Seasonal changes in oviposition activity, hatching and embryonation rates of eggs of *Aedes albopictus* (Diptera: Culicidae) on three islands of the Ryukyu Archipelago, Japan

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Abstract: An ovi-trap collection was made weekly both indoors and outdoors on three islands of the Ryukyu Archipelago, Japan, from May 2002 to May 2003. The total number of mosquito eggs collected from each of the islands was 17,757 in Okinawa, 11,190 in Ishigaki and 28,375 in Yonaguni. More than 99% of the pupae and adults emerging from the eggs were *Aedes albopictus*, while the rest were *Ochlerotatus togoi*. There were no *Ae. aegypti* eggs confirmed. Oviposition of *Ae. albopictus* was interrupted by heavy rains and typhoons but continued soon after weather recovery. No eggs were collected in January in Okinawa Is., but eggs were found even in winter in Ishigaki Is. and Yonaguni Is., although the number of eggs was small. *Aedes albopictus* oviposited more eggs in ovi-traps outdoors than indoors. The low hatching rates of *Ae. albopictus* eggs during winter in Okinawa Is. in spite of high embryonation rates suggest light dormant eggs. The egg biology of *Ae. albopictus* in the subtropical region is discussed.

Key words: *Aedes albopictus*, *Aedes aegypti*, Okinawa, Ryukyu Archipelago, oviposition, mosquito egg

INTRODUCTION

The eggs of *Aedes* mosquitoes play an important role in their survival during the drought and cold seasons. This has contributed to the expansion of their distribution to severe environments, where larvae and adults cannot survive during certain periods (Christophers, 1960; Gillett, 1972; Hawley, 1988). Since oviposition behavior and hatchability of *Aedes* mosquitoes vary among geographical strains, which is

a reflection of the differences in the local environments (Gillett, 1955; Mogi, 1982; Means et al., 1977), it is necessary to examine these biological characteristics at specific sites.

Aedes albopictus originated in the Indomalayan region, but has been expanding its geographical distribution by dispersion of eggs through international trade routes (Hawley et al., 1987; Hawley, 1988). In Japan, *Ae. albopictus* is common in populated areas, except for the northern part of Japan, and used to be incriminated

Fig. 1. Maps showing the Ryukyu Archipelago, Japan.

as a vector of dengue fever during the dengue epidemics in the early 20th century (Tanaka et al., 1979; Hotta, 1998; Kurihara, 2003). In the subtropical Okinawa Island of the Ryukyu Archipelago, the species breeds throughout the year, with some dormant eggs in winter (Toma and Miyagi, 1981, 1990; Toma et al., 1982). This is in contrast to the temperate region of Japan, where no eggs were collected in winter after diapause eggs were oviposited in autumn (Sasa et al., 1976; Mori et al., 1981). In the southern part of the Ryukyu Archipelago which is also subtropical and close to Taiwan and Southeast Asia, there is no information on the oviposition behavior and egg hatchability of *Ae. albopictus*.

Recent active international trade increases the possible introduction of emerging/re-emerging diseases from other parts of world. It is therefore important to

survey the vector situation in potential areas of introduction of the diseases in Japan. This study examined the seasonal changes in oviposition activity, hatching and embryonation rates of *Ae. albopictus* in residential areas near the coast of the Ryukyu Archipelago, and also determined whether there had been a re-introduction of *Ae. aegypti*, an effective vector of dengue/dengue hemorrhagic fever, which is considered to have disappeared from the Ryukyu Archipelago, Japan in the early 1970s.

MATERIALS AND METHODS

Study site

Mosquito egg collections were conducted on three islands in the Ryukyu Archipelago from May 2002 to May 2003 (Fig. 1). Okinawa Island (Okinawa, 1,202 km², 26°13'N, 127°40'E) is nearly at the

center of the Ryukyu Archipelago; it is the main island, with the Okinawa prefectural capital city of Naha and populated by about 1.3 million people. Ishigaki Island (Ishigaki, 222 km², 24°20'N, 124°09'E) is located 400 km southwest of Okinawa. Yonaguni Island (Yonaguni, 29 km², 24°27'N, 122°57'E) is the westernmost island of the Ryukyu Archipelago and Japan, and is situated about 100 km east of Taiwan. The climate of the Ryukyu Archipelago is subtropical, with several typhoons from May to October every year. The mean annual precipitation for the past 30 years is more than 2,000 mm. The inland area of each island is covered by natural subtropical rain forest.

