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# INSEMINATION RATES OF HYBRIDS BETWEEN *CULEX PIPIENS PIPIENS* AND *CULEX PIPIENS QUINQUEFASCIATUS* OR *CULEX PIPIENS PALLENS* AT HIGH TEMPERATURE

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ABSTRACT. Females of *Culex pipiens pallens* are rarely inseminated at temperatures above 30°C. Insemination and egg-hatching rates ($F_2$) were examined in females of hybrids ($F_1$) between *Culex pipiens pipiens* and *Culex pipiens quinquefasciatus* at 25°C and 30°C to examine temperature-dependent reproductive activity. Insemination rates were very high in hybrid females ($F_1$) at 25°C and 30°C, and egg-hatching rate ($F_2$) also was high at both temperatures. Crosses between *Cx. p. pipiens* and *Cx. p. quinquefasciatus* did not show insemination rates and egg-hatching rates similar to the rates observed in *Cx. p. pallens*, suggesting that the hybrids expressed reproductive activity characteristic of *Cx. p. quinquefasciatus*.

**KEY WORDS** *Culex pipiens pipiens, Culex pipiens quinquefasciatus,* hybrids, high temperature, insemination

## 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 stain 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-

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Table 1. Female insemination rates between *Culex pipiens* and *Culex pipiens quinquefasciatus* or *Culex pipiens pallens* and hatch rate of $F_1$ egg rafts at 25°C.

| Name of strain | Strain of parent: Female | Strain of parent: Male | No. (%) females inseminated: No. dissected | No. (%) females inseminated: No. (%) inseminated | Egg hatch rate of egg rafts: No. examined | Egg hatch rate of egg rafts: No. (%) with hatched eggs | Egg hatch rate of egg rafts: No. (%) with unhatched eggs |
|---|---|---|---|---|---|---|---|
| | *quinquefasciatus* × *pipiens* (*pipiens* × *quinquefasciatus*) | | | | | | |
| JH[1] | Ja | Ha | 15 | 10 (66.7) | 25 | 17 (68.0) | 8 (32.8) |
| HJ[1] | (Ha | Ja) | 15 | 4 (22.0) | 12 | 2 (16.7) | 10 (83.3) |
| OH[2] | Ok | Ha | 15 | 7 (50.0) | 59 | 32 (54.2) | 27 (45.8) |
| HO[2] | (Ha | Ok) | 15 | 0 (0.0) | 16 | 0 (0.0) | 16 (100.0) |
| | *pallens* × *pipiens* (*pipiens* × *pallens*) | | | | | | |
| NH[2] | Na | Ha | 15 | 8 (53.3) | 75 | 45 (60.0) | 30 (40.0) |
| NH[3] | (Ha | Na) | 15 | 2 (13.3) | 25 | 1 (4.0) | 24 (96.0) |

[1] Signficant between JH and HJ in either insemination rate ($\chi^2$-test; $P < 0.05$) or hatching rate ($\chi^2$-test; $P < 0.01$).
[2] Significant between OH and HO in either insemination rate or hatching rate ($\chi^2$-test; $P < 0.01$).
[3] Significant between NH and HN in either insemination rate ($\chi^2$-test; $P < 0.05$) or hatching rate ($\chi^2$-test; $P < 0.01$).

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 of each strain were put individually into 2 ml of water in glass tubes (5 cm in height and 2 cm in diameter) plugged with cotton wool and kept at 25°C until adult emergence. Newly emerged adults of each sex were isolated from each other for a further 2 days and maintained in a netted cage (20 × 20 × 30 cm) and provided a constant source of 2% sugar solution. When 2 days old, 100 male *Cx. p. pipiens* were released into a cage containing 100 females of *Cx. p. quinquefasciatus* or *Cx. p. pallens*. To set up a reciprocal cross, 100 females of the former were released into another cage together with 100 males of the latter 2 subspecies. Female insemination rates were examined 5 days later by observation of sperm in the spermathecae dissected in a drop of 0.8% saline under a binocular stereoscopic microscope. The females were regarded as inseminated when sperm was found in 1 or more spermathecae.

