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GEOGRAPHIC DISTRIBUTION OF *Aedes aegypti* AND *Aedes albopictus* COLLECTED FROM USED TIRES IN VIETNAM

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ABSTRACT. The spatial distribution of *Aedes aegypti* and *Aedes albopictus* in environmental and geographical zones, e.g., urban-rural, coastal-mountainous, and north-south, was investigated throughout Vietnam. Immature stages were collected from used tires along roads. The effects of regions, seasons, and the degree of urbanization on the density and the frequency were statistically analyzed. *Aedes aegypti* predominated in the southern and central regions, while *Ae. albopictus* predominated in the northern region, which may be related to climatic conditions (temperature and rainfall). Larval collection from used tires may be suitable to assess rapidly the current distribution of dengue mosquitoes for estimating health risks and implementing vector control measures.

KEY WORDS *Aedes aegypti*, *Aedes albopictus*, tires, Vietnam

INTRODUCTION

Among insect vector-borne diseases, recent epidemics of the dengue (DF)/dengue hemorrhagic fever (DHF) disease complex have had great impact on Southeast Asian countries and the Americas, where DF/DHF, as well as malaria, is serious public health threat (Gubler 1997; WHO 2009). The primary vector, *Aedes aegypti* (L.), is distributed in tropical countries worldwide. The secondary vector, *Aedes albopictus* (Skuse), originated in tropical to temperate Asia and has recently spread to the Americas, Europe, and Africa by tire trades (Hawley 1988; Rodhain and Rosen 1997).

The geographical distributions of *Ae. aegypti* and *Ae. albopictus* overlap in tropical Asia and the Americas. However, *Ae. aegypti* is highly adapted to the domestic environment, and therefore its abundance is positively correlated with increasing urbanization; whereas the distribution of *Ae. albopictus* is associated with vegetation throughout rural and urban areas, and its abundance is adversely affected by urbanization (Chan et al. 1971a, 1971b; Hawley 1988; Rodhain and Rosen 1997; Tsuda and Takagi 2001; Braks et al. 2003; Maciel-de-Freitas et al. 2006; Rey et al. 2006; Tsuda et al. 2006). The mosquito vectors are affected differently by environmental factors (Braks et al. 2003; Rey et al. 2006; Tsuda et al. 2006). The recent involvement of mosquito vectors in the pandemics of Chikungunya virus in many countries has increased the importance of examining the current vector situation for potential outbreaks (Senevir-

atne et al. 2007, Delatte et al. 2008, Pagès et al. 2009, Pistone et al. 2009, Yoosuf et al. 2009).

Since DF/DHF vaccine is unavailable for practical use, transmission can be prevented only by reducing human-vector contact. Thus, it is important to study the relationship between the distributions of *Ae. aegypti* and *Ae. albopictus* and environments in order to understand population trends in changing environments and the ecological basis of the spatial distribution in order to develop effective mosquito-control measures; it would be helpful to assess high-risk areas with high vector densities. The difference in the infestation of mosquito vectors along urban-rural gradient has been extensively studied owing to its ecological and epidemiological importance (Chan et al. 1971a, 1971b; Tsuda et al. 2002; Braks et al. 2003; Rey et al. 2006; Tsuda et al. 2006; Cox et al. 2007; Bagny et al. 2009). Habitat segregation between coastal and mountainous environment has also been recognized; the abundance of *Ae. aegypti* and *Ae. albopictus* was found to be high in coastal areas and forested/mountainous areas, respectively (Hawley 1988, Ishak et al. 1997). This was related to the introduction of *Ae. aegypti* from Africa to the new world by boats in seventeenth through nineteenth centuries (Gubler 1997) and preference of *Ae. albopictus* for environments with vegetation (Hawley 1988; Niebylski and Craig 1994; Takagi et al. 1995a, 1995b; Maciel-de-Freitas et al. 2006).

In the present study, we collected immature stages of mosquitoes from used tires, which recently drew attention as an important breeding site for dengue vectors (Simard et al. 2005, Roiz et al. 2007, Yee 2008). The spatial distributions of *Ae. aegypti* and *Ae. albopictus* in urban-rural, coastal-mountainous, and north-south, were investigated to understand the habitat segregation as well as the current distribution of the 2 species in Vietnam.