Ovi-trap collection

Ovi-trap collection sites were selected in residential areas close to the coast in each island. In each site, 2–3 and 3 ovi-traps with 18 cm diameter and 20 cm height were placed indoors and outdoors, respectively. The inside wall of the ovi-trap was lined with a piece of brown paper which was clipped to the wall to avoid being removed by snails, heavy rain and wind. As a rule, the papers were replaced every Monday with new ones. Water was added to ovi-traps to maintain a 2-liter level every week. The collected papers with eggs were dried gradually for 3–4 days at room temperature to complete embryogenesis. The collections and treatment of drying of papers in Ishigaki and Yonaguni were the same as those in Okinawa. The dried papers from Ishigaki and Yonaguni were mailed to the laboratory in Okinawa every 2 weeks. In Okinawa, a container with the same conditions as the ovi-trap (filter paper and water level) was also placed at each collection site to examine larval and pupal appearance. On the day of filter paper collections, the numbers of larvae and pupae in the containers were counted at each collection site and the larvae and pupae were returned to the containers.

Identification

The mosquito eggs laid on paper were examined under a stereomicroscope on the 2nd week after the collections. The number of eggs was counted, and the status of hatching (hatched or un-hatched/remained) was recorded. On the 3rd week after the collections, the remaining un-hatched eggs were submerged in water containing 0.1% of mouse pellet and dry yeast mixture at a ratio of 1:1 to stimulate hatching. The larvae that hatched within a week were reared to pupae or adults in an insectarium. The resulting individuals were identified to species by morphological characteristics, such as pupal paddle and scale pattern of adults. A week after submersion, the eggs were re-examined for hatching; un-hatched eggs were dissected under a stereomicroscope to examine embryonation. Both hatched and un-hatched eggs which formed larvae were regarded as embryonated. Hatching rate was calculated as the proportion of the hatched eggs (already hatched before filter paper collection + hatched in a week's submersion in the laboratory) to the number of the embryonated eggs (already hatched before filter paper collection + hatched in a week submersion + un-hatched in a week's submersion but embryonated). Embryonation rate was calculated as the proportion of the embryonated eggs to the total number of the eggs on filter papers. All the procedures for rearing were done in an insectarium at 25±0.5°C and 80% relative humidity.

Meteorological data

Meteorological data were obtained from monthly reports by a meteorological observatory in Okinawa Prefecture, Japan. In order to examine seasonal differences in the egg number, hatching rate and embryonation rate, the year was divided into seasons according to the average daily temperature: summer (average temperature >25°C, Okinawa: June to September, Ishigaki and Yonaguni: May to

September), winter ($t < 20^{\circ}\text{C}$, Okinawa: November to March, Ishigaki and Yonaguni: January and February) and other ($20^{\circ}\text{C} < t < 25^{\circ}\text{C}$).

RESULTS

Seasonal change in oviposition activity

A total of 72,377 eggs from 810 ovi-trap samples was collected during the study period, of which 57,322 (79.2%) were unhatched (the remained eggs). More than 99.9% of individuals (25,467) reared from the remained eggs were *Ae. albopictus*, and the rest were *Ochlerotatus togoi* (3), indicating that nearly all of the mosquito eggs collected by ovitraps in residential areas of the Ryukyus were *Ae. albopictus*. The island from which the most number of eggs was collected was Yonaguni (36, 871 eggs; average: $145 \pm 165/\text{trap}/\text{week}$) followed by Okinawa (22, 114; 71 ± 122) and Ishigaki (13, 392; 55 ± 84).