To examine egg-hatching rates, bloodfed females from each strain were kept individually in plastic bottles containing 30 ml of tap water, and allowed to oviposit. The percentage of egg rafts with 1 or more hatched eggs and overall percentage of embryos in all egg rafts laid (egg-hatching rate) was determined.

By following procedures described above, hybrid strains between *Cx. p. pipiens* and *Cx. p. quinquefasciatus* or *Cx. p. pallens* were established and allowed to interbreed. The 1st instars were raised to adults at 25°C and 30°C and a portion of the females were dissected to determine insemination rates. The remaining females were fed on mice and allowed to oviposit. The egg-hatching rate for each egg raft laid by the 2nd generation of hybrid strains was obtained by the same rearing method.

## 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* and males of *Cx. p. pipiens* 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 $F_1$ females between 3 hybrid strains of *Cx. p. quinquefasciatus* or *Cx. p. pallens* and *Cx. p. pipiens* (Table 2). The rate was high for each strain at both temperatures. However, the insemination rate in $F_1$ females between females of *Cx. p. pallens* and males of *Cx. p. pipiens* was high at 25°C but low at 30°C.

Insemination and egg-hatching rates also were determined at 25°C and 30°C in $F_4$ hybrids between females of *Cx. p. quinquefasciatus* and males of *Cx. p. pipiens*. Results were similar to those in Table 1. The insemination and egg-hatching rates were high even at 30°C.

After bloodfeeding and oviposition by $F_1$ females shown in Table 2, the egg-hatching rate in $F_2$ hybrids was calculated (Table 3). The hatching rate in eggs laid by $F_1$ females arising from crosses of *Cx. p. quinquefasciatus* and *Cx. p. pipiens* was considerably high at both 25° and 30°C. Unhatched eggs were not embryonated, showing that the females

Table 2. Female hybrid ($F_1$) insemination rates between *Culex pipiens pipiens* and *Culex pipiens quinquefasciatus* or *Culex pipiens pallens* at 25°C and 30°C.[1]

| Name of strain | Strain of hybrids ($F_1$) Female | Male | Rate of female insemination 25°C No. dissected | 25°C No. (%) inseminated | 30°C No. dissected | 30°C No. (%) inseminated |
|---|---|---|---|---|---|---|
| | *quinquefasciatus* × *pipiens* (*pipiens* × *quinquefasciatus*) | | | | | |
| JH | Ja | Ha | 15 | 13 (86.7) a | 15 | 14 (93.3) a |
| HJ | (Ha | Ja) | 15 | 14 (93.3) a | 15 | 13 (86.7) a |
| OH | Ok | Ha | 15 | 13 (86.7) a | 15 | 13 (86.7) a |
| | *pallens* × *pipiens* | | | | | |
| NH | Na | Ha | 15 | 11 (73.3) a | 15 | 1 (6.7) b |

[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.

that laid these eggs were not inseminated. The egg-hatching rate of $F_1$ females resulting from crosses between females of *Cx. p. pallens* and males of *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 *Cx. p. quinquefasciatus* or *Cx. p. pallens* from Japan with *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 *Cx. p. quinquefasciatus* or *Cx. p. pallens* with males of *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 *Cx. p. pallens* and *Cx. p. pipiens.* However, reproductive isolation did not occur between *Cx. p. pallens and Cx. p. quinquefasciatus.*

The insemination and egg-hatching rates in $F_1$ adults resulting from crosses between *Cx. p. pallens* and *Cx. p. pipiens* were high at 25°C but markedly low at 30°C. These results were similar to those in *Cx. p. pallens* (Oda et al. 2002). However, both the insemination and egg-hatching rates were high in the $F_1$ between *Cx. p. quinquefasciatus* and *Cx. p. pipiens* at both 25°C and 30°C, which exhibited characteristics of *Cx. p. quinquefasciatus* but not *Cx. p. pallens.* As for the origin of *Cx. p. pallens,* Laven (1967) proposed the interesting theory that *Cx. p. pallens* originated from hybrids between *Cx. p. pipiens* and *Cx. p. quinquefasciatus.* However, the present study suggests that this hybrid does not show physiological characteristics of *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 *Cx. p. pallens* and hybrid offspring.

