INTRODUCTION

In Japan, the Culex pipiens complex consists of 3 taxa: Culex pipiens quinquefasciatus Say, Culex pipiens pallens Coquillett, and Culex pipiens molestus Forskal. Culex pipiens quinquefasciatus is distributed throughout the Ryukyu and Ogasawara islands. Culex p. pallens and Cx. p. molestus are found north of Kagoshima (31°34′N), throughout the main Kyushu Islands and northward, but not in more southern parts such as Okinawa. Although these 3 mosquito taxa are morphologically similar, except for some minor details such as the structure of the male genitalia, they show marked differences in physiological and behavioral characteristics. Immature Cx. p. molestus are most frequently found in underground water pools and occasionally in open water, and the females exhibit autogeny. In contrast, the females of Cx. p. quinquefasciatus and Cx. p. pallens oviposit only after a blood meal, and their larvae are found in a wide variety of artificial containers with stagnant water such as ditches, gutters, and ground pools (Sasa et al. 1966; Tanaka et al. 1979; Oda et al. 1986, 1999). Female Cx. p. pallens exhibit imaginal diapause, but female Cx. p. quinquefasciatus and Cx. p. molestus lack a diapause (Oda 1992).

We previously compared reproductive activity (insemination rates and egg-hatching rates) and longevity of adults of Japanese Cx. p. pallens and Japanese Cx. p. quinquefasciatus at 25°C and 30°C. Egg-hatching rate was high for Cx. p. pallens at 25°C, but egg-hatching rate was very low at 30°C because very few females were inseminated at this temperature. In Cx. p. quinquefasciatus, the egg-hatching rates and insemination rates were very high even at 30°C. The longevity of both female and male Cx. p. pallens generally was shorter than that in Cx. p. quinquefasciatus at both temperatures. Because high temperatures may restrict the spread of Cx. p. pallens, we suggested that even if this subspecies spread to Okinawa, the possibility of it becoming established is very low. That is, high temperatures may be a limiting factor for the spread of this species to the southern part of Japan (Oda et al. 2002). In the present report, to further determine the reproductive activity of Cx. p. pallens at high temperatures, we examined reproductive activity at high temperatures in hybrids between Cx. p. pipiens and Cx. p. quinquefasciatus or Cx. p. pallens.

MATERIALS AND METHODS

An Indonesian strain and a Japanese strain of Cx. p. quinquefasciatus, a Japanese strain of Cx. p. pallens, and a German strain of Cx. p. pipiens were used in this study. The Jakarta strain (Ja) of Cx. p. quinquefasciatus was collected in Jakarta City (06°11′S), Indonesia, in July 1982, and was maintained for 55–57 generations. The Japanese strain (Ok) of Cx. p. quinquefasciatus was collected in Naha City (25°14′N), Okinawa, in March 1980, and was maintained for 64–66 generations. The Japanese strain (Na) of Cx. p. pallens was collected in Nagasaki City (32°48′N), Japan, in December 1979, and was maintained for 67–69 generations. Hiber-
nating females representing the German strain (Ha) of Cx. p. pipiens were collected in the cellar of a house in Hamburg (53°33'N), Germany, in February 1989. All colonies were maintained in insectaries at 25°C and relative humidity of 70–80% under a 16:8 h light:dark schedule, with 2,000-lux fluorescent lighting. These mosquitoes were fed on mice at night.

One hundred 1st instars were placed in an enamel tray (22 × 28 × 4 cm) with approximately 1,500 ml of water. Equal amounts of brewer's yeast and finely ground mouse-food pellet powder were mixed and given as larval food (Mori et al. 1988). A water suspension of 0.2 g of this mixture was added to each tray every day. About 700 larvae were reared to pupae at 25°C. Thereafter, the pupae were added to each tray every day. About 700 larvae were reared to pupae at 25°C. Thereafter, the pupae were added to each tray every day.

By following procedures described above, hybrid strains between Cx. p. pippens and Cx. p. quinquefasciatus or Cx. p. pipiens pallens and males of Cx. p. pippens but low in the reciprocal crosses (Table 1). In the reciprocal crosses, most unhatched eggs did not contain embryos, indicating that these eggs were from uninseminated females.

The insemination rates at 25°C and 30°C were determined in F1 females between 3 hybrid strains of Cx. p. quinquefasciatus or Cx. p. pallens and males of Cx. p. pippens but low in the reciprocal crosses (Table 1). In the reciprocal crosses, most unhatched eggs did not contain embryos, indicating that these eggs were from uninseminated females.

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RESULTS

The insemination rate as well as the egg-hatching rate in egg rafts were high in crosses between females of Cx. p. quinquefasciatus or Cx. p. pallens but low in the reciprocal crosses (Table 1). In the reciprocal crosses, most unhatched eggs did not contain embryos, indicating that these eggs were from uninseminated females.

