INTRODUCTION

Aedes japonicus (Theobald) is a mosquito distributed primarily in eastern Asia, including Japan, Korea, China, Russia, and Taiwan (1). Aedes j. japonicus, the original subspecies of this species, was first found to have invaded the eastern United States in 1998 (2) and has since expanded to other areas including Canada, France, and Belgium (3–6). Ae. j. japonicus has also been confirmed to be an important vector of West Nile virus, was established from larvae collected in Narita, Japan. The mosquitoes were maintained with induced insemination, blood-feeding on humans, and oviposition in water provided from the original collection site during the first few generations, then the colony was transferred to a large cage (40 × 40 × 100 cm in height) and adapted to conditions in which specimens were allowed to mate freely. White mice were provided as the blood source, and deionized water was available for oviposition. Approximately 185 eggs, most of which were tolerant to desiccation for at least 1 month, with some surviving for up to 2.5 months, were obtained per female following a single blood-feeding. The rate of successful emergence was nearly 90%, although this rate decreased significantly at high larval densities. The colony has been maintained for 5 years, and developmental profiles of the species have been obtained during that time.

MATERIALS AND METHODS

Origin of mosquito colony: The various instar larvae of Ae. j. japonicus (approximately 500 individuals) were collected from the stone basin of a shrine in Narita (35°38’/27°N, 140°28’/52°E), Chiba Prefecture, Japan, in June and July 2004, and they were used to establish the laboratory colony.

Maintenance of mosquitoes: The larvae were reared at a density of approximately 10 larvae per 100 mL and fed with fish food TetraMin (for the early instar larvae; Tetra, Melle, Germany) and SWIMMY BABY (for the late instar larvae; Nippon Pet Food, Tokyo, Japan) in a white plastic tray (W:L:D = 30 × 20 × 5 cm). An appropriate amount of larval food (10–20 and 30–50 mg/day during the 1st to 3rd and 4th larval stages, respectively) was supplied on the basis of the number and developmental stage of the larvae. Additional food was provided once the blocks of larval food had been con-
sumed and disappeared completely. Once the pupae appeared, they were carefully transferred into a cup filled with deionized water and placed inside a cage with stainless-steel supports covered with nylon-mesh net (70 × 30 × 20 cm) for eclosion. The emerging adults were supplied with cotton soaked in an approximately 3% sucrose solution. The adults were reared in a 20 × 30 × 20 cm cage with frequent opportunities to feed, which involved introducing a human arm into the cage up to the wrist as the first generation of mosquitoes was not attracted to white mice. Artificial mating was subsequently performed as described previously (15,16). A glass dish (110 mm in diameter, 60 mm in depth) was lined with Advantech filter paper Grade 2 (Toyo Roshi Kaisha, Tokyo, Japan) positioned with the rough side facing the inside for oviposition. This dish was filled to the halfway mark with water and placed stably in the cage of approximately 50 gravid females for 1 week. Water from the original larval habitat was used to stimulate oviposition. The eggs deposited on the filter paper were submerged in a tray filled with deionized water. A number of adults which had developed from larvae in the original habitat were subsequently collected and placed into the cage along with the second generation in July 2004. The next five generations of the colony were maintained under the same conditions described above. Then approximately 3,000 adults from the following generation were transferred into 40 × 40 × 100 cm cage, the top of which was covered with aluminum foil containing a few small holes. The adults were allowed to mate freely for a period of 3–4 weeks after emergence. Adult females were allowed to take a blood meal from anesthetized white mice (Japan SLC, Hamamatsu, Japan) for 2 h in the latter half of the light period in order to mimic the blood-feeding habit of this species in its natural environment. Additionally, the blood-feeding activity on mice was enhanced for the first few generations by exhaling over the mice for a few minutes, since these mosquitoes exhibited a low preference for white mice. Deionized water was used for oviposition. All developmental stages of mosquitoes were maintained in a controlled insectary at 50–70% relative humidity, a temperature of 25°C, and a light/dark photoperiod of 16/8 h, with no dawn or dusk light dimming.

**Basic biological properties of the established colony:**
The numbers of eggs per female, the hatching rate, the tolerance of eggs to desiccation, the larval development, the density effects of immature stages, and the adult survivorship were examined to describe the basic biological properties of the established colony. All data obtained are quoted as mean and standard deviation. Statistical analyses were performed by one-way analysis of variance (ANOVA) or Student’s t test, followed by Tukey’s honestly significant difference (HSD) test for multiple comparisons of means.

