The movement and dispersal of *Aedes albopictus* emerging at a cemetery to surrounding vegetation areas

Yoshihide Maekawa*1), Yoshio Tsuda1), Takeo Yamauchi2), Masato Igarashi3), Tohru Kazuma4), Yuzo Satou5), Ryuichi Kanayama6) and Kyoko Sawabe1)

* Corresponding author: maekawa@niid.go.jp

1) Department of Medical Entomology, National Institute of Infectious Disease, 1–23–1 Toyama, Shinjuku-ku, Tokyo 162–8640, Japan

2) Institute of Natural and Environmental Sciences, University of Hyogo/Museum of Nature and Human Activities, Hyogo, Yaoigaoka 6, Sanda, Hyogo 669–1546, Japan

3) Hohto Shoji Co., Ltd., 2–1 Kami-Ikedai 5-Chome, Ota-ku, Tokyo 145–0064, Japan

4) Japan Environmental Sanitation Center, 10–6 Yotsuyakami-cho, Kawasaki-ku, Kawasaki City, Kanagawa 210–0828, Japan

5) SC Environmental Science Co., Ltd., 2–8 Doshomachi 2-Chome, Chuo-Ku, Osaka 541–0045, Japan

6) SC Environmental Science Co., Ltd., Home & Environment Products Development Depart, Research & Development Sector, 2–1 Takatsukasa 4-chome, Takarazuka, Hyogo 665–0051, Japan

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Abstract: Cemeteries are suitable habitats for *Aedes albopictus*. The dispersal of adults emerging at a cemetery was studied by a mark-release-recapture experiment during the period July 11–15, 2016 in Okayama, Japan. Larvae and pupae of *Ae. albopictus* were collected from artificial containers found in the cemetery, reared to the adult stage, and used for the experiment. A total of 818 females and 810 males were sprayed with a 0.5% solution of Rhodamine B and released from a release site in a vegetation area surrounding the cemetery. Recapture of released *Ae. albopictus* was conducted for 4 days, by human-bait sweep net collection, at 21 collection sites located in vegetation areas. The recapture rate of females and males was 19.2% and 4.3%, respectively. The longest distance travelled by female and male mosquitoes observed 1 day post release was 99 m and 231 m, respectively. The coefficient of determination indicated that the variation in the number of recaptured females among collection sites was explained 58 and 63% by the distance from release site. The flight range of *Ae. albopictus* was considered to be 100 to 300 m in the urban area with vegetation.

Key words: *Aedes albopictus*, mark-release-recapture, cemetery, movement, dispersal, vegetation

Introduction

*Aedes albopictus* (Skuse) is distributed widely in Japan at a high density, except in the Hokkaido region (Kamimura, 1968; Tanaka et al., 1979; Maekawa et al., 2016). Among Japanese mosquitoes, *Ae. albopictus* is the species with the highest medical importance (Maekawa et al., 2016). *Aedes albopictus* was shown to be the vector of the dengue virus when dengue outbreaks occurred in Yoyogi Park and its vicinity, Tokyo, Japan in the summer of 2014 (Tsuda et al., 2016; Kobayashi et al., 2018). The larvae of this species occur in various types of small habitats, such as cans, buckets, used tires, catch basins, and tree holes. Adult mosquitoes rest and wait for blood sources passing near their natural shelters, such as in the vegetation of undergrowth, grass, and scrubs. It is thought that *Ae. albopictus* mostly obtain their bloodmeals not only from humans, but also from small animals, such as dogs, cats, rats, and birds (Sawabe et al., 2010; Tamashiro et al., 2011). If there is no chance to encounter the preferred blood-source, they move repeatedly to seek more suitable latent and blood-feeding places (Tsuda, 2013).