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that laid these eggs were not inseminated. The egg-hatching rate of \( F_1 \) females resulting from crosses between females of \textit{Cx. p. pallens} and males of \textit{Cx. p. pipiens} was high at 25°C but 0% at 30°C. Unhatched eggs did not contain embryos. All \( F_1 \) females that laid unhatched eggs were uninseminated.

**DISCUSSION**

Kruppa (1988) crossed various strains of \textit{Cx. p. quinquefasciatus} or \textit{Cx. p. pallens} from Japan with \textit{Cx. p. pipiens} from Germany to determine the insemination rate in the parent generation and the egg-hatching rate in \( F_1 \) adults. He found that both the insemination rate in the parent generation and the egg-hatching rate in \( F_1 \) adults were high after crossing females of \textit{Cx. p. quinquefasciatus} or \textit{Cx. p. pallens} with males of \textit{Cx. p. pipiens}, but low after reverse crossings. The results of the present study were consistent with those of the study by Kruppa (1988), suggesting some degrees of reproductive isolation between \textit{Cx. p. pallens} and \textit{Cx. p. pipiens}. However, reproductive isolation did not occur between \textit{Cx. p. pallens} and \textit{Cx. p. quinquefasciatus}.

The insemination and egg-hatching rates in \( F_1 \) adults resulting from crosses between \textit{Cx. p. pallens} and \textit{Cx. p. pipiens} were high at 25°C but markedly low at 30°C. These results were similar to those in \textit{Cx. p. pallens} (Oda et al. 2002). However, both the insemination and egg-hatching rates were high in the \( F_1 \) between \textit{Cx. p. quinquefasciatus} and \textit{Cx. p. pipiens} at both 25°C and 30°C, which exhibited characteristics of \textit{Cx. p. quinquefasciatus} but not \textit{Cx. p. pallens}. As for the origin of \textit{Cx. p. pallens}, Laven (1967) proposed the interesting theory that \textit{Cx. p. pallens} originated from hybrids between \textit{Cx. p. pipiens} and \textit{Cx. p. quinquefasciatus}. However, the present study suggests that this hybrid does not show physiological characteristics of \textit{Cx. p. pallens}. Moreover, in addition to this, it is necessary to examine whether short day length induces diapause in the hybrids in order to gain a better understanding of the relationship between \textit{Cx. p. pallens} and hybrid offspring.

**ACKNOWLEDGMENT**

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**REFERENCES CITED**


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**Table 2.** Female hybrid (\( F_1 \)) insemination rates between \textit{Culex pipiens pipiens} and \textit{Culex pipiens quinquefasciatus} or \textit{Culex pipiens pallens} at 25°C and 30°C.\(^1\)

<table>
<thead>
<tr>
<th>Name of strain</th>
<th>Female strain</th>
<th>Male strain</th>
<th>Rate of female insemination at 25°C</th>
<th>Rate of female insemination at 30°C</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>( F_1 )</td>
<td></td>
<td>No. dissected</td>
<td>No. (%) inseminated</td>
</tr>
<tr>
<td>( \textit{pallens × pipiens} )</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>NH</td>
<td>Na</td>
<td>Ha</td>
<td>15</td>
<td>13 (86.7) a</td>
</tr>
<tr>
<td>HJ</td>
<td>(Ha)</td>
<td>Ja</td>
<td>15</td>
<td>14 (93.3) a</td>
</tr>
<tr>
<td>OH</td>
<td>Ok</td>
<td>Ha</td>
<td>15</td>
<td>13 (86.7) a</td>
</tr>
</tbody>
</table>

\(^1\) Values within each row (by strain) followed by different letters are significantly different (\( \chi^2 \)-test; \( P < 0.01 \)) between 25°C and 30°C.

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**Table 3.** Hatch rate of \( F_1 \) egg rafts laid by hybrid females between \textit{Culex pipiens pipiens} and \textit{Culex pipiens quinquefasciatus} or \textit{Culex pipiens pallens} at 25°C and 30°C.\(^1\)

<table>
<thead>
<tr>
<th>Name of strain</th>
<th>Female strain</th>
<th>Male strain</th>
<th>Hatch rate of egg rafts at 25°C</th>
<th>Hatch rate of egg rafts at 30°C</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>( F_1 )</td>
<td></td>
<td>No. examined</td>
<td>No. (%) with hatched eggs</td>
</tr>
<tr>
<td>( \textit{pallens × pipiens} )</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>NH</td>
<td>Na</td>
<td>Ha</td>
<td>32</td>
<td>26 (81.0) a</td>
</tr>
</tbody>
</table>

\(^1\) Values within each row (by strain) followed by different letters are significantly different (\( \chi^2 \)-test; \( P < 0.01 \)) between 25°C and 30°C.
lgrades des Fachbereichs Biologie der Universität Hamburg, Hamburg, Germany.