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Table 1. Regional location and season of larval collection from used tires in Vietnam.

| Period | Region | Season |
|---------------------|--|--------|
| December 7–16, 2006 | Central and a part of the north (far south of Hanoi) | Dry |
| March 17–20, 2007 | North (far north of Hanoi) | Dry |
| May 15–20, 2007 | South and mountainous | Wet |
| July 1–12, 2007 | Central and a part of the north (far south of Hanoi) | Wet |
| January 7–16, 2008 | South and mountainous | Dry |

MATERIALS AND METHODS

Larval collections

Using the mosquito-collection method of Kawada et al. (2009), mosquito larvae were collected from used tires along the national road from the north end of Vietnam to the Mekong Delta. During the 5 larval collections, the region far north of Hanoi was visited once in the dry season; every other region was visited twice (Table 1). Whenever we encountered used tires, most of which were found around repair shops for vehicles, while driving along a systematically determined route, the geographical position (with a global positioning system [GPS]), number of tires, presence of water, and presence of mosquito larvae in the tires were recorded. The degree of urbanization at each collection site was determined on the basis of the distribution of houses (continuously distributed or gaps with vegetated areas between houses), road conditions (asphalt-surfaced, paved road), and traffic lights, indicating frequent transportation in the area. Each area was classified as urban (houses continuously distributed, asphalt-surfaced road, and traffic light present), transition (usually a little gaps with vegetated sites between houses, paved road, no traffic light), or rural (gaps with vegetated sites between houses, unpaved road, and no traffic light). Larvae were collected from 527 sites throughout Vietnam (Fig. 1). Mosquito larvae were collected from tires (6–20 tires per site, depending on desiccation) by netting (5 times per tire). The collected larvae were placed in 1.5-ml plastic vials containing absolute ethanol solution and were taken to the laboratory for identification. All late instars (3rd and 4th) collected were carefully identified to species under a microscope, using identification keys of Stojanovich and Scott (1966) and Rattananarithikul et al. (2005a, 2005b). The number and species of mosquito larvae from each tire were recorded. Due to identification difficulty, the early instars and pupae were not included in our analyses.

Statistical analysis

The mosquito density was calculated as the number of larvae per tire. The frequency of tires containing *Ae. aegypti*, or *Ae. albopictus*, or both species, was calculated as the number of tires

positive for *Ae. aegypti*, or for *Ae. Albopictus*, or both divided by the number of tires positive for water. On the basis of latitude and elevation, which were good parameters for division of geographical zones and climates, the country was divided into 4 regions for subsequent analyses: the northern region, the central region, the mountainous regions, and the southern region. The effect of regions, seasons, and degree of urbanization on the density of *Ae. aegypti* and *Ae. albopictus* was analyzed by analysis of variance (ANOVA). The frequency of the presence of *Ae. aegypti*, or *Ae. albopictus*, or both species, according to regions, seasons, and the degree of urbanization, was tested as presence or

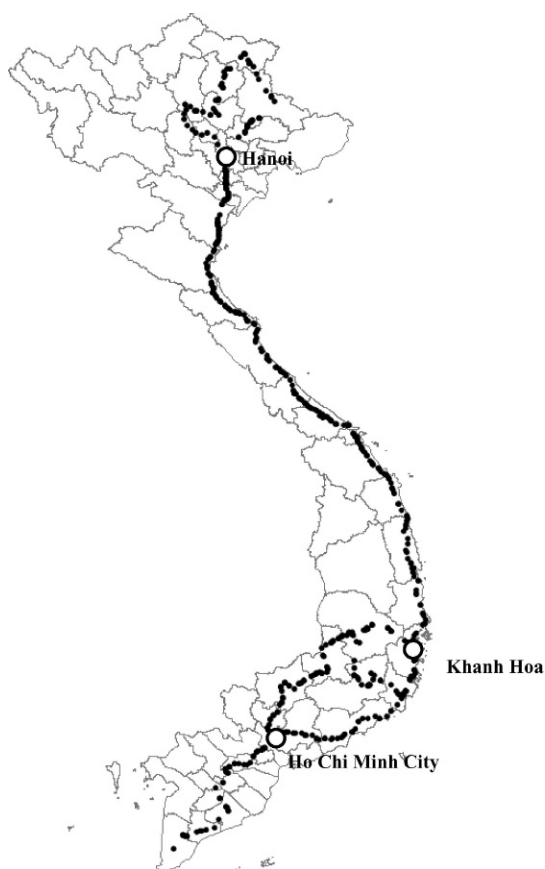


Fig. 1. Location of the mosquito collection from used tires in Vietnam.

