases, other smaller rooms. These houses had an opening between the wall and the roof for ventilation. The doors and windows were generally kept open until the people retired for the day.

Mosquito collection. Adult mosquitoes were collected from 50 adjacent compounds from June 16, 2003 to June 26, 2003. The compounds were selected such that they were located adjacent to each other as far as possible, and the compound at which we initiated collection was selected randomly. Sampling was essentially conducted twice from all compounds except one, from which sampling was conducted only once. Indoor and outdoor mosquito catches were carried out from each compound by two teams made up of four members each. The first collection was completed within 5 days, and the second collection was initiated after an interval of 5 days. The weather was stable (temperature range, 25.8–32.6°C) except a rainy day on which sampling was not performed. All the

sampled mosquitoes were morphologically studied, and their species or genus was identified.

Indoor collection. Pyrethrum-spray collections (PSCs)²⁰ were carried out from the compounds between 7:00 PM and 11:00 PM. Commercially available spray-type insecticide containing 0.08% prallethrin and 0.075% d-phenothrin was used. Before spraying, white sheets were spread out on the floor and were used to cover all the open doors. After spraying the insecticide for 1 minute and waiting for 5 minutes, all knocked-down mosquitoes were collected using aspirators or tweezers. Only the large room, which served as a living room, dining room, and bedroom, was sprayed. The abdominal contents of the fed mosquitoes were smeared on filter papers to determine their blood-meal hosts.

Outdoor collection. Centers for Disease Control (CDC)-type light trap collections were carried out from 7:00 PM to 9:00 AM without using dry ice. We hung one light trap in each compound at a point that was approximately equidistant from the host animals of various species in that compound. The mosquito collection time was divided into an early night period (7:00 PM to 11:00 PM) and a late night period (11:00 PM to 9:00 AM). However, because of sample desiccation, we could not identify the blood-meal hosts of the mosquitoes that were collected using the light traps.

Assessment of the stability of the mosquito count between the two collections. We assessed the stability of the mosquito count between indoor collections in each of 47 compounds and outdoor collections in each of 13 compounds and used the Kendall coefficient of rank correlation to determine the relative stability during the study period.

Identification of the *Cx. vishnui* subgroup species by polymerase chain reaction. A fraction of the samples of the *Cx. vishnui* subgroup comprising three morphologically similar species, namely, *Cx. tritaeniorhynchus*, *Cx. vishnui* Theobald, and *Cx. pseudovishnui* Colles, was maintained in 95% ethanol for further molecular verification.²¹ Based on the comparative analysis of the nucleotide sequence of the first internal transcribed spacer in the ribosomal DNA gene array, we used three species-specific primers for the polymerase chain reaction (PCR) assay.

Identification of the blood-meal hosts. To identify the blood-meal hosts of the mosquitoes that were collected indoors, we used the sandwich enzyme-linked immunosorbent assay (ELISA) method described by Chow and others²² with minor modifications. Each blood meal-smeared filter paper was tested for anti-human, anti-swine, anti-bovine, and anti-chicken antibodies using the commercially purchased anti-species IgG antibody as the capture antibody and horseradish-conjugated anti-species IgG antibody as the detector antibody. Each test plate was made up of wells wherein host sera were added as positive controls and heterologous sera were added as negative controls. The reactions were performed in duplicate.

Environmental factors. We counted the number of domestic animals and people residing in the 79 compounds, including the 50 compounds that were sampled for mosquitoes. Because the body size of pigs varies greatly depending on their age, the pigs were categorized based on their weight as follows: > 60 kg as Pig L, 20–60 kg as Pig M, and < 20 kg as Pig S. The location of each compound was recorded using a handheld global positioning system (GPS) and used as the representative location for analyzing both the indoor and outdoor

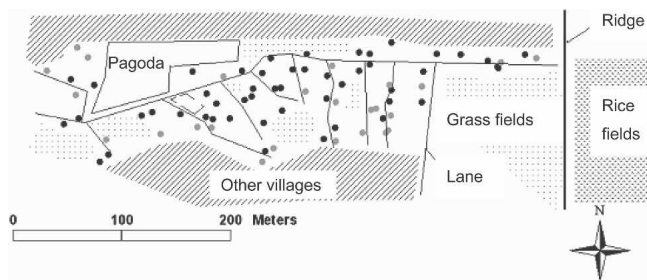


FIGURE 1. Map of the study area indicating the location of the sampled house compounds. The black dots indicate the sampled compounds, whereas the gray dots indicate the non-sampled compounds. The edge of the nearest rice field is represented by the reference line from which the minimum distance to the compound was measured. The *Cx. vishnui* subgroup was made up of the *Cx. tritaeniorhynchus* Giles and *Cx. vishnui* Theobald species.

collections from that particular compound. The minimum distance between each compound and the nearest ridge of a rice field (Figure 1)—considered to be the breeding site of the *Cx. vishnui* subgroup—was calculated using ArcView3.2 software (ESRI, Redlands, CA).

Data analysis. *Characterization of the spatial aggregation of each host and mosquito species.* The degree of aggregation of a specific host as well as that of each mosquito species was estimated using the crowding index $J = V/M^2 - (1/M)$, where V is the variance, M is the mean density of a species,²³ $J = 0$ indicates a random distribution; $J > 0$ indicates an aggregated distribution; and $J < 0$ indicates the tendency for a uniform distribution. The F test was used to determine whether the sample distribution differed significantly from the random distribution ($F = V/M$, $v_1 = n - 1$, and $v_2 = \infty$).²⁴ The mosquito abundance was standardized as one catch per compound to enable its application for the analysis of each of the three data sets. To increase the statistical reliability, only the species that could be collected from > 10 compounds were included in the analysis.

