Mark-release-recapture study on movement of mosquitoes: individual marking method and short-term study of *Aedes albopictus* and *Armigeres subalbatus* in residential area on Ishigaki island, Japan

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**Abstract:** An individual marking method by placing spots of paint at five different sites on the thorax of mosquitoes was applied and a short-term ecological study on movement of *Ae. albopictus* and *Armigeres subalbatus* was conducted during 18 to 27 March 2013 in a residential area on Ishigaki island, Japan. There were 2 preservation areas with vegetation, human dwellings, shops and buildings, and habitats of *Ae. albopictus* were distributed patchily in the study area of 230 m×250 m. Individual marking was carried out during the first 7 days, and 232 and 216 females of *Ae. albopictus* and *Ar. subalbatus*, respectively, were marked and released from 4 collection sites. The overall recapture rate of released females was significantly higher for *Ae. albopictus* (0.21=48/232) than *Ar. subalbatus* (0.09=20/216). The recapture rate of *Ae. albopictus* varied among collection sites. Analysis of the movements of released mosquitoes among collection sites indicated that the collection site inside the large preservation area was suitable for resting/searching *Ae. albopictus* and *Ar. subalbatus* and many females were accumulated through directional movements from the surrounding habitats. The individual marking method used in this study is applicable to field studies of the movements of *Ae. albopictus* and *Ar. subalbatus*.

Key words: individual marking, movement, *Aedes albopictus*, Armigeres subalbatus, Ishigaki Island

**INTRODUCTION**

Mark-release-recapture is a useful ecological method for population studies of mosquito vectors to estimate population density, survival rate, flight range, dispersion pattern etc., and various methods using different types of paints, inks and dyes have been applied to adult mosquitoes (Silver, 2008). Although the procedure is more time consuming than group marking methods, individual marking methods have been developed by placing spots of paint at different sites on the wing and/or thorax of mosquitoes in behavioral and demographic studies of wild mosquito populations (Conway et al., 1974; Sheppard et al., 1969; Trpis and Hausermann, 1986; Takagi et al., 1995a). The movement of adult mosquitoes is a primary ecological factor determining the dispersal range and spatial distribution of biting females and is important for understanding the spread of mosquito-borne diseases. The movement of biting *Aedes aegypti* (L.) and *Ae. albopictus* (Skuse) has been studied by individual marking in an African village and in Nagasaki City, Japan, respectively (Trpis and Hausermann, 1986; Takagi et al., 1995a), and the sequential movement of the released females among collection sites were analyzed.

*Aedes albopictus* is a most important urban mosquito in Japan because of its wide geographic distribution (Kobayashi et al., 2002; Tsuda et al., 2006a, b), high biting density during the summer (Makiya, 1974; Toma et al., 1982; Suzuki et al., 1993; Tsuda and Kim, 2012) and the high preference of biting females for humans (Hawley, 1988; Kobayashi et al., 2008; Kim et al., 2009; Sawabe et al., 2010). The geographic distribution of *Ae. albopictus* has been expanding from Southeast Asian countries to at least 28 other countries since the 1980s (Benedict et al., 2007) and its medical importance as a vector of chikungunya virus and dengue virus has been increasing in newly established areas in temperate regions (ECDC, 2007, 2009; Reiter, 2010). A variety of natural and manmade containers are used as their larval habitats (Hawley, 1988) and catch basins are productive larval habitats of *Ae. albopictus* in urban areas (Carrieri, 2011; Tsuda, 2012, 2013).

Although *Ae. albopictus* is found in urban areas, it is more commonly found in suburban and rural areas where open spaces with vegetation are prevalent (Chan, 1985; Hawley, 1988; Tsuda et al., 2002, 2006c). Spatial analysis of oviposition of *Ae. albopictus* in the United...
States suggested a significant association between oviposition intensity and the type of land cover; *Ae. albopictus* is most abundant in artificial containers in open, residential areas as opposed to wooded areas (Baker et al., 2003; Richards et al., 2006; Swanson et al., 2000). The results of spatial analysis of eggs, larvae and adults of *Ae. albopictus* found in the literature suggest the vegetation-related movement of females in residential areas.