absence by logistic regression analysis. The JMP 8 software was used for all statistical tests and models (SAS 2009).

RESULTS

Geographical distribution of mosquito species

Used tires are extensively and commonly distributed throughout the country (Fig. 1). Of the total of 24.8% (4,757/19,188) of all tires sampled, 51.9% (2,468) contained water. In total, 34.5% (852) tires contained larval mosquitoes, of which 26.5% (653) had *Ae. aegypti* and/or *Ae. albopictus*. In total, 8,771 *Ae. aegypti* and 5,916 *Ae. albopictus* larvae were collected, which accounted for 54.5% of all late instars collected from used tires. Non-dengue vectors collected from used tires included *Culex quinquefasciatus* Say (11,356), *Culex pallidothorax* Theobald (327), *Culex tritaeniorhynchus* Giles (158), *Armigeres* sp., *Anopheles* sp., *Toxorhynchites* sp., and *Uranotaenia* sp. In the northern part of Vietnam, *Ae. albopictus* was dominant, and this dominance gradually reduced toward the south (Fig. 2). *Aedes aegypti* was dominant in the southern area, whereas in the coastal areas far south of Khanh Hoa province, most of the larvae collected were those of *Ae. aegypti*. However, in the mountainous areas with forest vegetation, the proportion of *Ae. albopictus* was higher, indicating that each species is affected differently between the north-south and coastal-mountainous areas. *Aedes albopictus* individuals were collected from used tires in Ho Chi Minh City and Vinh Long, Soc Trang, and Ca Mau provinces in the southern Vietnam.

Effect of the regions, seasons, and urbanization on mosquito density

The density of *Ae. aegypti* was significantly higher in the southern region and lower in the northern region, thus exhibiting a strong effect of the geographical regions on the density ($P < 0.001$) (Fig. 3 and Table 2). Moreover, *Ae. aegypti* predominated over *Ae. albopictus* throughout urban-rural areas in the southern region (Fig. 3). Although the density of *Ae. aegypti* tended to be high in urban and transition areas, the effects of region and urbanization interactions were statistically significant ($P = 0.0197$), indicating that the effect of the degree of urbanization on density was not the same among the regions. For *Ae. albopictus*, the density was high in the northern region and low in the southern region. *Aedes albopictus* was likely to predominate over *Ae. aegypti* throughout urban-rural areas in the northern region (Fig. 3); however, urbanization strongly affected density when entire regions were considered ($P = 0.0157$)

i.e., high density in urban and transition areas. In mountainous areas, the density of *Ae. albopictus* was high in rural as well as urban areas (Fig. 3). The effects of the region \times season, region \times urbanization, and region \times season \times urbanization interactions were statistically significant ($P < 0.0001$, $P = 0.0006$, and $P = 0.0049$, respectively), indicating that the seasons and the degree of urbanization affected the density differently. The analyses revealed that the effect of region, season, and urbanization was greater on the density of *Ae. albopictus* than that of *Ae. aegypti*. The densities of *Ae. aegypti* and *Ae. albopictus* according to the regions, seasons, and urbanization (Fig. 3) were negatively correlated, suggesting adverse distributions of dengue vectors in Vietnam (Spearman rank order test: $\rho = -0.4235$, $P = 0.0392$).