Spatial autocorrelation analysis. We applied autocorrelation analysis to identify the optimum spatial resolution for correlating environmental factors and mosquito distribution. We used the Moran I correlogram to determine whether there was a significant clustering of mosquitoes in the study area.^{25,26} Moran I values were determined for the female culicine clusters over a series of distances by using the following three data sets: 1) the indoor collection between 7:00 PM and 11:00 PM (early night), 2) the outdoor collection between 7:00 PM and 11:00 PM, and 3) the outdoor collection between 11:00 PM and 9:00 AM (late night). This index compares the geographic neighbors in terms of their deviation from the mean of all observations. The Moran I for a given lag distance class is calculated as $I = n \sum \sum w_{ij} z_i z_j / S_0 \sum z_i^2$, where n is the number of sampling stations; $z_i = (x_i - M)$ and $z_j = (x_j - M)$, where x_i or x_j is the observation at the i^{th} or j^{th} station; w_{ij} is a weight that denotes the connection between the i and j stations (e.g., 1 for neighbors and 0 otherwise); and s_0 is the sum of the weights. When I is significantly positive, the observations that are obtained from the sampling stations separated by a distance falling within the analyzed lag distance class tend to be similar; when I is significantly negative, these observations tend to be dissimilar. In the absence of spatial autocorrelation, the expected value of I is close to 0. The

analyses were based on the following five lag classes (i.e., distances between compounds with mutually exclusive intervals): 0 to < 20, 20 to < 40, 40 to < 60, 60 to < 80, and 80 to < 100 m. The significance of the correlogram for spatial autocorrelation was tested using a permutation test ($N = 1,000$).

Relationship between mosquito abundance and environmental factors. We performed a redundancy analysis (RDA) as a direct linear gradient analysis by the canonical ordination method to detect patterns of variation in the female culicine clusters and to simultaneously analyze the complex correlation between them and several environmental factors. The spatial unit that was detected by the Moran I as having the maximum heterogeneity for *Culex* mosquito distribution was applied in the analysis. We used three data sets for RDA and applied the following explanatory variables: the number of animals of each host species, the minimum distance between a compound and the nearest rice field, and the number of male culicines. The variable of male abundance was used to represent the proximity of species-specific breeding sites. A number of breeding sites of *Cx. gelidus* and *Culex quinquefasciatus* Say were found around the houses. It was difficult to integrate the proximity of the breeding sites with their mosquito productivities. It is known that, generally, a high density of male mosquitoes is found in the proximity of a larval habitat. Therefore, we used the variable of male abundance instead of the integrated values of the distances from multiple breeding sites. The response variable used was the total number of female mosquitoes. CANOCO software version 4.5 (Microcomputer Power, Ithaca, NY) was used for RDA. The significance of the relationship between mosquito density and environmental variables was determined by Monte Carlo permutation tests ($N = 1,000$). To clarify the relationship between mosquito abundance and each environmental factor, linear correlation analyses and analyses of variance (JMP version 5.0.1.2; SAS Institute, Cary, NC) were conducted using variables that had shown significant correlations in the RDA.

RESULTS

Species composition of mosquitoes. A total of six mosquito genera, including four JE vector species, were collected (Table 1). The most dominant species found indoors was *Cx. quinquefasciatus*, followed by the *Cx. vishnui* subgroup and *Cx. gelidus* (Table 2). On the other hand, *Cx. gelidus* was the

TABLE 1

Total number of adult mosquitoes collected indoors (97 catches by the PSC) and outdoors (60 catches by light-trap) at 50 compounds in a rice-cultivating village in northern Vietnam

Species	Indoor		Outdoor			
	7:00 PM to 11:00 PM		7:00 PM to 11:00 PM		11:00 PM to 9:00 AM	
	Number	Percent	Number	Percent	Number	Percent
The <i>Cx. vishnui</i> subgroup*	611	16.0	276	29.2	398	18.9
<i>Culex gelidus</i> Theobald	515	13.4	549	58.0	1573	74.7
<i>Culex fuscocephala</i> Theobald	2	0.1	0	0	1	0.0
<i>Culex quinquefasciatus</i> Say	1894	49.5	16	1.7	15	0.7
<i>Anopheles</i> spp.	361	9.4	51	5.4	60	2.9
<i>Mansonia</i> spp.	329	8.6	32	3.4	28	1.3
<i>Armigeres</i> spp.	61	1.6	9	1.0	18	0.9
<i>Aedes albopictus</i> Skuse	49	1.3	5	0.5	2	0.1
<i>Culex (Lophoceraomyia)</i> spp.	8	0.2	8	0.8	10	0.5
Total	3,830		946		2,105	

* The *Cx. vishnui* subgroup was made up of *Cx. tritaeniorhynchus* Giles and *Cx. vishnui* Theobald.