*Armigeres subalbatus* (Coquillett) is common in central and western Honshu, Kyusyu and Ryukyu archipelago, and their breeding habitats and seasonal prevalence overlap with *Ae. albopictus* (Tanaka et al., 1979; Toma and Miyagi, 1981; Toma et al., 1983). Both *Ae. albopictus* and *Ar. subalbatus* bite human throughout the day (Tsuda, 2013) and numbers of biting females of both species in this study were large enough to conduct mark-release-recapture experiments. Since no mark-release-recapture study has been performed for *Ar. subalbatus* in Japan, females of both species were marked individually and released in this study.

This study focused on the vegetation-related movement of females of *Ae. albopictus* and *Ar. subalbatus* in residential areas where different sizes of open spaces with trees and shrubs distributed among human dwellings. An individual marking method by placing spots of paint at five different sites on the thorax was applied and the recapture rates of released females were calculated and compared among collection sites.

**MATERIALS AND METHODS**

**Study site and study period**

The study area, 230 m × 250 m, was chosen in a residential area of Ishigaki City on Ishigaki island, Japan (Fig. 1). The habitats of *Ae. albopictus* were distributed patchily in the study area. There were two preservation areas with vegetation, human dwellings, shops and local government buildings in the study area.

The preservation areas were dark and humid because of trees, shrubs and herbaceous plants, and were suitable for resting mosquitoes. Mark-release-recaptures of field collected mosquitoes were conducted during 18 to 27 March, 2013. Mosquitoes collected during the first 7 days were individually marked and released. Only recapture of the released mosquitoes were made during the last 3 days. The study period was at the beginning of the population increase of *Ae. albopictus* on Ishigaki island (Higa et al., 2007).

**Mosquito collection and individual marking**

Take into account of biting rhythm of mosquitoes and time period necessary for individual marking, mosquito collection was conducted basically twice a day, at 8:00 and 14:00. One collector visited a collection site and mosquitoes landing on his legs or arms were collected using a sucking tube for 10 min at each collection site. The collected mosquitoes were kept in a paper cup with a cotton pad soaked with a 3% sugar solution and were carried to the guest house where mosquito identification and marking were carried out.

Mosquitoes were collected at 4 collection sites besides shrubs or under trees located at a guest house (site A in Fig. 1), the entrance and within the large preservation area (site B and C, respectively) and at the entrance of the small preservation area (site D). All the collection sites were protected from direct sunshine and strong wind by shrubs and trees. The distance among the 4 collection sites varied between 48 m and 187 m (Fig. 1).

Collected mosquitoes were removed one by one from the cups using a sucking tube, anaesthetized using chloroform, the species was identified and they were checked for the presence of a mark. When a marked mosquito was found, the color and position of the spot was recorded with the collection site, and the mosquito was kept alive in the paper cup until it was released at the collection site on the same day.

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![Fig. 1. The study area (230 m × 250 m) for mark-release-recapture of *Ae. albopictus* and *Ar. subalbatus* located in a residential area of Ishigaki island, Japan.](image-url)
Unmarked mosquitoes were placed on filter paper that was wetted and cooled by a block of ice under the filter paper (Fig. 2a). Each mosquito was given a unique mark by placing spots of paint at five selected sites on the thorax under a stereo-microscope (Fig. 2b). A small spot of paint was applied with the head of an insect pin. The correction fluid “MISNON” (in white) (Lion Office Product Corp., Tokyo, Japan) was used as the paint, and the following three food colors were mixed to make 3 different colors of fluid: Food Blue No. 1 (F0147), Food Yellow No. 4 (F0144) and Food Red No. 2 (F0138) (Tokyo Chemical Industry Co., Tokyo, Japan). The number of unique marks obtained by combining 4 colors and 5 sites is $5^4 - 1 = 3,124$.

Meteorological data collection
Meteorological data, such as daily mean temperature, daily mean humidity and daily wind velocity, were obtained from the nearest meteorological station (24°20’2’’N, 124°9’8’’E) using the online database of the Japan Meteorological Agency (www.data.jma.go.jp/).

RESULTS AND DISCUSSION

The climate conditions during the study period were steady and suitable for a mark-release-recapture study. Rainfall of 1.5 to 18 mm/day was recorded in 4 days and total precipitation during the study period was 25 mm, and the mosquito collections were not disturbed by rain. The daily mean temperature ranged between 21.3 and 25.0°C, and the daily mean relative humidity ranged between 73 and 92% indicated suitable conditions for biting mosquitoes. The daily mean wind velocity varied from 3.2 to 6.2 m/s, however the biting activity of mosquito was not affected by wind mainly because of vegetation barrier.