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## REFERENCES CITED

Kruppa T. 1988. Vergleichende Untersuchungen zur Morphologie und Biologie von drei Arten des *Culex pipiens*-Komplex. Dissertation zür Erlangung des Dok-

Table 3. Hatch rate of $F_2$ egg rafts laid by hybrid females between *Culex pipiens pipiens* and *Culex pipiens quinquefasciatus* or *Culex pipiens pallens* at 25°C and 30°C.[1]

| Name of strain | Strain of hybrid ($F_1$) Female | Male | Hatch rate of egg rafts 25°C No. examined | 25°C No. (%) with hatched eggs | 25°C No. (%) with unhatched eggs | 30°C No. examined | 30°C No. (%) with hatched eggs | 30°C No. (%) with unhatched eggs |
|---|---|---|---|---|---|---|---|---|
| | *quinquefasciatus* × *pipiens* (*pipiens* × *quinquefasciatus*) | | | | | | | |
| JH | Ja | Ha | 15 | 14 (93.3) a | 1 (6.7) | 30 | 29 (96.7) a | 1 (3.3) |
| HJ | (Ha | Ja) | 21 | 21 (100.0) a | 0 (0.0) | 30 | 30 (100.0) a | 0 (0.0) |
| OH | Ok | Ha | 15 | 12 (80.0) a | 3 (20.0) | 30 | 21 (70.0) a | 9 (30.0) |
| | *pallens* × *pipiens* | | | | | | | |
| NH | Na | Ha | 32 | 26 (81.0) a | 6 (18.8) | 5 | 0 (0.0) b | 5 (100.0) |

[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.

tograder des Fachbereichs Biologie der Universität Hamburg, Hamburg, Germany.

Laven H. 1967. Speciation and evolution in *Culex pipiens*. In: Wright TW, Pal R, eds. *Insect vectors of disease* New York: Elsevier Publishing Company. p 251–275.

Mori A, Oda T, Zaitsu M, Ueda M, Kurokawa K. 1988. Studies on the developing period of larval stage of the *Culex pipiens* complex in Japan. *Trop Med* 30:155–161.

Oda T. 1992. Studies on overwintering of mosquitoes. *Akaieka Newsl* 15:1–15.

Oda T, Eshita Y, Uchida K, Mine M, Kurokawa K, Ogawa Y, Kato K, Tahara H. 2002. Reproductive activity and survival of *Culex pipiens pallens* and *Culex quinquefasciatus* (Diptera: Culicidae) in Japan at high temperature. *J Med Entomol* 39:185–190.

Oda T, Mori A, Fujita K. 1986. Observations on survival changes in feeding activity of *Culex pipiens molestus* in a house in Nagasaki City. *Z Angew Zool* 73:129–134.

Oda T, Uchida K, Mori A, Mine M, Eshita Y, Kurokawa K, Kato K, Tahara H. 1999. Effects of high temperature on the emergence and survival of adult *Culex pipiens molestus* and *Culex quinquefasciatus* in Japan. *J Am Mosq Control Assoc* 15:153–156.

Sasa M, Shirasaka A, Kurihara T. 1966. Crossing experiments between *fatigans, pallens* and *molestus* colonies of the mosquito *Culex pipiens*. 1. From Japan and southern Asia, with special reference to hatchability of hybrid eggs. *Jpn J Exp Med* 36:187–210.

Tanaka K, Mizusawa K, Saugstad ES. 1979. A revision of the adult and larval mosquitoes of Japan (including the Ryukyu Archipelago and the Ogasawara Islands) and Korea (Diptera: Culicidae). *Contrib Am Entomol Inst (Ann Arbor)* 16:1987.