TABLE 2
Total number of medically important culicine mosquitoes classified by sex and feeding status

		The <i>Cx. vishnui</i> subgroup			<i>Culex gelidus</i>			<i>Culex quinquefasciatus</i>		
		Indoor		Outdoor	Indoor		Outdoor	Indoor		Outdoor
		7:00 PM to 11:00 PM	7:00 PM to 11:00 PM	11:00 PM to 9:00 AM	7:00 PM to 11:00 PM	7:00 PM to 11:00 PM	11:00 PM to 9:00 AM	7:00 PM to 11:00 PM	7:00 PM to 11:00 PM	11:00 PM to 9:00 AM
Female	Unfed	325	146	247	194	301	916	458	3	5
	Fed	201	97	131	109	88	363	429	3	1
	Gravid	21	1	2	14	1	11	32	0	2
Male		64	32	18	198	159	283	975	10	17
Total		611	276	398	515	549	1,573	1,894	16	25

most dominant species found outdoors, followed by the *Cx. vishnui* subgroup (Table 2). Of the 275 PCR amplifications attempted, 130 were successful; based on these analyses, 79% of the captured specimens were identified as *Cx. tritaeniorhynchus* and 21% as *Cx. vishnui*. Some specimens that were morphologically identified as *Cx. tritaeniorhynchus* were shown to be *Cx. vishnui* (15 of 261) by PCR analyses; therefore, both *Cx. tritaeniorhynchus* and *Cx. vishnui* were considered as part of the *Cx. vishnui* subgroup in this study. The number of *Cx. vishnui* subgroup and *Cx. gelidus* mosquitoes was higher in the outdoor collections, whereas that of *Cx. quinquefasciatus* mosquitoes was higher in the indoor collections. Furthermore, the number of *Cx. vishnui* subgroup and *Cx. gelidus* mosquitoes collected between 11:00 PM and 9:00 AM was higher than that collected between 7:00 PM and 11:00 PM (two-sample Wilcoxon test; $Z = 9.46$ and $P < 0.001$ versus $Z = 4.11$ and $P < 0.001$; Table 2). For the indoor collections, the stability of the mosquito count between the two collections was found to be statistically significant, with a Kendall coefficient of 0.34 ($P = 0.004$) for female *Cx. gelidus* mosquitoes, 0.27 ($P = 0.012$) for female *Cx. vishnui* subgroup mosquitoes, 0.27 ($P = 0.012$) for female *Cx. quinquefasciatus* mosquitoes, and 0.28 ($P = 0.018$) for male *Cx. quinquefasciatus* mosquitoes. However, for the outdoor collections, the stability of the mosquito count showed no statistical significance for any of the species.

Identification of blood-meal hosts. A total of 780 engorged culicines were collected indoors; their blood-meal sampling accounted for 568 samples that tested positive for a single host. We observed that both the *Cx. vishnui* subgroup and *Cx. gelidus* had mainly fed on cattle and pigs (Table 3). Furthermore, a comparative analysis of the *Cx. vishnui* subgroup and *Cx. gelidus* revealed no significant difference in the proportion in which they fed on either cattle ($\chi^2 = 0.93$, $P = 0.33$) or pigs ($\chi^2 = 1.23$, $P = 0.27$). These species had also fed on human blood. *Cx. quinquefasciatus* had mainly fed on human and chicken blood; < 10% of these mosquitoes had fed on pig

and cattle blood. Mixed blood meals were detected in 15 (9%) of the 164 *Cx. vishnui* subgroup mosquitoes, 3 (4%) of the 70 *Cx. gelidus* mosquitoes, and 16 (5%) of the 299 *Cx. quinquefasciatus* mosquitoes. The mixed blood-meal combinations were as follows: 2 of the *Cx. vishnui* subgroup mosquitoes had ingested human and cattle blood, and 13 of these mosquitoes, along with 3 of the *Cx. gelidus* mosquitoes, had ingested cattle and pig blood. A variety of mixed blood meal combinations was detected in the *Cx. quinquefasciatus* mosquitoes as follows: three ingested human and pig blood; two ingested human and cattle blood; five ingested human and chicken blood; two ingested pig and cattle blood; one ingested pig and chicken blood; two ingested cattle and chicken blood; and one ingested human, pig, and chicken blood.

Host abundance and degree of aggregation. There were 370 humans, 787 pigs (Pig L, 131; Pig M, 554; and Pig S, 112), 48 cattle, 3,852 chickens, 144 dogs, and 141 ducks in the study area during the study period. With the exception of humans ($J = -0.1$, $F = 0.53$, $P > 0.05$), most of the hosts showed a significantly aggregated distribution ($P < 0.05$) to a certain degree (chicken: $J = 15.8$, $F = 773.7$; duck: $J = 8.4$, $F = 15.9$; Pig S: $J = 5.1$, $F = 8.3$; cattle: $J = 3.9$, $F = 3.3$; Pig L: $J = 3.5$, $F = 6.9$; Pig M: $J = 2.5$, $F = 18.7$; dog: $J = 0.3$, $F = 1.52$).

Female mosquito distribution. Mosquitoes collected indoors. The *Cx. vishnui* subgroup, *Cx. gelidus*, and *Cx. quinquefasciatus* mosquitoes were significantly aggregated ($F = 11.10$, 8.64, and 23.08, respectively; $J = 0.88$, 1.12, and 1.12, respectively; $P < 0.05$).

Mosquitoes collected outdoors. The *Cx. vishnui* subgroup and *Cx. gelidus* were significantly aggregated throughout the night (7:00 PM to 11:00 PM: $F = 13.9$, $J = 3.20$ versus $F = 15.3$, $J = 2.12$, $P < 0.05$; 11:00 PM to 9:00 AM: $F = 61.85$, $J = 10.23$ versus $F = 28.11$, $J = 1.10$, $P < 0.05$).