Cemeteries have many flower vases, catch basins, cups, and buckets that act as breeding habitats of *Ae. albopictus* and are important habitats in and out of Japan (Kamimura, 1968; Schultz, 1989; O’mera et al., 1992; Vezzani, 2007). In Japan, cemeteries were once located in the suburbs, but they have been located adjacent to residential areas in recent years because of expansion of the outskirts of city. It is highly possible that *Ae. albopictus* emerging in cemeteries spread extensively into surrounding residential areas, causing serious biting risk to inhabitants. The question is how large areas around the cemeteries are at risk of
mosquito bite.

The movement and dispersal of adult *Ae. albopictus* has been studied by mark-release-recapture experiments using fluorescent dyes in Japan (Mori, 1979; Takagi et al., 1995a, b; Tsuda and Kamezaki, 2014). The experiments were carried out in a vegetation area in residential area, and a specimen moved to 67 m away from the release point within 24 hours post-release (Takagi et al., 1995a). Among 1,000 female adults of *Ae. albopictus* released in the same area, 10 adults were recaptured in the first 1–5 days post-release at 100 m from the release site, and one adult was recaptured at 4 and 11 days post-release at collection sites with bushes located at 300 m from the release site (Mori, 1979). One of the 48 recaptured females repeated the movement to 187 m between the preservation areas with vegetation and human dwellings with shrubs and trees from the collection site (Tsuda and Kamezaki, 2014). The repeated movements of released mosquitoes among collection sites indicated that the collection site inside the large preservation area was suitable for resting/searching of *Ae. albopictus* and that many females were accumulated through directional movements from surrounding habitats (Tsuda and Kamezaki, 2014). Based on the results of these studies, some mosquitoes could perform sequential searching movement of at least 67 m/day in bushes and accumulated in suitable resting places. It was considered that some individuals could move to different bushes 300 m away. Therefore, in the situations of neighboring human dwellings and habitats of *Ae. albopictus* such as cemeteries where a large number of mosquitoes occur, it is possible that emerging *Ae. albopictus* move to surrounding bushes and residential areas. However, movements of *Ae. albopictus* from cemeteries to the surrounding residential areas have not been studied.

We surveyed the occurrence of *Ae. albopictus* in the urban area of Okayama city in 2015 and 2016. High densities of *Ae. albopictus* adults were observed at a public facility and a neighboring park but breeding habitats are hardly found in these premises. The hill located behind the public facility was covered by secondary forest, face eastward and received a lot of sunshine, opened to the north side facing some dwellings and fields. The secondary forest was dark, with herbaceous plants and shrubs, with accumulation of fallen leaves. A part of the secondary forest surrounding the cemetery extended to the CCLA and the neighboring park.

**Study sites**

A park (Taiyou-no-Oka Kouen) neighboring the public facility (Community Center for Lifelong Learning Activities: CCLA), and the cemetery located on the hillsides behind CCLA, in Okayama city, Japan was chosen for the study site. Okayama city is a temperate area, where annual sunlight hours exceed 2,000 hours, average precipitation is 1,100 mm, mean daily temperature is 16.2°C; we performed this study in June when *Ae. albopictus* typically shows exponential population growth.

The park has *Cerasus* (cherry trees) and *Pinus* (pine trees), and some *Ae. albopictus* come flying to feed in a part of park because there were bushes of *Rhododendron simsii* (azaleas) and *Hydrangea macrophylla* (hydrangeas) suitable for resting/searching. The cemetery contained 121 graves over 1,894 m², located on the middle of the hill which was covered by secondary forest, faced eastward and received a lot of sunshine, opened to the north side facing some dwellings and fields. The secondary forest was dark, with herbaceous plants and shrubs, with accumulation of fallen leaves. A part of the secondary forest surrounding the cemetery extended to the CCLA and the neighboring park.

