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EFFECTS OF HIGH TEMPERATURE ON THE EMERGENCE AND SURVIVAL OF ADULT *CULEX PIPIENS MOLESTUS* AND *CULEX QUINQUEFASCIATUS* IN JAPAN

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ABSTRACT. The emergence rate and adult survival (longevity) of Japanese strains of *Culex pipiens molestus* and *Culex quinquefasciatus* were compared at temperatures of 21, 25, and 30°C. The pupation and emergence rates in both strains were higher at 21 and 25°C than at 30°C. The adult emergence rate, especially in females, was lower in *Cx. p. molestus* than in *Cx. quinquefasciatus*. Longevity of females and males was lower in *Cx. p. molestus* at 25°C and above. The survival of *Cx. p. molestus* was adversely affected by temperatures of 28°C and higher. High temperature may restrict the distribution of this species. Therefore, if *Cx. p. molestus* infests the Okinawa region, the likelihood that it will become established is minimal.

KEY WORDS *Culex pipiens molestus, Culex quinquefasciatus,* high temperature, longevity, sex ratio, Japan

INTRODUCTION

The *Culex pipiens* complex in Japan consists of 3 taxa, *Culex quinquefasciatus* Say, *Culex pipiens pallens* Coquillett, and *Culex pipiens molestus* Forskal. *Culex quinquefasciatus* is distributed throughout the Ryukyu Islands and Ogasawara Islands. *Culex p. pallens* and *Cx. p. molestus* are found throughout the main Kyushu Islands and northwards, that is, they are found in the parts of Japan north of Kagoshima (31°34′N), but not in more southern parts, such as Okinawa (Fig. 1). Larvae of *Cx. p. molestus* occur most frequently in underground waterpools and occasionally in open water, and the adult females exhibit autogeny. In contrast, females of *Cx. quinquefasciatus* and *Cx. p. pallens* lay eggs only after taking a bloodmeal, and the larvae are found in a wide variety of artificial containers and various types of stagnant water such as ditches, gutters, and ground pools (Sasa et al. 1966, Tanaka et al. 1979, Oda et al. 1980). Female *Cx. p. pallens* exhibit imaginal diapause, but those of *Cx. quinquefasciatus* and *Cx. p. molestus* lack the mechanism of inducing diapause (Oda 1992). Oda et al. (1980) found that the hatching rate of *Cx. p. molestus* became lower at 28°C and the oviposition rate of autogenous females and hatching rate became very low at 30°C, but in *Cx. quinquefasciatus* the rates of oviposition and of egg hatching were high even at 30°C. We speculated that high temperatures may negatively influence physiologic characteristics such as survival of the immature stages and adults of *Cx. p. molestus* and may affect the distribution of this species. In our study, we compare the effects of temperature on emergence and survival (longevity) of *Cx. p. molestus* and *Cx. quinquefasciatus*.

MATERIALS AND METHODS

Strains of *Cx. p. molestus* and *Cx. quinquefasciatus* from Japan were used. The former strain was collected in Nagasaki City (32°47′N), Nagasaki in November 1979 and maintained for 6–10 generations. The latter strain was collected in Naha City (26°14′N), Okinawa, in March 1980 and thereafter bred for 2–6 generations. Details of the collection sites and breeding methods were previously reported by Mori et al. (1988). Mosquitoes were maintained in an insectary at a temperature of 25°C and relative humidity of 70–80% under a 16-h day (light : dark = 16:8 h). Adult *Culex quinquefasciatus* were fed bloodmeals from mice and adult *Cx. p. molestus* were not fed bloodmeals.

Immature stages of each strain were reared from newly hatched larvae at room temperatures of 21, 25, and 30°C, and the rates of pupation and adult emergence were determined. One hundred newly hatched larvae were placed in an enamel tray (22 × 28 × 4 cm) with approximately 1,500 ml water. Five replicates of each treatment were prepared. Equal amounts of brewer's yeast and finely ground mouse pellet powder were mixed and given as larval food. A water suspension of 0.2 g of this mixture was added to each tray every day.