Spatial autocorrelation. No significant spatial autocorrelation was detected in the three *Culex* species at any lag distances, except for the distribution of the *Cx. vishnui* subgroup specimens collected indoors that showed the highest spatial autocorrelation at lag distance < 20 m (Moran $I = 0.57$, $P = 0.001$; Figure 2). Mosquito clusters were not observed at scales larger than the house compound unit. Therefore, for further analysis, we used the mosquito density determined for each compound.

Relationship between mosquito abundance and environmental factors. To analyze the manner in which environmental factors influenced the aggregation of female mosquitoes, we conducted an RDA. For this purpose, we used the mosquito and host animal densities that were obtained for each compound based on the results of the spatial autocorrelation

TABLE 3

Blood-meal hosts of engorged medically important culicines collected indoors

Species	Number of positive reactions	Host species (%)			
		Human	Cattle	Pig	Chicken
The <i>Cx. vishnui</i> subgroup	179	4	59	35	2
<i>Culex gelidus</i>	73	4	66	27	3
<i>Culex quinquefasciatus</i>	316	76	5	4	15

The percentage was calculated with a number of positive reactions for one host species divided by total number of positive reactions in each mosquito species.

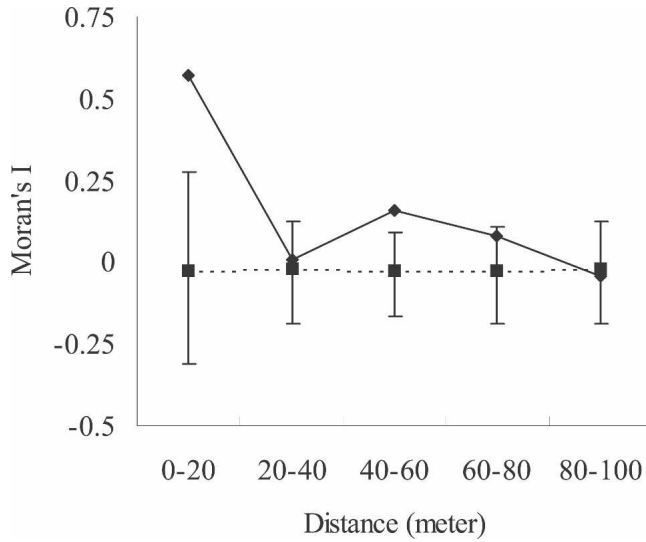


FIGURE 2. A spatial autocorrelation correlogram for female mosquitoes of the *Cx. vishnui* subgroup that were collected indoors. The solid line represents the observed value, whereas the dotted line represents the expected value under the null hypothesis of a random distribution; 95% confidence intervals are also shown.

analysis. We used the mosquito count per mosquito catch in a compound as the response variable. We analyzed 97 mosquitoes for the indoor collection and 60 for the outdoor collection. We performed a preliminary RDA using either the absolute number or the square-transformed value of the explanatory variables; the RDA performed using the latter enabled a more accurate prediction of variation than that performed using the former (eigenvalue: 0.347 versus 0.380, *F*: 12.2 versus 14.1). Therefore, only the results of the RDA performed using the square-transformed value of the variables have been presented in this paper.

Mosquitoes collected indoors. Based on the RDA, the factors that were significantly related to the number of female mosquitoes were the number of male *Cx. quinquefasciatus* and *Cx. gelidus* mosquitoes, the number of cattle, and the

minimum distance between a compound and the nearest rice field (Table 4). The first canonical axis predicted 26% of the total variation in the mosquito distribution, whereas all the canonical axes together predicted 38% variation. The number of male *Cx. quinquefasciatus* and *Cx. gelidus* mosquitoes contributed positively to the first canonical axis (Figure 3A). The number of cattle contributed negatively, and the minimum distance between a compound and the nearest rice field contributed positively to the second canonical axis (Figure 3A). The number of female *Cx. gelidus* mosquitoes correlated positively with the host proximity to the breeding sites (i.e., the number of its male abundance) and the number of cattle, whereas the number of female *Cx. vishnui* subgroup mosquitoes correlated positively with the number of cattle and their proximity to the rice field (i.e., it correlated negatively with the minimum distance between a compound and the nearest rice field; Figure 3A). For bivariate analyses, we used four environmental variables (the number of cattle, male *Cx. gelidus* mosquitoes, male *Cx. quinquefasciatus* mosquitoes, and the minimum distance between a compound and the nearest edge of a rice field). Figure 4 shows the bivariate scatter plot, which revealed a significant correlation. The statistical values are summarized in Table 5.

Mosquitoes collected outdoors. The numbers of the *Cx. vishnui* subgroup and *Cx. gelidus* mosquitoes were used as response variables for the outdoor collections. The early- and late-night collections were analyzed separately because indoor collections were carried out only during the early-night period. RDA of the early-night outdoor collection revealed that the number of male *Cx. gelidus* mosquitoes and the minimum distance between a compound and the nearest edge of a rice field were significantly related to the number of female mosquitoes (Table 4). As shown in Figure 3B, the number of female *Cx. gelidus* mosquitoes correlated positively with the proximity to the breeding sites. The first canonical axis predicted 25% of the total variation in the mosquito distribution during the early-night period, whereas all the canonical axes together predicted 31% variation. In the late-night outdoor collection, the numbers of male *Cx. gelidus* mosquitoes and large pigs were related to the number of female mosquitoes