Effect of regions, seasons, and urbanization on the frequency of occurrence

As shown in Figure 4 and Table 3, *Ae. aegypti* was rare in the northern region, especially during the dry season. Its frequency of occurrence gradually increased from the northern to the southern regions. The effects of the regions, urbanization, and region \times season \times urbanization interaction on the frequency of occurrence of only *Ae. aegypti* were statistically significant ($P < 0.0001$, $P = 0.0112$, and $P = 0.0444$, respectively), indicating that the frequency varied greatly according to the regions and the degree of urbanization. In contrast to the case of *Ae. aegypti*, *Ae. albopictus* was frequent in the northern region throughout urban-rural areas, especially in the wet season, and its frequency decreased dramatically in the central and southern regions (Fig. 4). The effects of seasons and urbanization on the frequency of occurrence of this species were not statistically significant. However, significant effects on the frequency were indicated by the region \times season interaction ($P = 0.0008$). The frequency of co-occurrence of both species was high in the central and mountainous regions (Fig. 4), and the effect of the region \times urbanization interaction on the frequency was significant, indicating that the degree of urbanization differently affected the frequency ($P = 0.0051$). The frequencies of occurrence of both species according to the regions, seasons, and urbanization (Fig. 4) were negatively correlated (Spearman rank order test: $\rho = -0.6111$, $P = 0.0015$).

DISCUSSION

The present study clearly showed the difference in the spatial distributions between *Ae. aegypti* and *Ae. albopictus* in the north-south and coastal-mountainous areas; the former seemed to have