In addition to this experiment, about 600 newly hatched larvae were reared to adults by the method

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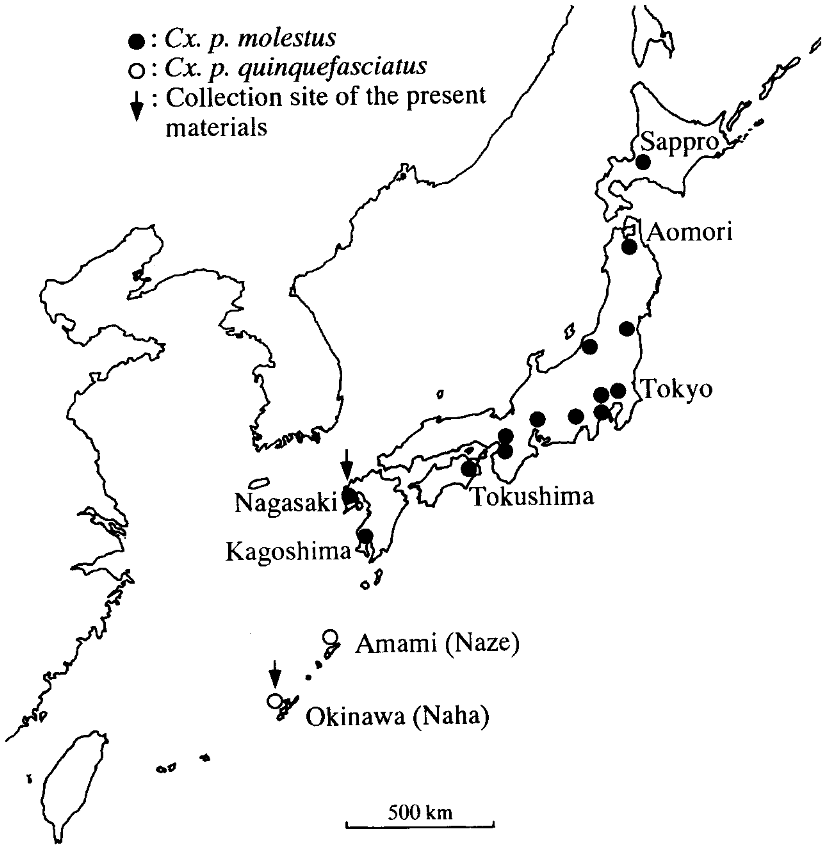

Fig. 1. Map of sites where *Culex pipiens molestus* and *Culex quinquefasciatus* were collected in Japan (from Sasa et al. 1966; Ishii 1978, 1980, 1991; Nishio et al. 1979; Tanaka et al. 1979; Mori et al. 1988).

described above at temperatures of 25 and 30°C. The females and males that emerged at 25°C were maintained at 21°C in a cloth cage (20 × 20 × 30 cm) and given 2% sugar solution. Furthermore, 100 females and 100 males of each strain were maintained in the same manner at 30°C. Survival was observed and the dead females and males were counted daily.

RESULTS

Pupation and emergence rates

We calculated percent pupation and percent emergence. Both were based on 100 larvae per pan. Table 1 shows the mean pupation and emergence rates for 5 trays of *Cx. p. molestus* and *Cx. quinquefasciatus* maintained at 21, 25, and 30°C. Pu-

Table 1. Pupation and emergence rates and sex ratio of *Culex p. molestus* and *Culex quinquefasciatus* at temperatures of 21, 25, and 30°C.

Larval rearing temperature (°C)	*Cx. p. molestus* Pupation and (emergence) rates (% ± SD)	*Cx. p. molestus* No. overall adults	*Cx. p. molestus* Sex ratio (% females)	*Cx. quinquefasciatus* Pupation and (emergence) rates (% ± SD)	*Cx. quinquefasciatus* No. overall adults	*Cx. quinquefasciatus* Sex ratio (% females)
21	91.6 ± 4.5 (90.6 ± 4.1)	453	48.8	81.0 ± 5.5 (78.2 ± 5.3)	391	44.8
25	90.0 ± 1.9 (89.0 ± 1.6)	445	47.9	81.0 ± 2.9 (79.6 ± 4.0)	398	43.0
30	78.0 ± 3.5[1] (50.2 ± 9.5)[1]	251	29.5[2]	96.4 ± 5.4[1] (95.4 ± 5.0)[1]	477	50.3[2]

[1] Significant ($P < 0.001$ by t-test) within species comparisons.
[2] Significant ($P < 0.001$ by χ^2-test) within species comparisons.