The average number of eggs deposited by an individual female after a single blood meal was estimated by using randomly selected gravid females. To evaluate the hatching rate under free mating conditions, the number of hatched larvae per 100 eggs of 7 days post-oviposition were counted within 2 days after submersion in water. The desiccation tolerance of colony laid eggs was measured by examining the hatch rate after allowing the eggs to dry in an airtight container for between 0 and 11 weeks after oviposition. The survival rate of eggs was determined by counting the number of hatched larvae and confirming the completely collapsed non-hatching eggs by optical microscopy in November 2005.

To examine the developmental period of the colony, hatched larvae were reared individually in 20 mL of pure water with 5 mg of larval food, which were changed daily, and their developmental stage recorded twice a day at the beginning and end of the light period.

The developmental and survival effects of the density of immature stages were determined by measuring the adult emergence rate and the mortality of immature stages at four different initial densities of hatched larvae with a fixed amount (25 mg per day) of larval food. A glass dish (110 mm in diameter, 60 mm in depth) filled with 100 mL of water was used, and both the water and food were changed daily.

Adult survivorship and longevity of the established colony were investigated by recording the number of deaths every day for three experimental cohorts of approximately 20 pairs of newly emerged adults reared with a 3% sucrose solution in a cage.

**RESULTS AND DISCUSSION**

**Colony maintenance:** To improve free mating, the large cage was designed to have a wide vertical space with no direct light other than a few beams of light, produced by covering the top of the cage with aluminum foil containing several small holes, to simulate sunbeams passing through tree leaves in the mosquito’s natural wooded habitat. We observed active flight with phototaxis toward the light beam from the top of the cage. Such environmental conditions have been suggested to be essential for the successful mating of some *Aedes* and *Toxorhynchites* mosquitoes in their native habitat (woodland) (16,17). Deposited eggs were found on the filter paper 5 days after blood-feeding. These eggs subsequently hatched into neonate larvae. The hatching process was observed within 2 days. The eggs on the filter paper were submerged in the water and any air bubbles attached to them were removed by pipetting the water. A number of eggs hatched, indicating that they were fertilized and deposited by females which had mated successfully under our laboratory conditions. We confirmed that the hatched larvae grew and metamorphosed into adults, with the next generation being obtained in the same way. The colony was successfully maintained under the same conditions thereafter.

**Basic biological properties of the colony:** The average number of deposited eggs per individual was 185.3 ± 24.0 (range, 165–218) in June 2008 (n = 4). Oviposition of fertilized eggs was rarely observed (1 out of 40 females) in the initial phase, immediately after the change to free mating, but took place frequently (4 out of 6 females) in June 2008, indicating that the rate of successful mating increased with successive generations.

The successful hatching rate was only 2.0 ± 0.7% in the initial colonization phase in June 2005, and it subsequently increased to 9.6 ± 2.3%, 32.8 ± 6.4%, 46.6 ± 6.2%, and 51.4 ± 11.5% in November 2005, June 2006, June 2007, and June 2008, respectively. This increase in successful hatching rate with increasing gener-
ation number was also reflected in an increase in the free mating success rate. The successful mating of *Ae. j. japonicas* in our colony was also observed. Based on these observations, *Ae. j. japonicus* appears to have a mating system comprised of swarming-like synchronized up-down flight and mating flight, similar to that of other mosquitoes (16). The increase in the successful hatching rate may therefore be largely due to adaptation of the male mating activity to the laboratory conditions, which was suggested to be important for free-mating in *Anopheles* and *Culex* spp. in laboratory colonies (18-20).

Figure 1 shows the tolerance of eggs to desiccation. The hatching rate decreased slightly after desiccation for 1 week with respect to the rate for eggs which had not been desiccated, although this difference may be due to errors arising from the loss of larvae that hatched prior to desiccation. The rate did not change further for up to 5 weeks, but then started to decrease after 6 weeks; and no hatching was detected after desiccation for 9 weeks. However, a very low hatching rate (probably $< 10^{-2}$) was observed up to a maximum of 11 weeks when extremely large numbers of eggs ($>10^6$) were examined (data not shown). These results show that eggs of *Ae. j. japonicus* have a similar tolerance to desiccation as those of other *Aedes* mosquitoes (21). This is consistent with the life history of this species, particularly as regards overwintering in the egg stage.

The developmental periods of the mosquitoes differed between males and females (Table 1). Thus, following hatching, the shortest time necessary for the male and female insects in the colony to grow into adults was 10.5 and 12.0 days, respectively. The females spent significantly longer (1.05 and 0.51 days; Student’s *t* test, $P < 0.001$) in the 4th instar larval and pupal period, respectively. Interestingly, the laboratory colony of the New Jersey strain of *Ae. j. japonicus* (14) also spent a longer time in the 4th instar larval and pupal period, whereas the periods for other instar larvae were consistent with those of our laboratory strains. In addition, the deaths observed tended to be concentrated during the 4th instar larval and pupal stages; none were found in either the 1st or 2nd instar larval stages in our Narita strain, thus suggesting that any developmental fragility may occur during these specific stages.