TABLE 4

Summary of RDA for female mosquito assemblage in each compound in the three data sets: indoor, early-night outdoor, and late-night outdoor collections

Location (time)	Indoor (7:00 PM to 11:00 PM)			Outdoor (7:00 PM to 11:00 PM)			Outdoor (11:00 PM to 9:00 AM)		
Response variable	Female CV, CG, CQ			Female CV, CG			Female CV, CG		
Eigenvalue of first axis	0.255			0.250			0.243		
Species-environment correlation of first axis	0.704			0.613			0.585		
Monte Carlo test of significance of first canonical axis									
Eigenvalue	0.255			0.250			0.243		
<i>F</i> ratio	31.427			19.007			13.318		
<i>P</i> value	0.002			0.002			0.002		
Environment variable	(F value > 1)								
	Variable	<i>F</i>	<i>P</i>	Variable	<i>F</i>	<i>P</i>	Variable	<i>F</i>	<i>P</i>
	Male CQ	23.31	0.002	Male CG	19.33	0.002	Male CG	16.97	0.002
	Male CG	10.92	0.002	D	4.98	0.03	Pig L	3.78	0.03
	Cattle	7.48	0.002	Male CV	3.1	0.068	Male CV	3.41	0.058
	D	6.82	0.002	Pig S	2.41	0.09	Human	1.59	0.218
	Duck	1.72	0.162	Cattle	1.19	0.32	Dog	1.47	0.236
	Pig M	1.38	0.252	Dog	1.61	0.208	D	1.07	0.318
	Chicken	1.32	0.306						
	Male CV	1.05	0.332						

CV, the *Cx. vishnui* subgroup; CG, *Cx. gelidus* Theobald; CQ, *Cx. quinquefasciatus* Say; D, the minimum distance between a compound and the nearest rice field; Pig S, pig weighing < 20 kg; Pig M, pig weighing between 20 and 60 kg; Pig L, pig weighing > 60 kg.

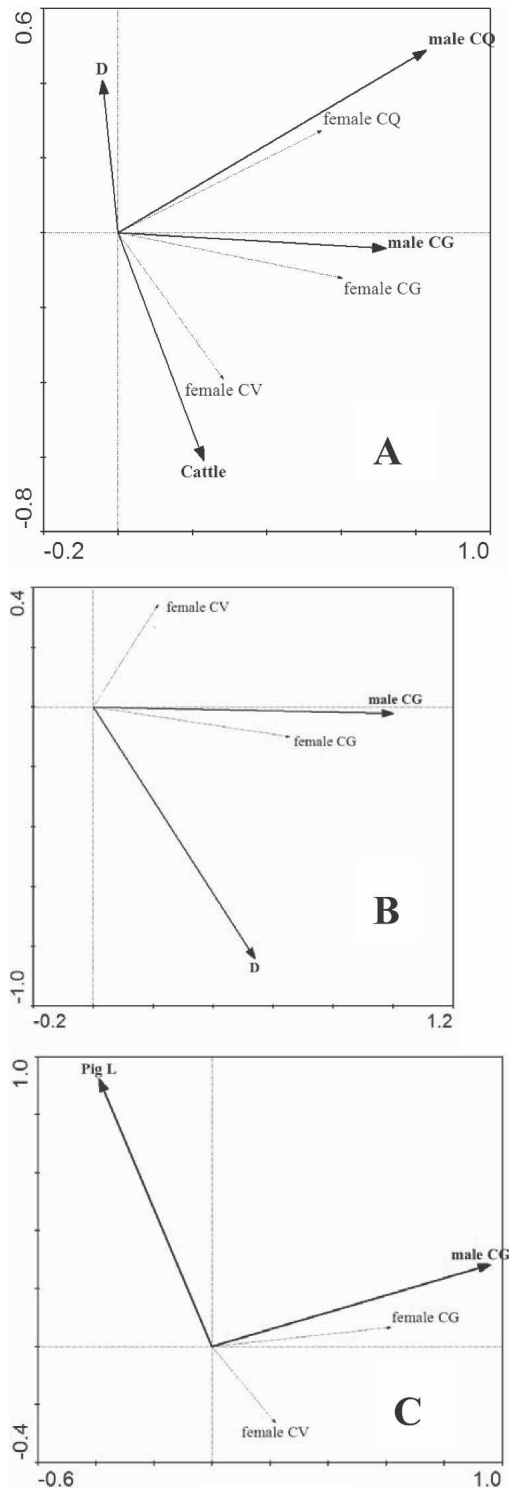


FIGURE 3. RDA ordination diagrams showing the association between the environmental variables and the number of female *Culex* mosquitoes collected indoors (A), outdoors from 7:00 PM to 11:00 PM (B), and outdoors from 11:00 PM to 9:00 AM (C). CV, the *Cx. vishnui* subgroup; CG, *Cx. gelidus* Theobald; CQ, *Cx. quinquefasciatus* Say; D, minimum distance between a compound and a rice field. The first and second axes represent linear combinations of the most significant composition variables, which are indicated by solid arrows (explanatory variables). Dashed arrows indicate values of weighted averages of the female mosquito species (response variables). The relative importance of individual variables is indicated by the length of the arrows and the relationship between the variables, by the angle between the arrows. Statistical values are summarized in Table 4.