**Larval survey at the cemetery**

Preliminary surveillance of breeding habitat was carried out in the CCLA and the park in June 2015 and found 54 containers most of which were catch basins. Of these, only one catch basin contained *Ae. albopictus* larvae. On the other hand, *Ae. albopictus* larvae were found in 61 of 180 artificial containers in the cemetery in the same period. We examined the larvae and pupae occurring in all the artificial containers at each grave using pipettes and strainers on June 21, 2016. The larvae and pupae were carried to the laboratory and number of larvae and pupae were counted. Identification was done for the late instar larvae (3–4 instar) and emerged mosquitoes from young instar larvae (1–2 instar) and pupae. Collected larvae and pupae were reared to adult stage and the emerged *Ae. albopictus* were used for the mark-release-recapture experiments.

**Mosquito rearing at insectarium**

Collected larvae and pupae were carried to the insectary room at Okayama Prefectural Institute for Environmental Science and Public Health. Larvae and pupae were transferred and reared in a tray (373×273×63 mm) with water and were fed with fish food. Aged-tap water was used for rearing.
and an aeration pump was used for preventing death of larvae and pupae by deterioration of water quality. Emerged adults were transferred into a cage (200×200×300 mm) daily and were provided with 3% sugar solution. Wet towels were put on the top and sides of the cages to avoid drying and reduce external stimulus causing physical exhaustion. The temperature and humidity in the rearing room was not controlled and allowed to change naturally similar to the outdoor conditions; the mean room temperature was 26.8°C (23.3–32.0°C) and mean room humidity was 71.9% (56.5–80.9%) during the rearing period.

**Mark-release-recapture experiment**

Mosquitoes were released from a release site (RS) in the secondary forest beside the cemetery, 20 recapture sites in residential areas, such as vegetation of trees, shrubs and herbaceous plants, were settled skirting the secondary forest and park within a 300 m radius from the RS (Fig. 1).

In total, 1,784 adults of *Ae. albopictus* were obtained by rearing and a total of 1,628 adults comprising 818 unfed females of 5–10 days old that seemed to be sexually matured and strongly motivated for feeding and 810 males in the same age that to be active individuals were used for the mark-release recapture experiment. A 0.5% solution of fluorescent dye (Rhodamine B, Wako Pure Chemical Industries, Ltd., Osaka, Japan) were sprayed onto the mosquitoes on the release day, and after drying, the mosquitoes were released at RS at 16:00, July 11, 2016. The mark-release recapture experiment was carried out for 5 days from July 11 to 15, 2016. Mosquitoes were recaptured by human-bait sweep net collection for 8 min at 21 collection sites. Mosquito recapture was done at 10:00 and 16:00 on July 12, at 10:00 on July 14 and 15, and collection on July 13 was cancelled because of rain.

Meteorological data (daily precipitation, daily mean temperature, mean relative humidity, and daily wind velocity) were obtained from the nearest meteorological station (N34°41.1′, E133°55.5′) using the online database of Japan Meteorological Agency (https://www.data.jma.go.jp/). Collected mosquitoes were killed using chloroform, identified for species and sex, and the numbers at each collection site were counted. Collected *Ae. albopictus* were placed on filter paper and rinsed with a drop of 99.5% ethanol, for detection of the presence of fluorescent dye on recaptured mosquitoes under ultraviolet light. Collectors wore long-sleeved shirts, long trousers, and a cap, and applied a repellent to the bare skin of their hands and face for avoiding mosquito bites. After the experiment, an ultra-low volume spray of 10% Fenitrothion emulsion (Kincho Sumithion emulsion; Dainihon Jochugiku Co. Ltd., Osaka, Japan) was carried out in the secondary forest surrounding the cemetery to kill the released and resting mosquitoes.
**Results and Discussion**

The larval survey at the cemetery (Table 1) showed that this cemetery was one of the important habitats of *Ae. albopictus* in the study area. Upon larval collection, a total of 3,033 larvae and pupae were collected, of which 2,012 late instar larvae belonged to 5 genera and 5 species. The dominant species was *Ae. albopictus*, which occupied 71% (1,432) of the collected late instar larvae. The secondary dominant species numbered 261 (13%) of *Ae. japonicus* (Theobald), followed by 193 (10%) of *Tripteroides bambusa* (Yamada).

