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Characteristics of oviposition behavior of *Aedes aegypti* and *Aedes albopictus* to different shapes of bamboo stump

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Abstract
Many people on low income in developing countries choose bamboo to build their houses. However, many reports mention that mosquitoes from the genus *Aedes* breed in bamboo stumps, although ecological detail of oviposition behavior to bamboo stumps has not been well studied, especially in human residences. This preliminarily laboratory study investigated the oviposition behavior of *Aedes* mosquitoes to bamboo cylinders of four different shapes: Φ4 × 10, Φ4 × 15, Φ6 × 10, Φ6 × 15 cm. Preferences were calculated based on egg oviposition rate (%) for each shape of the cylinders. We concluded that *Aedes aegypti* preferred the wide and deep shape and that *Aedes albopictus* preferred the wide and shallow shape. These results indicate that the oviposition behavior of mosquitoes may change significantly depending on the shape of the bamboo cylinders.

Keywords: Oviposition, Bamboo stump, Shape preference, *Aedes albopictus*, *Aedes aegypti*

1. Introduction

Mosquitoes in the genus *Aedes* are distributed in tropical and subtropical regions and they transmit many infectious diseases (Dengue, Chikungunya, Zika, and others). This genus shows a high preference for humans and adapts well to their environment. The mosquitoes' habit of breeding in bamboo stumps is well reported in India [1, 2], Puerto Rico [3], Philippines [4], Reunion Island [5], Madagascar [6], South Senegal [7], and Mayotte Island [8]. These reports investigated the ecological distribution of one or more species of mosquitos, including *Ae. aegypti* and/or *Ae. albopictus*. Additionally, studies using bamboo cylinders, which are used as a standard collection trap, were reported in Brazil [9], Nigeria [10], Puerto Rico [11], and South Africa [12]. These studies shows that bamboo cylinders are preferred by both species for breeding purposes. Conversely, several groups have investigated the ability of bamboo leaf infusions and bacteria isolated from bamboo to attract *Aedes* mosquitoes [13-15].The possibility that the bamboo stumps is the breeding site of mosquito is considered, but people on low income living in developing countries often choose bamboo as a building material for their residence and outbuildings (Fig. 1). It is breathable, light and durable, and is preferred in the tropics and subtropics. Sometimes it is recommended officially by the authorities [16-20], and it is estimated that one billion people live in bamboo houses around the world [20]. The method of using bamboos as a building material varies according to the community and its location. The use of longitudinally sliced bamboo does not create a problem; however, problems may arise when bamboos are horizontally cut without slicing. In latter case, mosquitoes may be breeding in the cut ends of bamboo stumps, and infection efficiency is expected to increase when humans and such bamboos are in very close proximity. However, such an ecological interaction has not been investigated in detail. Investigation is difficult since one cannot break into the fabric of a house; examinations are only possible on exposed cut edge of bamboo stumps. To date, therefore, research has generally taken place only on a limited portion of the available residential materials, and the additional details on mosquito breeding in bamboo houses remain unclear. But, Several paper already reported that preference behavior of oviposition depend on the surface area of the spawning place [21, 23], and wider areas are preferred in all reports. Therefor in this research, we decided to investigate not only the confirmation of oviposition to bamboo cylinders but also shape preference. This study aim that this experiment clarified the mosquito shape preference in oviposition behavior to bamboo cylinders at the laboratory level and we provide reference information on vector control in bamboo residential area.

2. Materials and Methods

2.1 Mosquito rearing

Laboratory colonies of *Ae. aegypti* and *Ae. albopictus* provided by Institute of Tropical Medicine, Nagasaki University were used in this study. *Ae. aegypti* were collected in Singapore and *Ae. albopictus* were collected in Nagasaki. After collection, the mosquitoes were maintained in the laboratory at 27 °C, at ambient relative humidity, and under a photoperiod of 12:12 h (light:dark). This study used 30 female mosquitoes 7–10 days old for each times test. They were given their first blood feed and then released into the test cage (65 × 65 × 50 cm) (Fig. 2A).