(Table 4). Furthermore, as shown in Figure 3C, the number of male *Cx. gelidus* mosquitoes showed a positive correlation with the number of female mosquitoes of the same species. The first canonical axis predicted 24% of the total variation in the mosquito distribution during the late-night period, whereas all the canonical axes together predicted 27% variation. This means that the second axis, mainly contributed by large pigs, at the most explained only 3% of the total variation. Therefore, the effect of the large pigs is meager (Figure 3C). Figure 5 shows the bivariate scatter plots obtained for the early- and late-night collections, which present a significant correlation. The statistical values are summarized in Table 5.

DISCUSSION

Our results showed that host distribution affects the distribution of mosquitoes indoors but not outdoors. The number of cattle was a significant factor, whereas the number of pigs was not. Our results are based on limited data obtained by sampling once or twice from 50 compounds during a 2-week-long period. However, it is difficult to experimentally test 50 patterns of host distribution. Although the results were location-specific and temporary, we could avoid the influence of climatic or geographical factors on the distribution of the mosquito and focused on the phenomenon that occurred when the mosquitoes approached in close proximity to a host. The conditions of the study area were suitable to examine the influence of host distribution on vector abundance. Host-seeking *Culex tarsalis* Coquillett mosquitoes congregated at specific landscape features that were not necessarily associated with a high density of potential blood-meal hosts.²⁷ Our study area was made up of a monotonous flat landscape surrounded by rice fields. Significant spatial autocorrelation within a lag distance of < 20 m was observed for the *Cx. vishnui* subgroup only. The absence of significant spatial autocorrelation in the rest of the groups suggested that none of the geographical features included a spatial scale of mosquito clustering larger than a house compound. Thus, it is unlikely that landscape features influenced the vector distribution within the study area.

Culex gelidus, *Cx. tritaeniorhynchus*, and *Cx. vishnui* are exophilic species, i.e., they remain outdoors. The blood-meal hosts of the JE vectors collected indoors were identified to predominantly be cattle and pigs (Table 3). This suggests that the structure of the houses in the study area permitted the entry of the JE vectors before and after feeding.

The number of cattle hosts was a significant factor for the indoor collections of the *Cx. vishnui* subgroup (Table 4), whereas the proximity of these hosts to the breeding sites (the minimum distance between a compound and the nearest rice field) was less significant for both indoor and outdoor collections of the *Cx. vishnui* subgroup during the early night period (Table 4; Figure 3A). This indicates that the distribution of the *Cx. vishnui* subgroup was not constrained by their breeding site in the village. This result is consistent with our previous results that revealed that this species prefers cows rather than pigs or chickens as hosts.^{17,18}

The number of *Cx. gelidus* mosquitoes was mainly affected by the proximity to the breeding sites (Table 4; Figure 3A–C) and slightly affected by the number of cattle hosts (Table 4;