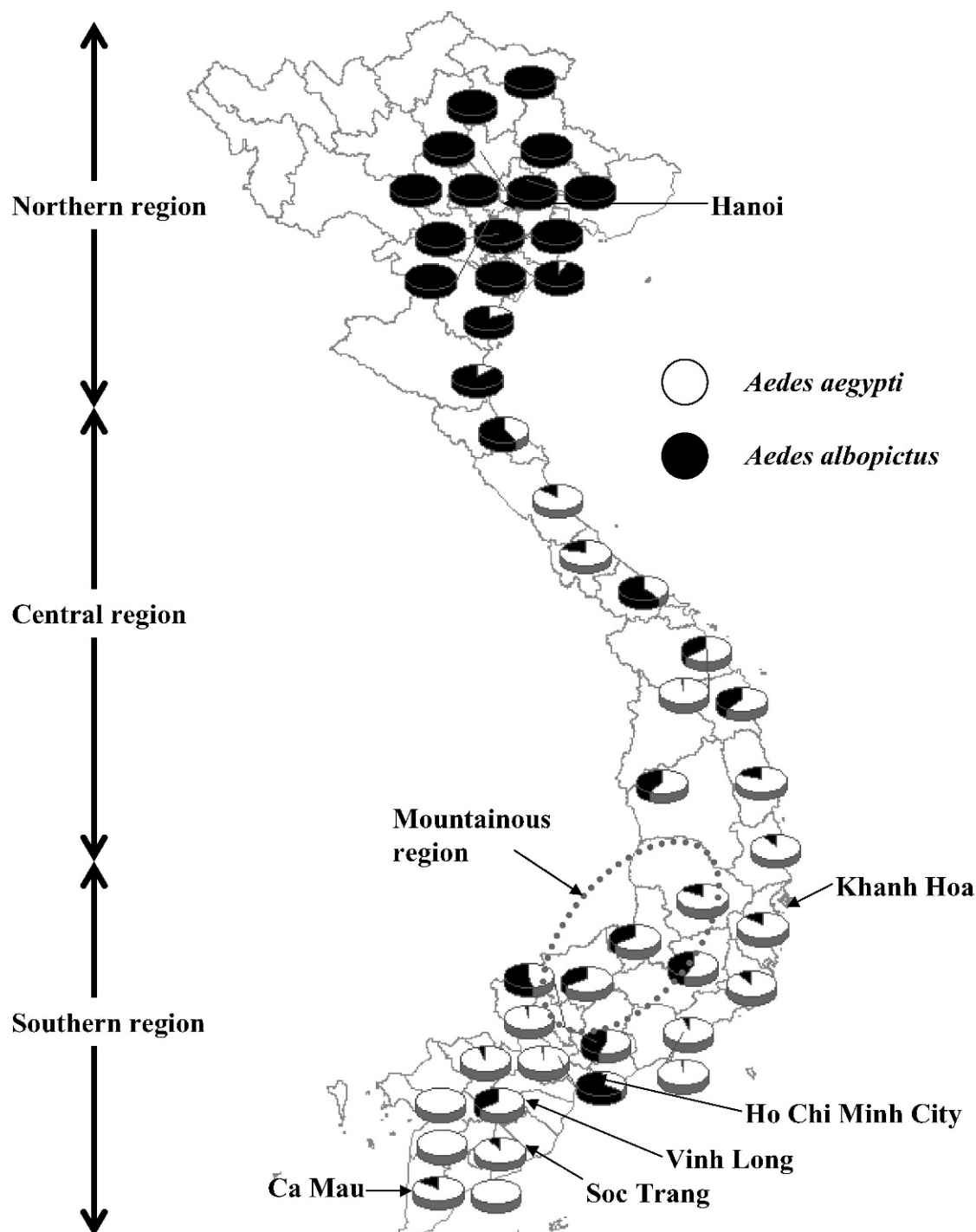


Fig. 2. Relative abundance of *Aedes aegypti* and *Aedes albopictus* larvae collected from used tires in Vietnam.

the most significant effect on the distribution of the dengue vectors. However, the effect of urbanization on the spatial distribution was rather unclear and not consistent throughout the regions.

In general, *Ae. aegypti* moves short distances, prefers dark places, is anthropophilic, endophagous, and endophilic, and favors urbanized environments (Christophers 1960, Service 1993, Kuno 1997, Kawada et al. 2005b). On the other