2.2 Bamboo cylinders design

Bamboo (*Phyllostachys bambusoides*) was cut from two diameters to two lengths (four variations: Φ4 × 10, Φ4 × 15, Φ6 × 10, and Φ6 × 15 cm) with a nodal ring positioned at the base. The four bamboo cylinders were arranged close together in the center of the cage floor, each containing water to a depth of ~1 cm (Fig. 2A).

2.3 Egg collection and counting

The mosquitoes were released into the cage (Fig. 2A) and fed on 3% sugar water. The bamboo cylinders were collected after 1 week. While flushing with additional water, the eggs laid inside the bamboo cylinders were scraped off with a metal rod (Fig. 2B) and the suspension poured into a funnel containing filter paper (Fig. 2C). The filter paper bearing the eggs was then dried for ~1 week. After drying, the eggs were transferred from the filter paper to a disposable petri dish and counted with a microscope (Fig. 2D). For *Ae. aegypti*, seven replicates were performed, and for *Ae. albopictus*, six replicates were performed.

2.4 Data analysis

The average value of the oviposition number, and standard deviation was statistically calculated using the real number (Table1). The oviposition rate for each shapes was calculated for each measured and the mean oviposition was obtained by further averaging the oviposition rate of each measurement. The mean oviposition rates (with standard deviation) for each cylinders shape were calculated using Excel (Excel 2010, Microsoft) (Fig. 3).

3. Results and Discussion

3.1 Actual number of oviposition eggs

The total number of oviposition varied widely between each cylinders shape and between the two species (Table 1). The mean number for *Ae. aegypti* was 978.9 ± 683.4 (Max: 2020, Min: 214), the mean number for *Ae. albopictus* was 1289 ± 315.0 (Max: 1822, Min: 858). The difference between the totals was quite large, possibly due to the state of colonies and whether the blood feed was satisfactory. Generally, mosquitoes oviposit in the temperature range 15–35°C, with the optimum being 28–30°C [24, 25]. Even within the optimum temperature range, oviposition rate varies widely, so if the temperature is not controlled, the number of eggs laid changes dramatically. It is thought that temperature changes influenced the number of eggs laid per measurement. The intention had been to control the environment to preclude this effect, but this could not be fully achieved in our laboratory. Therefore, in order to derive means, the egg oviposition rate (%) was calculated for each shape in each test and the following comparisons were considered.

3.2 Tendency of oviposition behavior of *Ae. aegypti*

In this study, *Ae. aegypti* laid eggs preferentially in the deeper, larger diameter cylinders (Fig. 3). Surface area has been shown to be important for oviposition in *Aedes* mosquitoes [21, 23], and our results reflected this. However, since the range of bamboo cylinders shapes was limited in this study, comparisons using a wider range of shapes (especially wider and deeper) is recommended in future studies.

3.3 Tendency of oviposition behavior of *Ae. albopictus*

In this study, *Ae. albopictus* laid eggs preferentially in the larger diameter, shorter cylinders (Fig. 3), showing a difference from *Ae. aegypti*. The difference in behavior probably arises from physiological differences between the two species, and other factors may also be involved. In several survey reports [4, 10], the two species show different behavioral trends, and our results also may related to this natural state.

From these results, it is clear that oviposition behavior changes depending on the shape of bamboo cylinders in laboratory level, and also we assume that too these mosquitos will choose to oviposition in specific bamboo stump shapes on natural state. But, we suspect that this study, limited as it was to a small selection of cylinder diameters and depths, has not revealed the full extent of the environmental factors involved, and we believe it is important to investigate the material of buildings in detail. If the mosquitoes' preferred oviposition location can be found, this would likely be useful information for vector control in bamboo residential areas. Since buildings and people are in close proximity, it is important to investigate the breeding situation in these special building materials. According to a previous study that examined vectors from bamboo stumps, *Ae. albopictus* is mainly reported from Asian regions [1, 2, 4] and *Ae. aegypti* from African [7, 10, 12] , Latin America [3, 9, 11] and other regions [5, 6, 8]. Considering that they showed different behaviors in two species, when applying this result to the field and interpreting it, it is necessary to clarify the species distributed in each region. And, We believe that it would be advantageous to investigate these aspects carefully in the future of vector control in bamboo residential area and it need more investigation.