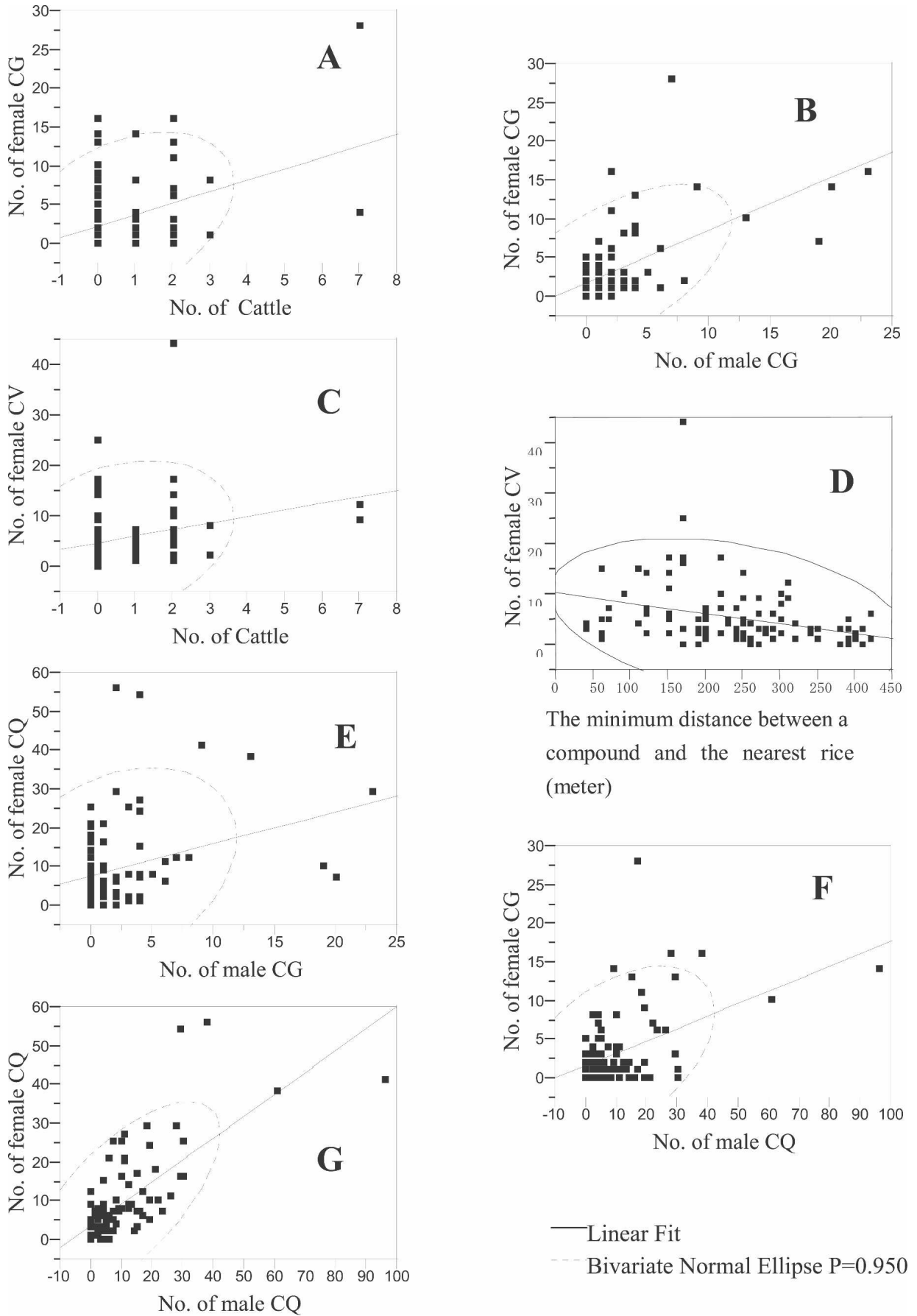


FIGURE 4. Continued on next page.

TABLE 5
Summary of bivariate analyses

Environment		Response						
		Indoor (7:00 PM to 11:00 PM)			Outdoor (7:00 PM to 11:00 PM)		Outdoor (11:00 PM to 9:00 AM)	
		Female CV	Female CG	Female CQ	Female CV	Female CG	Female CV	Female CG
Cattle	<i>r</i>	0.25	0.40					
	<i>P</i>	0.012	0.000					
	<i>R</i> ²	0.06	0.16	NS	NT	NT	NT	NT
	<i>F</i>	6.6	17.8					
	<i>P</i>	0.012	< 0.0001					
Male CG	<i>r</i>		0.60	0.31		0.62		0.50
	<i>P</i>		0.000	0.002		0.000		0.000
	<i>R</i> ²	NS	0.35	0.096	NS	0.39	NS	0.25
	<i>F</i>		52.2	10.2		36.6		19.3
	<i>P</i>		< 0.0001	0.002		< 0.0001		< 0.0001
Male CQ	<i>r</i>		0.47	0.70				
	<i>P</i>		0.000	0.000				
	<i>R</i> ²	NS	0.22	0.486	NT	NT	NT	NT
	<i>F</i>		26.5	89.6				
	<i>P</i>		< 0.0001	< 0.0001				
D	<i>r</i>	-0.31				0.29		
	<i>P</i>	0.002				0.027		
	<i>R</i> ²	0.10	NS	NS	NS	0.08	NT	NT
	<i>F</i>	10.2				5.2		
	<i>P</i>	0.002				0.026		
Pig L	<i>r</i>							
	<i>P</i>							
	<i>R</i> ²	NT	NT	NT	NT	NT	NS	NS
	<i>F</i>							
	<i>P</i>							

CV, the *Cx. vishnui* subgroup; CG, *Cx. gelidus* Theobald; CQ, *Cx. quinquefasciatus* Say; D, the minimum distance between a compound and the nearest rice field; Pig L, pig weighing > 60 kg; NT, not tested; NS, not significant.

Figure 3A); this result is also consistent with our previous result that revealed that *Cx. gelidus* mosquitoes prefer cows rather than pigs or chickens as hosts.^{17,18} Our results implied that the proximity between the available hosts and the breeding sites is a more critical factor than host preference for *Cx. gelidus*. It has been reported that this species breeds in a variety of habitats, including ditches, drains, small streams, ponds, temporary ground pools, artificial containers, and rice fields in Malaysia.²⁸ In the study area, people washed animal sheds and thus created polluted ground pools that served as a larval habitat for *Cx. gelidus*. In cases where there is proximity between the hosts and the mosquito breeding sites, the mosquitoes are not required to cover long distances; this may be an important factor because of the limited flight ability of the mosquitoes and/or the occurrence of polluted water bodies around animal sheds in Asian countries.

The number of female *Cx. quinquefasciatus* mosquitoes positively correlated with the proximity to the breeding sites. *Cx. quinquefasciatus* is reported to breed in any type of habitat that contains water ranging from fresh and clear water to polluted water with decayed organic matter from garbage and human wastes accumulated in ground pools, ditches, drains, sewers, dumping areas, and various types of artificial containers.²⁹ In the study area, the larval habitats of *Cx. quinquefas-*

ciatus were assumed to be located within the village, similar to those of *Cx. gelidus*. The host proximity to the breeding sites may be an important factor in the case of species whose larval habitats are located in close proximity to the hosts. The number of female *Cx. quinquefasciatus* mosquitoes did not correlate with the abundance of animal hosts (Table 4; Figure 3A). Human blood made up 76% of the diet of this species (Table 3). Because the distribution of humans in the study area was not aggregated, we could not study its effect on the mosquito distribution.

In this study, *Cx. tritaeniorhynchus* and *Cx. vishnui* were considered under the *Cx. vishnui* subgroup. Therefore, the results for this subgroup are applicable to both species. Precise data are not available to prove that these species exhibit only slight behavioral differences. However, no reports have revealed a difference between the host preferences of these two species.^{9,18} Therefore, we assumed they have similar feeding behaviors. However, the results of this study were obtained from a mixed community made up of both of these species.

In conclusion, this study revealed that 1) the number of female JE vectors exhibited a positive relationship with the number of cattle hosts and 2) the critical factor affecting the number of mosquitoes varied with the mosquito species.