The seasonal change in the number of eggs oviposited is shown in Fig. 2a-c. In Okinawa, a large number of eggs was collected from May to November with a sudden decrease in number during unusual weather such as typhoons or heavy rains shown by arrows in Fig. 2a-c; during the period the average precipitation and wind velocity was 4–255 mm/day and 2.5–19.3 m/s, respectively. However, oviposition restarted soon after weather recovery. The egg number then gradually decreased after December, and no eggs were collected in January, during which the average air temperature was the lowest in the year. Oviposition was observed from early February, and the egg number increased with temperature. A similar seasonal change in egg number was observed in Ishigaki and Yonaguni, except for a small number of eggs in winter (January and February). The average egg number was higher in summer than in winter in all islands (one-way ANOVA, Tukey's HSD test: Okinawa, $F = 20.729$, $P < 0.001$; Ishigaki, $F = 12.571$, $P < 0.001$; Yonaguni, $F = 19.940$, $P < 0.001$).

Oviposition site

The average number of eggs collected indoors and outdoors is shown in Table 1. The number of eggs was significantly influenced by seasons and oviposition sites (two-way ANOVA, $P < 0.01$). *Aedes albopictus* oviposited significantly more eggs outdoors than indoors throughout the year on all the islands (t -test, $P < 0.05$).

Hatching rate

The overall hatching rate was significantly higher in Yonaguni (83.6%), followed by Okinawa (79.9%) and Ishigaki (78.3%) ($X^2 = 409.111$, $\text{df} = 2$, $P < 0.001$). The seasonal differences in the hatching rate was not significant in Ishigaki and Yonaguni, but significant in Okinawa (summer > winter, Fig. 3a). The hatching rate of eggs from outdoor ovi-traps was higher than that from indoor ovi-traps in both summer and winter in all islands except for Ishigaki and Yonaguni in winter (summer: Okinawa, $X^2 = 26.626$, $P < 0.001$; Ishigaki, $X^2 = 33.274$, $P < 0.001$; Yonaguni, $X^2 = 11.283$, $P < 0.01$; winter: $X^2 = 55.390$, $P < 0.001$; $X^2 = 0.348$, $P > 0.05$; $X^2 = 0.186$, $P > 0.05$, respectively). The percentage of the eggs hatched before filter paper collections is shown in Table 2. Except for eggs collected from indoor ovi-traps in Ishigaki, the percentage was significantly higher in summer followed by intermediate between summer and winter, and in winter (X^2 test, $P < 0.001$).

Embryonation rate

The difference in embryonation rate had no relationship with oviposition sites and islands. The embryonation rate from outdoor ovi-traps was higher in winter than in summer in Ishigaki and Yonaguni (Fig. 3b).

Larval and pupal appearance

From the identification of eggs in ovi-traps, it was clear that nearly all larvae and pupae observed in the containers should be *Ae. albopictus*. Larvae and pupae in Okinawa were present through-

Fig. 2. Seasonal changes in the number of eggs of *Aedes albopictus* (closed circle) and weekly average temperature (open circle) on three islands of the Ryukyu Archipelago, Japan from May 2002 to May 2003.

out the year for both indoor and outdoor containers (Fig. 4). However, in outdoor containers, younger instar larvae were observed in winter as well as in summer, while in indoor containers, only old instar and pupae were detected in February and March, 2003.

DISCUSSION

Some geographical differences in egg biology during winter among 3 islands in the Ryukyu Archipelago, Japan are suggested by the present study. As sam-

Table 1. Average number of eggs (/trap/collection) of *Aedes albopictus* collected by ovi-traps in Okinawa, Ishigaki and Yonaguni islands of the Ryukyu Archipelago, Japan.