The adult emergence rates were affected by the rearing density of the larvae (Table 2). An extremely high density of 500 larvae per 100 mL led to both developmental delay and a significant decrease in the numbers of larvae. We also observed that these larvae began to

**Table 1. Developmental periods of immature stage for the established colony of *Aedes japonicus japonicus***

<table>
<thead>
<tr>
<th>Developmental stage</th>
<th>Male (n = 30)</th>
<th>Female (n = 23)</th>
<th><em>P</em> value$^{(1)}$</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Period (days)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Larva</td>
<td>mean ± s.d.</td>
<td>range</td>
<td>mean ± s.d.</td>
</tr>
<tr>
<td>1st instar</td>
<td>2.08 ± 0.19</td>
<td>2.0–2.5</td>
<td>2.04 ± 0.14</td>
</tr>
<tr>
<td>2nd instar</td>
<td>1.65 ± 0.27</td>
<td>1.0–2.0</td>
<td>1.54 ± 0.21</td>
</tr>
<tr>
<td>3rd instar</td>
<td>2.10 ± 0.20</td>
<td>2.0–2.5</td>
<td>2.35 ± 0.35</td>
</tr>
<tr>
<td>4th instar</td>
<td>3.15 ± 0.48</td>
<td>2.5–4.5</td>
<td>4.20 ± 0.45</td>
</tr>
<tr>
<td>Pupa</td>
<td>2.51 ± 0.09</td>
<td>2.5–3.0</td>
<td>3.02 ± 0.10</td>
</tr>
<tr>
<td>Larva + pupa</td>
<td>11.50 ± 0.71</td>
<td>10.5–12.5</td>
<td>13.15 ± 0.80</td>
</tr>
</tbody>
</table>

$^{(1)}$: The value was estimated by *t* tests between both sexes in the same developmental stage.

**Table 2. Effect of rearing density of larvae on adult emergence and mortality in *Aedes japonicus japonicus***

<table>
<thead>
<tr>
<th>Density (Larvae/100 mL)</th>
<th>Adult emergence rate (%) mean ± s.d.$^{(1)}$</th>
<th>Mortality (larva/pupa) (%) mean ± s.d.</th>
<th>Replication</th>
</tr>
</thead>
<tbody>
<tr>
<td>10</td>
<td>88.0 ± 8.7a</td>
<td>8.0 ± 4.5a/4.0 ± 5.5a</td>
<td>5</td>
</tr>
<tr>
<td>50</td>
<td>78.8 ± 6.1a</td>
<td>8.8 ± 2.3a/12.4 ± 3.8b</td>
<td>5</td>
</tr>
<tr>
<td>100</td>
<td>55.0 ± 10.2b</td>
<td>N.D.</td>
<td>5</td>
</tr>
<tr>
<td>500</td>
<td>4.7 ± 1.5c</td>
<td>N.D.</td>
<td>5</td>
</tr>
</tbody>
</table>

$^{(1)}$: Values followed by the same letter are not significantly different in each vertical line ($P < 0.05$, Tukey’s HSD test).

N.D., not determined.
Bite solid foods, the surface of the tray, and even other larvae, suggesting that the rapid decrease in the numbers of larvae observed at high rearing densities could be a result of the increased likelihood of the larvae biting each other. A rearing density of < 50 larvae per 100 mL should therefore be sufficient to provide enough adult mosquitoes to maintain a stable colony. The increase in mortality observed during the pupal stage in the same experiments was also due to this biting habit, although it could be avoided by transferring the pupae to a separate cup, which is the usual method for maintaining a colony.

The maximum lifespan of the adults in the colony was 101 days for females and 89 days for males under the conditions described in the Materials and Methods section. According to the data in the survivorship curve (Fig. 2), adults of both sexes in the colony lived for at least 2 months under these conditions. The mean longevity of male and female mosquitoes was 76.9 ± 9.9 and 84.5 ± 10.8 days, respectively, which is sufficient to be able to carry out infection experiments with mosquito-borne pathogens.

In conclusion, we have succeeded in adapting Ae. j. japonicus to laboratory conditions, and have stably maintained a colony for approximately 35 full generations over a 5-year period. In addition, we have confirmed that a sufficient number of eggs to maintain the colony can be obtained by a single blood-meal, and that additional oviposition as a result of secondary or further blood-feedings can also be successful. This new colony of Ae. j. japonicus will be used to provide material for further studies concerning virus susceptibility and many other basic characteristics of this organism.

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Conflict of interest None to declare.

Fig. 2. Survivorship curves for a cohort of adult Aedes japonicus japonicus. Adults of both sexes, male (n = 56) and female (n = 55), were examined.

REFERENCES