Table 2. Mean longevity of adult *Culex p. molestus* and *Culex quinquefasciatus* maintained at 21, 25, and 30°C.

Maintenance temperature (°C)		Mean longevity in days for *Cx. p. molestus*				Mean longevity in days for *Cx. quinquefasciatus*			
		Females		Males		Females		Males	
Immature	Adult	No. replicates (total no.)	Mean longevity (min.–max.) (days)	No. replicates (total no.)	Mean longevity (min.–max.) (days)	No. replicates (total no.)	Mean longevity (min.–max.) (days)	No. replicates (total no.)	Mean longevity (min.–max.) (days)
25	21	1 (100)	54.3[1] (8–102)	1 (100)	33.4 (5–73)	1 (100)	54.3[1] (2–91)	1 (100)	41.1 (4–67)
25	25	2 (196)	29.0[1] (1–40)	2 (202)	27.6[1] (2–36)	2 (190)	64.4[1] (2–100)	2 (198)	39.8[1] (2–72)
30	30	2 (207)	14.2[1] (1–33)	2 (198)	6.9[1] (1–20)	2 (200)	30.1[1] (5–50)	2 (200)	24.7[1] (2–41)

[1] Significant difference ($P < 0.01$ by Mantel–Cox test) for longevity of females or males between *Cx. p. molestus* and *Cx. quinquefasciatus* at each temperature.

pation and emergence rates were statistically the same and were highest in mosquitoes maintained at 21 and 25°C. At 30°C, the pupation and emergence rates were significantly lower for *Cx. p. molestus.* The total number of emerging adults for each species is shown in Table 1. The sex ratio (% emerged females) was treatment-dependent and ranged from 43 to 50% in *Cx. quinquefasciatus* and from 30 to 48% in *Cx. p. molestus.* At 30°C, few female *Cx. p. molestus* emerged, suggesting that high temperatures can result in a skewed sex ratio at emergence for this species.

Adult survival

Table 2 shows the mean longevity of *Cx. p. molestus* and *Cx. quinquefasciatus* at 3 different larval rearing temperatures. Mean longevity time was reduced for females and males of both species maintained at 25 and 30°C with the exception of female *Cx. quinquefasciatus* maintained at 25°C, and the longevity was generally shorter in females and males in *Cx. p. molestus* than in *Cx. quinquefasciatus* at temperatures of 25 and 30°C.

We here summarize the effect of high temperatures on survival and reproductive activity of *Cx. p. molestus* and *Cx. quinquefasciatus* in Japan on the basis of our present and previous results (Oda et al. 1980). In *Cx. p. molestus,* survival was markedly decreased at 25°C and above, and hatching rate of the eggs as well as survival became lower at 28°C. The longevity and emergence rates were significantly lower at 30°C, and the hatching and oviposition rates also became very low. Emergence rate, longevity, hatching rate, and oviposition rate were higher in *Cx. quinquefasciatus* than in *Cx. p. molestus.*

DISCUSSION

Our findings suggest that the reproductive activity and survival of *Cx. p. molestus* are reduced by temperatures above 28°C. In contrast, no significant decrease in reproductive activity or survival rate was recorded in *Cx. quinquefasciatus* at any temperature.

These differences may account for the distribution of these species and for seasonal patterns in population density of *Cx. p. molestus.* In the Okinawa region of Japan, temperatures often rise above 30°C from June to August, and the daily mean temperature stays above 28°C for weeks (Table 3). Therefore, if *Cx. p. molestus* infests more southern parts of Japan, such as Okinawa, the likelihood that this mosquito species will proliferate and establish a permanent population in the region is considered to be minimal.