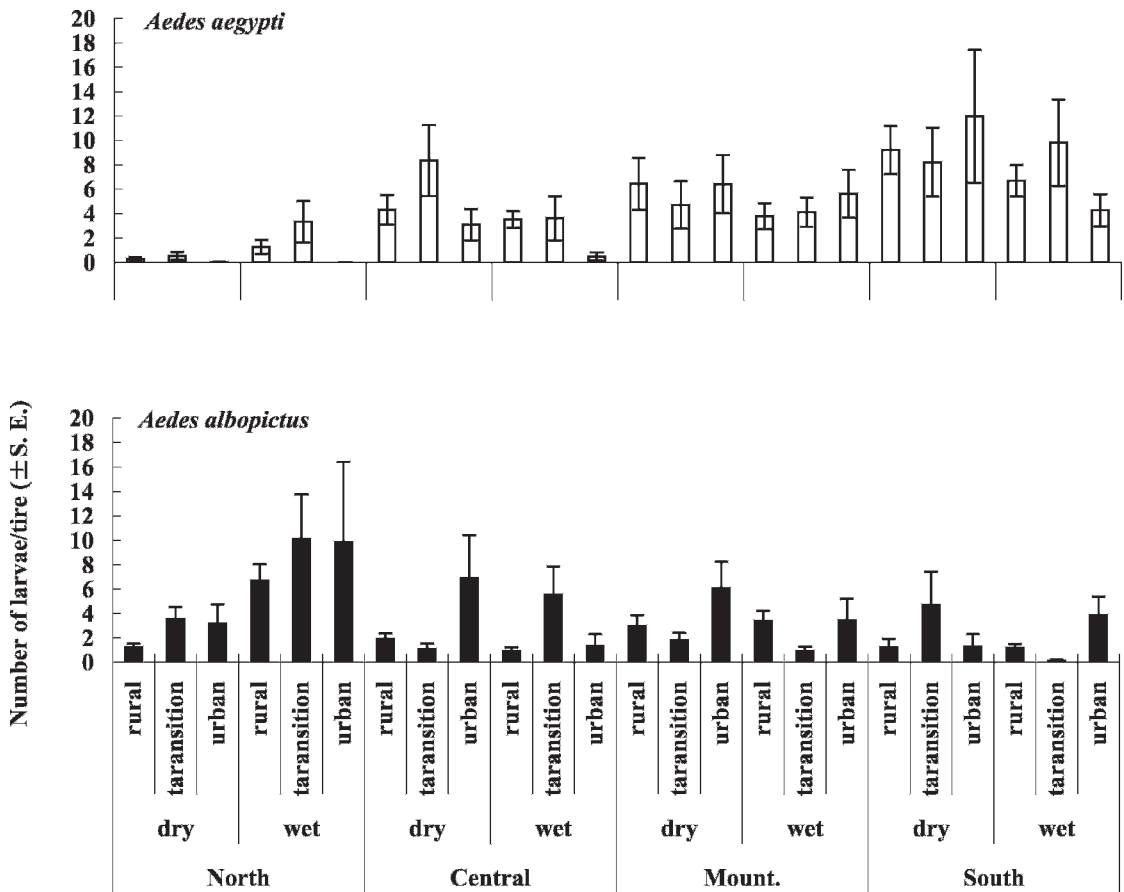


Fig. 3. The number of *Aedes aegypti* and *Aedes albopictus* collected from used tires in Vietnam.

hand, *Ae. albopictus* travels longer distances than *Ae. aegypti*, prefers vegetated environments, is an opportunistic feeder, and is exophagous and exophilic (Hawley 1988, Niebylski and Craig 1994, Niebylski et al. 1994, Higa et al. 2001). These characteristics imply that the latter species has a wider activity range and can adapt more easily to outdoor environments than the former species. The differences between the adult niches of *Ae. aegypti* and *Ae. albopictus* were related to different responses to environments; this partially explained the difference in their spatial distribution between the urban-rural and coastal-mountainous environments and seemed to contribute to the coexistence within a region (Ishak et al. 1997, Maciel-de-Freitas et al. 2006, Tsuda et al. 2006).

In Vietnam, the effect of the north-south geographical difference on the spatial distributions of *Ae. aegypti* and *Ae. albopictus* indicated that the distribution of these mosquitoes was strongly affected by climatic factors e.g., temperature, humidity, and precipitation. In the region where 1 species dominates over the other, climatic conditions would highly favor the former species.

For example, *Ae. albopictus* is distributed in temperate regions as well as tropical regions, and thus, it is more adaptive to cooler climates than *Ae. aegypti*; while the eggs and adults of *Ae. aegypti* are more resistant to desiccation than those of *Ae. albopictus*, and thus, *Ae. aegypti* is more adaptive to hot and dry environments than *Ae. albopictus* (Hawley 1988, Sota and Mogi 1992, Sota 1993, Juliano et al. 2002). The meteorological data of the average temperature and precipitation in Vietnam over the last half century seemed appropriate for explaining this phenomenon (Weatherbase 2009). The average temperature was below 20°C from December to March in the northern region and in January in the mountainous region. On the other hand, the temperature was above 24°C throughout the year in the southern region. The average lowest temperature, which influenced the distribution of *Ae. aegypti* (Christophers 1960), was different among the regions; it was less than 15°C from December to February in the northern region and December in the mountainous regions, while it was more than 15°C throughout the year in the central and southern regions. The monthly

Table 2. Analysis of variance (ANOVA) table for the effects of region, season, and the degree of urbanization on density of *Aedes aegypti* and *Aedes albopictus* collected from used tires in Vietnam.

| Species | Source | df | F value | P |
|-----------------------|--------------------------------|-------|---------|----------|
| <i>Ae. aegypti</i> | Model | 23 | 11.9579 | <0.0001* |
| | Region | 3 | 25.3033 | <0.0001* |
| | Season | 1 | 2.1323 | 0.1444 |
| | Urbanization | 2 | 0.9860 | 0.3732 |
| | Region × season | 3 | 1.9880 | 0.1137 |
| | Region × urbanization | 6 | 2.5169 | 0.0197* |
| | Season × urbanization | 2 | 0.9364 | 0.3922 |
| | Region × season × urbanization | 6 | 0.8284 | 0.5478 |
| | Error | 2,439 | | |
| <i>Ae. albopictus</i> | C. total | 2,462 | | |
| | Model | 23 | 7.0427 | <0.0001* |
| | Region | 3 | 14.2588 | <0.0001* |
| | Season | 1 | 3.7348 | 0.0534 |
| | Urbanization | 2 | 4.1618 | 0.0157* |
| | Region × season | 3 | 11.6485 | <0.0001* |
| | Region × urbanization | 6 | 3.9928 | 0.0006* |
| | Season × urbanization | 2 | 0.4031 | 0.6683 |
| | Region × season × urbanization | 6 | 3.1115 | 0.0049* |
| | Error | 2,439 | | |
| | C. total | 2,462 | | |