The climate conditions during the mark-release-recapture experiment periods are shown in Table 2. The climate was steady on the day of release and 1 day post-release, but rainfall was recorded on 2 days post-release and the mean wind velocity was stronger on 2-4 days post-release than on 1 day post-release. Little precipitation was observed on 3-4 days post-release. Wind velocity of 3 m/s or more tends to suppress the flight activity of *Ae. albopictus* (Bonnet and Worcester, 1946; Clements, 1999). There was a possibility that the bad weather condition suppressed the movements and dispersals of marked mosquitoes in this study after 2 days post-release. The mean numbers (±S.D.) of unmarked female and male *Ae. albopictus* collected before the rain (1st and 2nd collection) was 24.9±28.8 females/person/8 min and 8.9±9.9 males/person/8 min, whereas those after the rain (3rd and 4th collection) was 16.5±15.8 females/person/8 min and 4.5±4.5 males/person/8 min. Differences in mean adult density of unmarked mosquitoes before and after rain were compared and tested by Tukey’s HSD test. The mean number of unmarked females before and after rain was not significantly different (*t*=1.634, *p*=0.106), but the males showed a significant difference (*t*=2.617, *p*=0.011), indicating that the activity of unmarked mosquitoes was suppressed by bad weather conditions.

Recapture was conducted 4 times at 21 collection sites including the release site, and 192 and 2,304 marked and unmarked *Ae. albopictus*, respectively, were collected in total (Table 3). Five *Tp. bambusa*, 4 *Armigeres subalbatus* (Coquillet), 3 *Culex pipiens* group, and 2 *Ae. japonicus* were collected as well. The number of recaptured/captured *Ae. albopictus* at each collection sites is shown in Fig. 1. The number of recaptures was the largest at RS, following No. 9 and No. 11 (Fig. 1a). At collection sites No. 18, No. 2 and No. 15 over 200 unmarked adult mosquitoes were collected (Fig. 1b). The recapture rate of released marked females and males was 19.2% and 4.3%, respectively. Recapture rates in the similar experiments were 9.7% of females and 8.9% of males (Mori, 1979) and 23.6% of females (Takagi, et al., 1995a).

In the first (10:00) and second (16:00) recapture on 1 day post-release, marked females and males were collected at 6 sites and 4 sites, respectively. The farthest collection sites of marked females and males were 99 m and 231 m from the release site, respectively. The farthest distance of recaptured female *Ae. albopictus* was 67 m from the release site on 1 day post-release, and Mori (1979) recaptured 2 individuals of both sexes of marked *Ae. albopictus* in different bushes at 100 m from the release site in 1 day post-release. The longest flight distance of female and male *Ae. albopictus* in the mark-release-recapture experiment at La Reunion Island was 100 m, and male *Ae. albopictus* used for mark-release-recapture experiment in a scrap tire yard in Missouri was 225 m (Niebylski and Craig, 1994). Bonnet and Worcester (1946) reported the maximum elapsed time was 21 days involving a distance of 212 m. The

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**Table 1.** Results of larval collection from 121 water samples found at a cemetery on June 21, 2016.

| Species         | Total |
|-----------------|-------|
| *Aedes albopictus* | 1432  |
| *Ae. japonicus*  | 261   |
| *Tripteroides bambusa* | 193  |
| *Armigeres subalbatus* | 109  |
| *Culex sp.*      | 17    |
| pupae            | 535   |
| Young larvae     | 486   |
| **Total**        | 3033  |

**Table 2.** Precipitation, air temperature, relative humidity and wind velocity recorded during the mark-release-recapture study conducted in July 2016, Okayama, Japan.