In the Nagasaki region, the oviposition activity and bloodfeeding behavior of *Cx. p. molestus* was examined during July and August in 1982 by Oda et al. (1986). They found that the number of larvae in aeration tanks that served as the primary oviposition sites decreased during this period. Because the daily mean temperature in this region was higher than 28°C during the above observation (Table 4), much higher than the usual temperature in the

Table 3. Air temperature[1] in Okinawa (Naha), Japan.

Temperature (°C)	Jan.	Feb.	March	April	May	June	July	Aug.	Sept.	Oct.	Nov.	Dec.
Maximum	18.6	19.0	20.8	23.9	26.5	28.8	31.1	30.7	29.9	27.5	24.0	20.6
Mean	16.0	16.3	18.1	21.1	23.8	26.2	28.3	28.1	27.2	24.5	21.4	18.0
Minimum	13.6	13.9	15.6	18.6	21.5	24.2	26.1	25.8	25.0	22.3	19.1	15.7

[1] Mean temperature in each month for the period from 1961 to 1990. The data are from National Astronomical Observatory (1998).

Table 4. Air temperature[1] in Nagasaki, Japan.

	May	June	July	Aug.	Sept.	Oct.	Nov.
Mean air temperature (°C)	18.9	21.1	25.6	28.7	25.2	19.8	14.6

[1] From Oda et al. (1986).

Table 5. Air temperature[1] in Nagasaki, Japan.

Temperature (°C)	Jan.	Feb.	March	April	May	June	July	Aug.	Sept.	Oct.	Nov.	Dec.
Maximum	9.9	10.6	14.1	19.4	23.1	25.8	29.7	31.3	28.1	23.3	17.9	12.5
Mean	6.4	7.0	10.1	15.4	19.5	22.9	26.9	27.6	24.0	18.1	12.3	7.0
Minimum	3.1	3.7	6.3	11.3	15.4	19.3	24.0	24.7	21.1	15.3	10.1	5.3

[1] Mean temperature in each month for the period from 1961 to 1990. The data are from National Astronomical Observatory (1998).

same season (Table 5), the decreased number of *Cx. p. molestus* in Nagasaki was likely the result of reduced reproductive activity and decreased survival caused by high temperature. The hypothesis that high temperatures limit distribution of *Cx. p. molestus,* as suggested by our previous study, is supported by the present results. These findings have implications in studies of intraspecies differentiation of the *Cx. pipiens* complex, and also are relevant to any changing geographic distribution of *Cx. p. molestus* due to global warming.

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REFERENCES CITED

Ishii, T. 1978. Chikaieka wo meguru mondai (Problems on *Culex pipiens molestus*). Akaieka Newsl. 3:1–14. [In Japanese.]

Ishii, T. 1980. Nihon ni okeru chikaieka no bunpu no kakudai (Expansion of distribution of *Culex pipiens molestus* Forskal in Japan). Akaieka Newsl. 5:7–12. [In Japanese.]

Ishii, T. 1991. Integrated study on the *Culex pipiens* complex: Species diversion in the *Culex pipiens* complex. Akaieka Newsl. 14:5–40.

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

National Astronomical Observatory (editor). 1998. Rika nenpyo (chronological scientific tables), Volume 71. Maruzen Co. Ltd., Tokyo, Japan. [In Japanese.]

Nishio, Y., M. Ikuzawa, S. Yonemoto and S. Yamamoto. 1979. Kagoshima-shinai ni seisoku suru chikaieka ni tsuite (*Culex pipiens molestus* found in the city of Kagoshima). Jpn. J. Sanit. Zool. 30:8. [In Japanese.]

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

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

Oda, T., A. Mori, M. Ueda and K. Kurokawa. 1980. Effects of temperatures on the oviposition and hatching of eggs in *Culex pipiens molestus* and *Culex pipiens quinquefasciatus.* Trop. Med. 22:167–172.

Sasa, M., A. Shirasaka and T. Kurihara. 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., K. Mizusawa and E. S. Saugstad. 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) 16:1–987.