* Statistically significant.

average precipitation was high i.e., >180 mm, in the mountainous region. In other regions, the monthly average precipitation was 160 mm, not markedly different; however, the dry period, which was characterized by precipitation below 30 mm, was longer in the southern region (3 months long, from January to March) than in the northern region (1 month long, in January). These parameters were intermediate in the central region. The sequential change of responses to climatic conditions was possibly one of the

factors for the difference in infestation of *Ae. aegypti* and *Ae. albopictus* along the north-south geographical divide in Vietnam. On the other hand, in the region where both *Ae. aegypti* and *Ae. albopictus* breed, climates may be moderate for both species, and neither one would dominate the other. In such regions, habitat heterogeneity along the urban-rural gradient and interspecific interaction were considered to be more important than climate for spatial distribution. From previous studies, it is assumed that the former

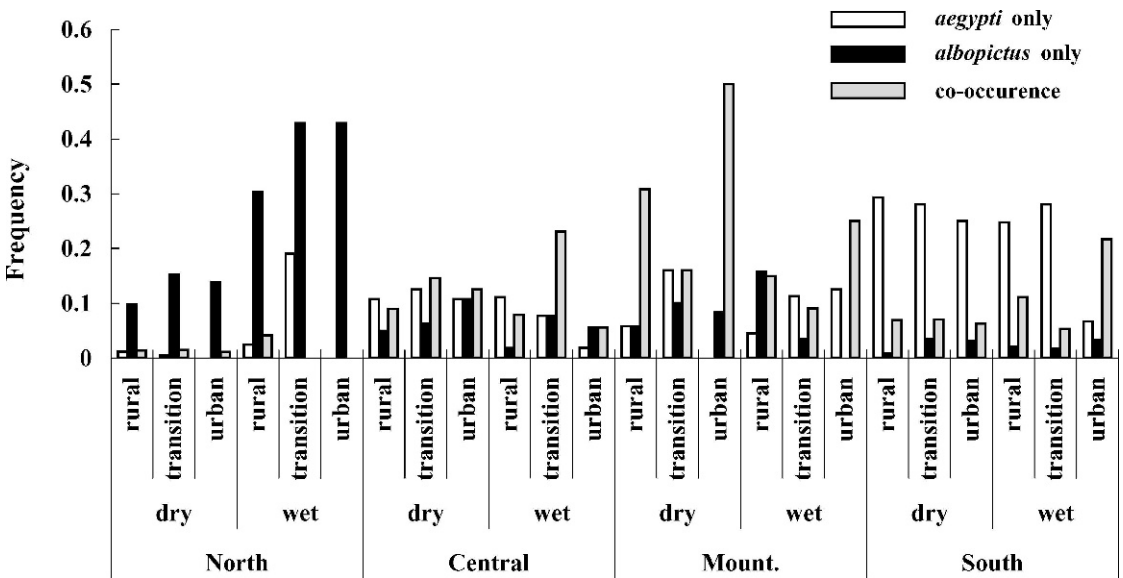


Fig. 4. Frequency distribution of the number of larvae-positive tires in Vietnam. Each frequency was for *Aedes aegypti*, or *Aedes albopictus*, or both.

Table 3. Logistic regression table for the effects of region, season, and the degree of urbanization on frequency of *Aedes aegypti*, *Aedes albopictus*, and co-occurrence from used tires in Vietnam.