A total of 309 and 300 females of *Ae. albopictus* and *Ar. subalbatus*, respectively, were collected in the study (Table 1). Among them 232 and 216 females were marked and released during the first 7 days for *Ae. albopictus* and *Ar. subalbatus*, respectively. The proportion of recaptured females was 0.21(=48/232) and 0.09 (20/216) for *Ae. albopictus* and *Ar. subalbatus*, respectively, and the difference was statistically significant (Chi-square = 11.351, $p=0.001$). The lower recapture rate of *Ar. subalbatus* may indicate their higher dispersal ability and larger dispersal range than *Ae. albopictus*. In the literature, the recapture rate of released *Ae. albopictus* marked with fluorescent dye solution varied between 7.7 to 38.7% and the average recapture rate was calculated as 25%, slightly higher

| Date       | *Aedes albopictus* |   | *Armigeres subalbatus* |   |
|------------|--------------------|---|------------------------|---|
|            | No. collected      | No. released | No. recaptures | No. collected | No. released | No. recaptures |
| 18-Mar-2013| 17                 | 17            |               | 19            | 19            |              |
| 19-Mar     | 43                 | 43            | 1             | 32            | 32            | 0             |
| 20-Mar     | 68                 | 68            | 9             | 34            | 34            | 3             |
| 21-Mar     | 22                 | 22            | 6             | 27            | 27            | 2             |
| 22-Mar     | 39                 | 39            | 8             | 56            | 56            | 5             |
| 23-Mar     | 23                 | 23            | 8             | 23            | 23            | 2             |
| 24-Mar     | 20                 | 20            | 9             | 25            | 25            | 3             |
| 25-Mar     | 6                  | 0             | 1             | 2             | 0             | 0             |
| 26-Mar     | 32                 | 0             | 2             | 45            | 0             | 3             |
| 27-Mar     | 39                 | 0             | 4             | 37            | 0             | 2             |
| Total      | 309                | 232           | 48            | 300           | 216           | 20            |
than in this study (Takagi et al., 1995c; Tsuda, 2013). When individual marking was applied to the wings of *Ae. albopictus* the recapture rate was 13% (Takagi et al., 1995a), significantly lower than that obtained in this study (Chi-square=7.265, *p*=0.007). These results suggested that damage caused by the individual marking on thorax used in this study is not heavy and the marked *Ae. albopictus* probably show normal biting behavior.

The recapture rate of released females was calculated for each collection site and is shown in Table 2. The recapture rate of *Ae. albopictus* observed at Site B was 0.4, significantly higher than that observed at Site C (Chi-square=4.211, *p*=0.04) or Site A+D (Chi-square=7.547, *p*=0.006). The differences in the recapture rate of *Ar. subalbatus* among the 4 collection sites were not statistically significant (Chi-square test, *p*>0.05).

Movements of released *Ae. albopictus* among the 4 collection sites are summarized in Table 3(a). The numbers shown in the same row (for example, Site A) mean that 3, 2, 3 and 0 females released from Site A were recaptured at Site A, B, C and D, respectively. Therefore, 37% (=3/8) of females released from Site A were recaptured at the release site. The results for Site B and D showed a similar tendency to Site A, and the percentage of released females collected at the release site was low, 30 and 25%, respectively, whereas at Site C, 88% (=23/26) of released females were recaptured at the release site, indicating that Site C was a suitable place for resting and/or searching *Ae. albopictus*.

The numbers shown in the same column (for example Site A) in Table 3(a) mean that 3, 0, 1, and 1 females recaptured at Site A had been released from Site A, B, C, and D, respectively. This result indicated that Site A received females from Site C and D; however, the frequency was very low. The numbers of females that moved into Site B and D from other release sites shown in the 2nd and 4th columns were also small, 1 and 2. By contrast, 35 out of 48 recaptured females in this study were caught at Site C and 34% (12/35) of the recaptured females had moved from the other 3 sites. As shown in Table 2, the recapture rate of *Ae. albopictus* released from Site B was higher than other collection sites, but 7 out of 10 females released from Site B were recaptured at Site C. This means that after *Ae. albopictus* reached Site B, 70% moved to and stayed at Site C and were recaptured there. These results indicated the directional movement of *Ae. albopictus* from Site B to Site C.