| Days post release (dpr) | Precipitation | Air temperature | Relative humidity | Wind |
|-------------------------|---------------|-----------------|-------------------|------|
|                         | Total (mm)    | Mean (°C)       | Mean (%)          | Mean velocity (m/s) | Max. velocity (m/s) |
| 0 (release day)         | 0             | 27.5            | 84                | 2.3             | 6.7                |
| 1                       | 1.5           | 27.8            | 85                | 2               | 5.6                |
| 2                       | 17            | 26.7            | 88                | 3.5             | 7.1                |
| 3                       | 1.5           | 28              | 76                | 3.6             | 9.4                |
| 4                       | 0             | 24.8            | 70                | 4.7             | 9.6                |
The farthest distance of recaptured female *Ae. albopictus* at 1 day post-release observed in the present study (99 m) agreed with the previous studies. The mosquito population has the potential to expand its distribution 100 m every mosquito generation and more than 10% dispersed further 100 m (Niebylski and Craig, 1994). It is considered that the flight range of *Ae. albopictus* was generally 100 to 300 m (Bonnet and Worcester, 1946; Mori, 1979; Niebylski and Craig, 1994).

Most of marked males and females were collected in and around the release site (Fig. 2a). Distance between release site and recaptured site and number of female mosquitoes recaptured showed negative correlation (Fig. 2b) as reported by Takagi et al. (1995a). Log-regression analysis was done only for females because the number of recaptured male mosquitoes was small and were collected at only 4 sites. Moreover, 8 collection sites further than 150 m from the release site were ignored because no mosquito was recaptured in those sites. The log-regression analysis was

**Table 3. Number of marked and unmarked *Aedes albopictus* caught by human landing-sweep net collection at 21 collection sites and distance from the release site to each collection site.**

| Site No. | Female | Male | Male | 1 day-post-release 2 dpr 3 dpr 4 dpr Total | Unmarked *Ae. albopictus* | Distance from RS (m) |
|---------|--------|------|------|------------------------------------------|--------------------------|---------------------|
|         |        |      |      | 10:00 16:00 10:00 16:00 10:00 16:00 10:00 16:00 | 10:00 16:00 10:00 16:00 10:00 16:00 10:00 16:00 | |
| Release site | 36 | 34 | 6 | 4 | 80 | 29 | 25 | 13 | 20 | 87 | 0.0 | |
| 11 | 6 | 10 | 0 | 4 | 20 | 5 | 12 | 9 | 9 | 35 | 20.6 |
| 9 | 4 | 21 | 8 | 3 | 36 | 1 | 9 | 19 | 13 | 42 | 27.7 |
| 8 | 3 | 3 | 3 | 3 | 12 | 14 | 23 | 19 | 12 | 68 | 33.9 |
| 6 | 0 | 0 | 1 | 2 | 3 | 27 | 21 | 17 | 11 | 76 | 48.8 |
| 12 | 0 | 0 | 0 | 0 | 0 | 1 | 8 | 2 | 3 | 14 | 56.6 |
| 5 | 1 | 2 | 0 | 0 | 0 | 3 | 6 | 45 | 14 | 11 | 76 | 59.0 |
| 20 | 0 | 0 | 0 | 0 | 0 | 2 | 2 | 5 | 9 | 18 | 62.9 |
| 10 | 0 | 0 | 0 | 0 | 0 | 7 | 36 | 40 | 2 | 85 | 90.3 |
| 7 | 0 | 1 | Rainy day | 0 | 2 | 3 | 12 | 12 | 15 | 12 | 51 | 98.9 |
| 4 | 0 | 0 | No collection | 0 | 0 | 0 | 6 | 33 | 11 | 10 | 60 | 116.0 |
| 13 | 0 | 0 | 0 | 0 | 0 | 3 | 7 | 1 | 11 | 3 | 7 | 18 | 117.8 |
| 14 | 0 | 0 | 0 | 0 | 0 | 2 | 1 | 11 | 1 | 27 | 41 | 128.3 |
| 17 | 0 | 0 | 0 | 0 | 0 | 7 | 4 | 2 | 1 | 14 | 180.3 |
| 19 | 0 | 0 | 0 | 0 | 0 | 8 | 45 | 17 | 16 | 86 | 191.8 |
| 16 | 0 | 0 | 0 | 0 | 0 | 16 | 7 | 6 | 6 | 35 | 201.2 |
| 2 | 0 | 0 | 0 | 0 | 0 | 118 | 50 | 36 | 54 | 258 | 211.7 |
| 15 | 0 | 0 | 0 | 0 | 0 | 44 | 33 | 47 | 29 | 153 | 212.1 |
| 18 | 0 | 0 | 0 | 0 | 0 | 88 | 82 | 79 | 30 | 279 | 231.6 |
| 3 | 0 | 0 | 0 | 0 | 0 | 16 | 48 | 31 | 16 | 111 | 234.8 |
| 1 | 0 | 0 | 0 | 0 | 0 | 110 | 11 | 5 | 7 | 133 | 312.8 |
| Total | 50 | 71 | 18 | 18 | 157 | 520 | 524 | 391 | 305 | 1740 | |