| Species | Source | df | Chi square | P |
|----------------------------|--|----|------------|----------|
| Only <i>Ae. aegypti</i> | Region | 3 | 68.3594 | <0.0001* |
| | Season | 1 | 0.0005 | 0.9823 |
| | Urbanization | 2 | 8.9801 | 0.0112* |
| | Region \times season | 3 | 3.7457 | 0.2903 |
| | Region \times urbanization | 6 | 6.5404 | 0.3654 |
| | Season \times urbanization | 2 | 2.0122 | 0.3656 |
| | Region \times season \times urbanization | 6 | 12.9149 | 0.0444* |
| Only <i>Ae. albopictus</i> | Region | 3 | 58.3860 | <0.0001* |
| | Season | 1 | 0.1065 | 0.7442 |
| | Urbanization | 2 | 1.7815 | 0.4103 |
| | Region \times season | 3 | 16.8306 | 0.0008* |
| | Region \times urbanization | 6 | 7.7256 | 0.2589 |
| | Season \times urbanization | 2 | 2.7792 | 0.2492 |
| | Region \times season \times urbanization | 6 | 9.7126 | 0.1373 |
| Co-occurrence | Region | 3 | 60.7649 | <0.0001* |
| | Season | 1 | 0.5983 | 0.4392 |
| | Urbanization | 2 | 1.3989 | 0.4969 |
| | Region \times season | 3 | 7.8274 | 0.0497* |
| | Region \times urbanization | 6 | 18.5210 | 0.0051* |
| | Season \times urbanization | 2 | 1.2738 | 0.5289 |
| | Region \times season \times urbanization | 6 | 7.2661 | 0.2969 |

* Statistically significant.

species may be abundant in urban areas, and the latter in the rural areas, according to the differences in the preferred sites of *Ae. aegypti* and *Ae. albopictus* for resting, feeding, oviposition, and breeding. Habitat segregation between the urban-rural areas would play an important role for the coexistence of the 2 species within the region, even if the larvae have similar habitat requirements (Chan et al. 1971a, 1971b; Hawley 1988). In that case, the frequency of larval co-occurrence may be high at intermediate sites such as transition areas. However, although *Ae. aegypti* and *Ae. albopictus* coexisted in the central region of Vietnam, the high frequency of co-occurrence through urban to rural areas suggested that there was no obvious habitat segregation between the urban-rural areas in our study. This may be explained as follows. First, because we collected larvae along roads, the environments might not be too heterogeneous to exhibit a significant difference along the urban-rural gradient as compared to the environments inside villages, towns, and cities. Second, although our larval collections were limited to used tires, the higher density and frequency of *Ae. albopictus* in urban and transition areas than rural sites suggested an increase in the abundance of the species in domestic environments in Vietnam. This may suggest the abundance of dengue mosquitoes is changing to some extent in Vietnam. Braks et al. (2003) mentioned that the coexistence of the 2 species seems to be possible when the local environment favors a nonaquatic stage of 1 species and larval competition favors the other. Further study concerning both larvae

and adults is needed to examine habitat segregation of these 2 species along the urban-rural gradient in central Vietnam.

The difference in spatial distribution of *Ae. aegypti* and *Ae. albopictus* seems to be significant for dengue occurrence in Vietnam. Out of 25,269–234,920 annual dengue cases from 1996 to 2000, 50.8–75.6% were reported from southern Vietnam (Ministry of Health 2001), incriminating *Ae. aegypti* as a major vector and a priority for vector control in Vietnam. Although *Ae. aegypti* was not collected from used tires in northern Vietnam, the species was observed in urbanized cities such as Hanoi city and Hai Phong Province of northern Vietnam (Vu et al. 1998, Kawada et al. 2005a), where dengue cases were still reported. Therefore, it should be important to monitor the infestation of *Ae. aegypti* and *Ae. albopictus* for potential of dengue outbreaks.

Tires provide habitats to various container-inhabiting mosquitoes, including the dengue vectors (Roiz et al. 2007, Yee 2008). The aquatic environment for larvae basically originated from rainfall; therefore, water qualities contained in tires did not fluctuate throughout the study areas, indicating that tires would be undisturbed and would serve as a relatively stable environment for mosquito larvae without severe predation pressure in Vietnam (Higa et al., unpublished data). The sequential difference in the spatial distribution of *Ae. aegypti* and *Ae. albopictus* indicates that larval collection from used tires is a reliable way to assess the distribution of dengue vectors. The newly established method in the present study makes it possible to rapidly investigate the

current nationwide distributions of vectors, especially mosquitoes breeding in artificial containers, and apply the results to geographic information systems (GIS) (Kawada et al. 2009).

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