Island	Season	Indoor		Outdoor	
		No. samples	Ave.	No. samples	Ave.
Okinawa	Summer	52	30.7 ^a ±43.3	52	178.0 ^b ±161.3
	Other	44	35.6 ^a ±46.7	37	192.0 ^b ±183.4
	Winter	66	5.6 ^a ±21.3	62	35.9 ^b ±57.5
	Total	162	21.8 ^a ±39.6	151	123.1 ^b ±154.5
Ishigaki	Summer	38	18.6 ^a ±28.3	60	124.3 ^b ±112.1
	Other	42	24.0 ^a ±53.6	62	60.1 ^b ±69.7
	Winter	16	2.4 ^a ±9.2	24	19.0 ^b ±30.7
	Total	96	18.3 ^a ±40.6	146	79.7 ^b ±94.7
Yonaguni	Summer	46	65.5 ^a ±73.9	69	301.7 ^b ±190.4
	Other	40	26.6 ^a ±36.5	60	168.5 ^b ±122.0
	Winter	16	5.9 ^a ±11.6	24	73.9 ^b ±88.7
	Total	102	40.9 ^a ±59.6	153	213.7 ^b ±175.4

Average numbers in the same row followed by the same letter are not significantly different (*t*-test, *P*> 0.05).

Table 2. Seasonal variations in the percentage (No. hatched/total no.) of *Aedes albopictus* eggs already hatched before paper collections on Okinawa, Ishigaki and Yonaguni islands of the Ryukyu Archipelago, Japan.

Island	Season	Indoor	Outdoor
Okinawa	Summer	20.7 ^a (331/1,596)	24.2 ^a (2,240/9,254)
	Other	16.0 ^b (253/1,582)	21.3 ^b (1,510/7,104)
	Winter	0.3 ^c (1/372)	1.8 ^c (41/2,225)
Ishigaki	Summer	3.4 ^a (24/706)	19.0 ^a (1,416/7,455)
	Other	2.7 ^a (27/1,009)	19.7 ^a (734/3,729)
	Winter	0 ^a (0/38)	0.2 ^b (1/455)
Yonaguni	Summer	19.4 ^a (586/3,013)	31.4 ^a (6,547/20,818)
	Other	9.1 ^b (97/1,065)	12.2 ^b (1,232/10,107)
	Winter	1.1 ^c (1/95)	1.9 ^c (33/1,773)

Values in the same column for each island followed by the same letter are not significantly different (*X*² test, *P*>0.05).

pling and identification of the collected eggs were conducted under the same conditions among the studied islands, the observed differences should be due to the local ecological differences. Hatching rate in Okinawa was significantly lower in winter than in summer, whereas there was no significant seasonal difference in Ishigaki and Yonaguni. Shorter day length, drought and coldness could be considered as factors affecting the winter diapause of *Aedes albopictus* egg stage in temperate regions (Imai and Maeda, 1976; Mori et al., 1981; Hawlay, 1988). Lower hatching rate during winter in Okinawa might be the result of a combination of

factors. Toma and Miyagi (1990) showed that some of the embryonated eggs laid in mid-October to December did not hatch even after a 2-month period of submersion. In the present study, it was also indicated that *Ae. albopictus* females in Okinawa oviposited dormant eggs rather than diapause eggs in winter.

Dormancy of insects is considered as a strategy to avoid high mortality rates in adverse environments. *Aedes albopictus* was collected from both tropical and temperate regions in the summer; however, the species enters diapause (in the strict sense) during winter in temperate regions (Hawlay, 1988). Eggs of temperate *Ae.*

Fig. 3. Hatching and embryonation rates of eggs of *Aedes albopictus* by season and collection site on three islands of the Ryukyu Archipelago, Japan (Chi-square test: ***, $P < 0.001$; *, $P < 0.05$; ns, not significant; ID, indoors; OD, outdoors).

albopictus populations do not hatch even in submersion during winter (Mori et al., 1981), while those of tropical populations reproduce all year long without entering diapause (Ho et al., 1971). In this study, although the hatching rate decreased in winter, 38% of the eggs was hatched in Okinawa. Further, Toma and Miyagi (1990) showed that eggs of the species gradually hatched if the climate was warm in Okinawa. From the results of this study and previous observations of other researchers described above, eggs of *Ae. albopictus* in Okinawa may be in a condition of light dormancy during the winter season. These observations likewise suggest that variations in depth of dormancy according to latitudes were also present in *Ae. albopictus* in the Ryukyu Archipelago, Japan, as Hawley et al. (1987) showed by experiment.