FIGURE 4. Bivariate scatter plots obtained for the variables that were significantly correlated in the RDA analyses of the indoor collections. The number of female *Cx. gelidus* (CG) mosquitoes collected correlated positively with the number of cattle hosts (A), male CG mosquitoes (B), and male *Cx. quinquefasciatus* (CQ) mosquitoes (F). The number of female *Cx. vishnui* subgroup mosquitoes correlated positively with the number of cattle (C) and with the minimum distance between a compound and the nearest rice field (D). The number of female CQ mosquitoes correlated positively with the number of male CG (E) and CQ mosquitoes (F). Statistical values are summarized in Table 5.

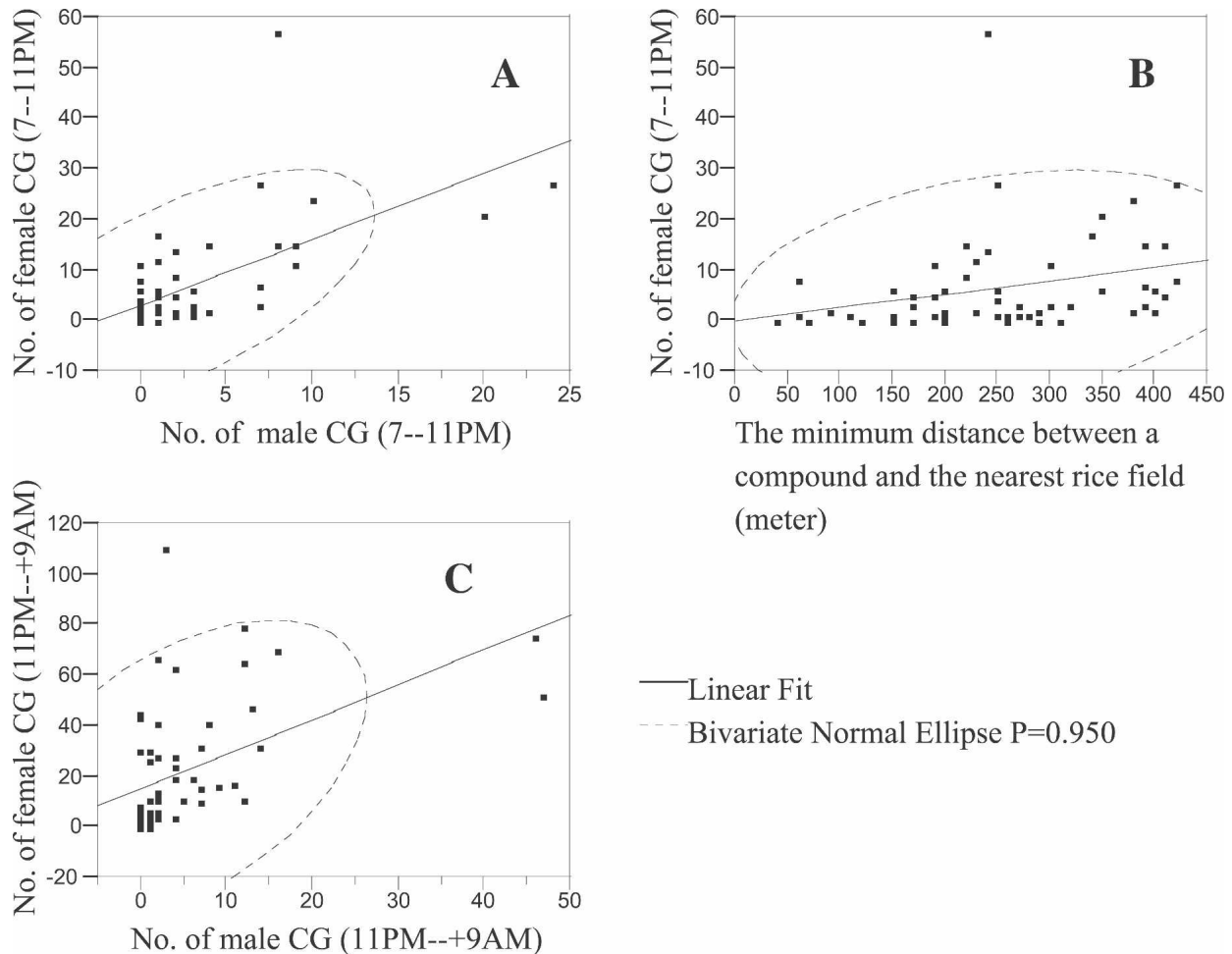


FIGURE 5. Bivariate scatter plots obtained for the variables that were significantly correlated in the RDA analyses of the outdoor collections. The number of female *Cx. gelidus* (CG) mosquitoes collected between 7:00 PM and 11:00 PM correlated positively with the number of male CG mosquitoes (A) and with the minimum distance between a compound and the nearest rice field (B). The number of female CG mosquitoes collected between 11:00 PM and 9:00 AM correlated positively with the number of male CG mosquitoes (C). Statistical values are summarized in Table 5.

Received December 4, 2006. Accepted for publication September 24, 2007.

Acknowledgments: The authors thank T. Yanagi for technical advice; T. Sunahara for constructive criticism on the manuscript; and the staff of the entomology laboratory, National Institute of Hygiene and Epidemiology in Hanoi, the health staff in CatQue village, and Truong Dinh Bac at Preventive Medicine Center of Hatay province, Vietnam, for help in the field study.

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