3.1 Tables and Figures

Fig 1: Bamboo housing in the Philippines. A: Bamboo windbreak. B: Bamboo livestock enclosure

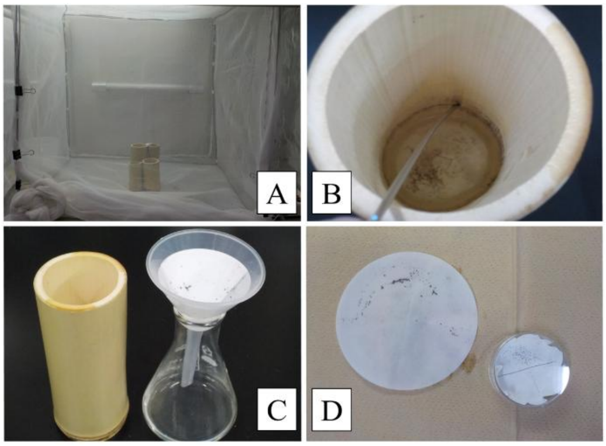

Fig 2: Egg collection and counting. A: Cage with bamboo cylinders. B: Scraping eggs off with a metal rod. C: Egg collection from the infundibulum. D: (Left) Collected sample placed for drying before transferring it to a disposable petri dish. (Right) Collected dried eggs on a petri dish for counting.

Table 1: Mean oviposition rates for each species and each bamboo cylinders shape

Mosquito species	Total oviposition (Max, Min)	Oviposition for each bamboo cylinders shapes (Max, Min)			
		Φ4 × 10 cm	**Φ4 × 15 cm**	**Φ6 × 10 cm**	**Φ6 × 15 cm**
Ae. Aegypti (N = 7)	978.9 ± 683.4 (2020, 214)	101.7 ± 102.0 (299, 9)	158.7 ± 79.1 (234, 28)	264.1 ± 246.1 (710, 31)	477 ± 359.0 (985, 116)
Ae. Albopictus (N = 6)	1289 ± 315.0 (1822, 858)	228 ± 160.0 (277, 102)	265.8 ± 214.8 (634, 111)	654.3 ± 216.0 (201, 265)	185.2 ± 124.4 (375, 81)
					mean ± SD

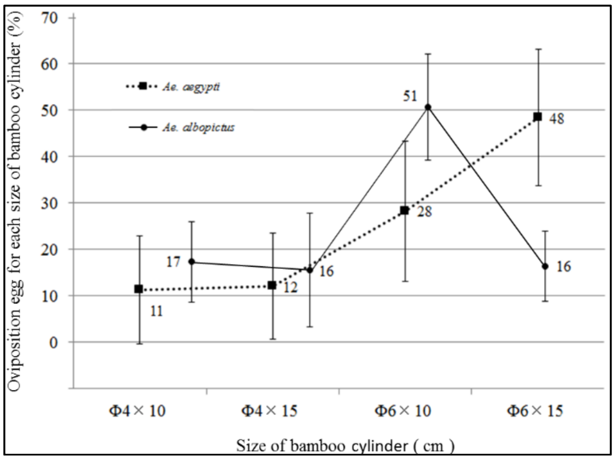

Fig 3: Percentage of oviposition by bamboo cylinder shapes

4. Conclusions

Results showed that oviposition behavior of the two species varied depending on the shape of the bamboo cylinders, with *Ae. aegypti* preferring to oviposit in the wider, deeper bamboo cylinder and *Ae. albopictus* preferring the wider, shallower cylinder.

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