Mori et al. (1981) concluded that the

advantage of diapause eggs of *Ae. albopictus* in temperate Japan was to prevent autumnal hatching of eggs and hibernar larval mortality by lethal cold water in habitats, rather than to tolerate low temperatures. In outdoor conditions of winter in Okinawa, eggs hatched, larvae slowly developed and adults fed blood and oviposited on warm days (Toma and Miyagi, 1990). However, the fact that no eggs were collected during winter in the present study shows that the previous observation probably varies from year to year. Thus, the hypothesis for egg dormancy as concluded in the temperate regions (Mori et al., 1981) was unlikely. Due to reduced precipitation (100–125.2 mm from December to February; 176.1–247.2 mm from June to September for the last 30 years in Okinawa) and lower temperatures in winter, drying up of habitats should be a severe and rather significant mortality factor of autumnal hatching in Okinawa. Therefore, gradual hatching of eggs in light dormancy according to weather conditions seemed necessary to prevent high larval mortality brought about by drought, as indicated by Toma and Miyagi (1990).

In Ishigaki and Yonaguni Islands, the percentage of eggs that hatched before collection of filter papers in outdoor conditions during winter was very low, (0–1.9%), whereas that in summer was high (19.0–31.4%), showing seasonal variations. Nevertheless, the final hatching rates of remaining un-hatched eggs in laboratory conditions were not significantly different (Fig. 3 and Table 2), showing that winter eggs in Ishigaki and Yonaguni would have ability to hatch under warm conditions. The results suggested that winter eggs in Ishigaki and Yonaguni ceased activity to some extent although it was unclear whether the eggs were in a condition of dormancy like in Okinawa. Studies on the physiology of eggs in the subtropical region and the transition area between the tropics and temperate regions are important to understand the mechanism of ac-

Fig. 4. Proportion of larvae and pupae observed in outdoor and indoor containers on Okinawa Island of the Ryukyu Archipelago, Japan, from May 2002 to May 2003.

quisition of dormancy for species such as *Ae. albopictus* which is widely distributed throughout the tropics and the temperate regions.

The embryonation rates of outdoor ovi-traps in Ishigaki and Yonaguni were significantly lower in summer than in winter. Mating or insemination probably related to the embryonation rate may be influenced by high temperature, although laboratory experiment is required to clarify the relationship between mating/insemination and temperature with various geographical strains of *Ae. albopictus*. It is known that *Culex pipiens pallens* is sensitive to temperature, and the percentage of inseminated females was reduced under high temperatures experienced during larval and adult stages (Oda et al., 2002).

In this study, *Ae. albopictus* oviposited significantly more eggs outdoors than indoors, and the hatching rate outdoors was higher than that of indoors. The large number of eggs collected outdoors should

be attributed to females reluctant to enter indoors as indicated by Higa et al. (2001). Additionally, outdoor temperature is higher than indoor temperature, which helps outdoor eggs to develop faster than indoor eggs, resulting in higher hatching rates for outdoor eggs.

During the breeding season, ovi-trap collections are very effective in detecting oviposition activity of *Ae. albopictus* as indicated by the rapid recovery after typhoons and heavy rains in this study. In temperate Japan, ovi-trap has often been used to study the distribution and density of *Ae. albopictus* (Moriya, 1974; Mori et al., 1981; Kikuchi et al., 1990). *Aedes aegypti* eggs were not found in the present study; this species also readily oviposits in artificial containers in the domestic environment of tropical countries (Chan et al., 1971; Service, 1993; Ishak et al., 1997). The reestablishment of *Ae. aegypti* in the Ryukyu Archipelago is possible by repetitive introductions of the adults/eggs

through international trades, and also because of the abundance of water-holding artificial containers around residences. In order to study the biology and to monitor the introduction of vector mosquitoes such as *Ae. aegypti* in potential areas of vector borne diseases, the ovi-trap collection can be useful as an easy, inexpensive and effective method for identifying sub-genus *Stegomyia* species.

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