The collection site attractive to searching females and suitable for resting females in this study was Site C, which was located inside the large preservation area covered with vegetation. The association between vegetation and the distribution of *Ae. albopictus* has been reported in many studies (Baker et al., 2003; Chan, 1985; Hawley, 1988; Richards et al., 2006; Swanson et al., 2000; Takagi et al., 1995a, b, c; Tsuda et al., 2002, 2006c). Most of the previous studies examined the spatial distribution of eggs of *Ae. albopictus* by using ovitraps, and studies focusing on the spatial distribution of biting *Ae. albopictus* are limited. In Japan, the movements of biting *Ae. albopictus* were examined within an isolated, grassy and scrub area by mark-release-recapture (Takagi et al., 1995a, c). The dispersal of released females was found to be uneven and collection sites characterized by the accumulation and/or frequent exchange of females through movements were noted. The present study was designed to examine the movements of *Ae. albopictus* in residential areas where patchy mosquito habitats are distributed. The results showed that *Ae. albopictus* moved among different habitats, such as the guest house (Site A), the small preservation area (Site D) and the large preservation area (Site B and C) in addition to frequent within-habitat movements observed within

| Collection site | No. recaptures | No. released | Recap. Rate | No. recaptures | No. released | Recap. Rate |
|-----------------|---------------|-------------|-------------|---------------|-------------|-------------|
| Site A          | 8             | 54          | 0.15        | 0             | 15          | 0           |
| Site B          | 10            | 25          | 0.40        | 2             | 33          | 0.06        |
| Site C          | 26            | 125         | 0.21        | 16            | 129         | 0.12        |
| Site D          | 4             | 28          | 0.14        | 2             | 40          | 0.05        |
| Total           | 48            | 232         | 0.21        | 20            | 216         | 0.09        |

Table 2. Differences in recapture rate of released *Ae. albopictus* and *Ar. subalbatus* among 4 collection sites.

| Release site | Recapture site | Total |
|--------------|----------------|-------|
| Site A       | Site B         | Site C | Site D |
| Site A       | 3              | 3      | 0      | 8    |
| Site B       | 0              | 3      | 7      | 10   |
| Site C       | 1              | 1      | 23     | 26   |
| Site D       | 1              | 0      | 2      | 4    |
| Total        | 5              | 6      | 23     | 48   |

Table 3. Movements of released mosquitoes among 4 collection sites.

(a) *Ae. albopictus*

(b) *Ar. subalbatus*
the large preservation area. The movements of biting females among different habitats are the main factors determining the dispersal range of the biting *Aedes albopictus* population within residential areas where patchy mosquito habitats are distributed, and extensive mark-release-recapture studies will be required in the future to obtain a reliable estimation of the dispersal range.

Movements of *Ar. subalbatus* were confined to Site C where 90% (=18/20) of recaptured females were collected (Table 3(b)). The total number of *Ar. subalbatus* collected in this study was 300 nearly equal to that of *Ae. albopictus*, as shown in Table 1, and *Ar. subalbatus* were collected from all the collection sites (Table 2). Therefore, the density and spatial distribution of *Ar. subalbatus* in this study area were similar to *Ae. albopictus* and only the recapture rate of marked females was different between the two species. Details of movement behavior are not known for *Ar. subalbatus* and more field studies will be necessary to estimate the dispersal ability of the species.

The present study revealed that the individual marking method used in this study is applicable to field studies of the movement of *Ae. albopictus* and *Ar. subalbatus*. Multiple recaptures of individually marked mosquitoes make it possible to trace sequential movements of individuals from place to place. Among 232 *Ae. albopictus* marked and released in this study, only 5 females were collected 3 times and the following 4 different patterns of sequential movements were found: Site C→C→B, Site B→B→C, Site A→D→A, and 2 females collected 3 times at Site C, C→C→C. Although the number of sequential movements found in this study was too small to analyze, the accumulation of sequential movement patterns provides useful information to find out movement of mosquitoes in particular directions. To understand the vegetation-related and/or directional movement and estimate the dispersal range of *Ae. albopictus* and *Ar. subalbatus* in residential areas, long-term individual marking studies will be required in the future to collect and analyze sequential movements among vegetation areas.

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