A total of 818 females and 810 males of marked *Aedes albopictus* were released from the release site.
performed for 2 periods, 1 day post-release (1st and 2nd recaptures) and 3–4 days post release (3rd and 4th recaptures), respectively (Fig. 2b). The log-regression line showed a significant difference against distance effect at both periods (1 day post release, \( p < 0.001 \); 3 to 4 days post-release, \( p = 0.002 \)). The coefficient of determination indicated that the variation in the number of recaptured females among collection sites was explained 58 and 63% by the distance from RS in 1 day-post release and 3 to 4 days-post release, respectively. Takagi et al. (1995a) reported that the number of recaptured mosquitoes on the collection point after release depended on the distance from the release point, because a higher number of mosquitoes tended to be collected in areas close to the release point, and this “Distance effect” was slowly weakened in the first 3 days post-release. The slope of the regression line were smaller at 3–4 days post-release (0.0082) than those at 1 day post-release (0.0127), thus, it was indicated that the distance effect weakened with elapsed time. The distance effect became smaller against the number of marked \( Ae. \ albopictus \) according to the days post-release as reported by Takagi et al. (1995a), and the results of the present study supported their finding.

Proportions of recaptured mosquitoes at the release site against the total number released were significantly lower in female (50.9%, 80/157) than in male (77.1%, 27/35) (Chi-square = 6.92, \( p = 0.008 \)) indicating that the tendency of staying at the release site was stronger in males than in females. Furthermore, the sex ratio (proportion of females) of collected unmarked mosquitoes was calculated for each collection site, and the distances from the release site (=cemetery) were found to have a significantly positive correlation (Fig. 3, \( r = 0.577 \), \( p < 0.01 \), \( r^2 = 0.333 \)). This result suggests that the cemetery in the study area was the main source of \( Ae. \ albopictus \) and females dispersed at a higher rate than males from the cemetery to the surroundings. The value of the coefficient of determination is 0.333, indicating that one third of the variance of the sex ratio of unmarked mosquitoes was explained with the distance from the cemetery. The present results also indicate the possibility of detecting major breeding habitats of \( Ae. \ albopictus \) based on the information on the ratio of captured males and females.

The cemetery was important breeding habitat of \( Ae. \ albopictus \) in this study area and the maximum flight distance of marked females and males observed in this study was 99 m and 231 m from the release site, respectively. Although the maximum flight distance seems to be long enough to move from the cemetery to the park, we didn’t confirm their movement between them. Because we examined only the early stage of dispersal in this study, it is expected that the released mosquitoes repeat movement day by day and some of them will reach the park and stay there. An additional mark-release-recapture study will be required to get conclusive results in the future.

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