

Human Blood Feeding Activity of Female Hybrids between *Culex pipiens pipiens* and *Culex pipiens quinquefasciatus* (Diptera: Culicidae)

Manabu YOSHII¹, Mariko MINE², Kenji KUROKAWA³,
Tsutomu ODA¹, Katsutomo KATO⁴, Yasunori OGAWA⁵,
Yuuki ESHITA⁶, Keikichi UCHIDA⁷

Abstract Human blood feeding activity was examined in females of hybrids between *Culex pipiens pipiens* and *Culex pipiens quinquefasciatus* during long photoperiod at 25 °C. Blood feeding rates of hybrids were lower than in *Culex pipiens quinquefasciatus* and *Culex pipiens pallens*, and higher than in *Culex pipiens pipiens*, because no females fed on human blood in *Culex pipiens pipiens*.

Health Science Research 20(1): 91-93, 2007

Key Words : *Culex pipiens pipiens*, *Culex pipiens quinquefasciatus*, hybrids, human blood feeding

Received 7 Jun 2007

Accepted 27 August 2007

INTRODUCTION

Human Bancroftian and dog filariae (*Dirofilaria immitis*) are transmitted by the mosquito of the *Culex pipiens* complex in Japan. The *Culex pipiens* complex consists of three 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^{1,2)}. Although these three 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. We have studied those characteristics such as diapause, the insemination rates, and survival at high temperature with various strains^{4,6)}. *Culex p. pallens* is assumed to

originate from hybrids between *Cx.p. pipiens* and *Cx.p. quinquefasciatus*³⁾. We have studied insemination rate and diapause state in hybrids between *Cx. p. pipiens* and *Cx. p. quinquefasciatus*^{7,8)}, as first step helping to clarify the physiological characteristics in the hybrids, because the characteristics were scarcely found. In the present paper, we examined human blood feeding rates by hybrids.

MATERIALS AND METHODS

An Indonesian strain of *Cx. p. quinquefasciatus* and a German strain of *Cx. p. pipiens* were used in this study. The strain (Ja) of *Cx.p. quinquefasciatus* from Jakarta was collected in Jakarta City (06°11'S), Indonesia in July 1982, and maintained for 55 to 57 generations. Hibernating females representing *Cx. p. pipiens* (Ha) were collected in the cellar of a house in Hamburg (53°33'N), Germany in February 1989. The *Cx. p. quinquefasciatus* Japanese strain (Ok) was col-

1 Kyushu College of Medical Technology

2 Division of Scientific Data Registry, Atomic Bomb Disease Institute, Nagasaki University Graduate School of Biomedical Sciences

3 Division of Cellular and Molecular Biology, Nagasaki University Graduate School of Biomedical Sciences

4 Department of Physical and Occupational Therapy, Health Sciences, Nagasaki University Graduate School of Biomedical Sciences

5 Nagasaki City Health Center

6 Department of Infectious Disease Control, Oita Medical University

7 Department of Biology, Juntendo University School of Medicine

lected in Naha City (25°14'N), Okinawa in March 1980 and maintained for 64 to 66 generations. The *Cx. p. pallens* strain (Na) was collected in Nagasaki City (32°48'N), Japan in December 1979 and maintained for 67-69 generations. The name of hybrids were written with the abbreviation of OH or JH when the hybrid was produced from Ok strain and Ha strain or when the hybrid from Ja strain and Ha strain. The name of HN shows hybrid between Ha and Na in strain.

All colonies were maintained in insectaries at 25°C and relative humidity of 70-80% under a 16-hr lighting schedule (L:D = 16:8) using 2000 LUX fluorescent lighting. These mosquitoes were fed on mice at night.

One hundred first instar larvae were placed in an enamel tray (22 × 28 × 4 cm) with Ca 1,500 ml water. Equal amounts of brewer's yeast and finely ground mouse-food pellet powder were mixed and given as larval food⁸⁾. 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 two days, maintained in a netted cage (20 × 20 × 30 cm), and provided with a constant source of 2% sugar solution. When two days old, 100 male *Cx. p. pipiens* were released into a cage containing 100 females of *Cx. p.*

quinquefasciatus. In this experiment, the hybrid of the 3rd generation was used.

Following the procedures described above, hybrid strains between *Cx. p. pipiens* and *Cx. p. quinquefasciatus* or *Cx. p. pallens* were established and allowed to interbreed. The first instar larvae were bred to adults in a long photoperiod (L:D = 16:8) and a short photoperiod (L:D = 10:14) at 21°C. The mosquitoes were fed on the arm of a man for 30 minutes at 7 PM to 7:30 PM at night 8 days after emergence, and thereafter, the number of fed females was counted by using aspirator.

RESULTS AND DISCUSSION

Table 1 shows the human blood sucking and egg-laying rates in each line. *Culex p. pallens* and *Cx. p. quinquefasciatus* sucked blood well from humans, and most mosquitoes that sucked blood laid eggs, while *Cx. p. pipiens* did not suck human blood at all. Hybrids between *Cx. p. pipiens* and *Cx. p. quinquefasciatus* or between *Cx. p. pipiens* and *Cx. p. pallens* sucked human blood, though the blood-sucking rates were low. However, the blood-sucking rates in these hybrids markedly differed from those in *Cx. p. quinquefasciatus* and *Cx. p. pallens*. Many hybrid mosquitoes laid eggs. *Culex p. quinquefasciatus* and *Cx. p. pallens* are known to have a taste for human blood, that is, they are anthrophilic. This taste for human blood is a factor associated with major vector mosquitoes for *Wuchereria bancrofti*¹⁰⁾. On the other hand, *Cx. p.*

Table 1 . Human Blood Feeding Activity in Females among *Culex pipiens pallens*, *Cx. p. pipiens*, *Cx. p. quinquefasciatus*, and hybrids between *Cx. p. pipiens* and *Cx. p. quinquefasciatus*

Name of Strain	Female	Male	No. (%) [*] of females feeding	(%)	No. (%) ^{**} of females oviposited after feeding	(%)
Na	<i>pallens</i>		35	35 ¹⁾	26	74.3
Ok	Na <i>quinquefasciatus</i>	Na Ok	45	45 ²⁾	38	84.3
Ha	<i>pipiens</i>		0	0 ³⁾	-	-
	Ha Ha	Ha Ha				
OH	<i>quinquefasciatus</i> × <i>pipiens</i>		19	19	12	63.2
	Ok Ok	Ha Ha				
JH	Ja	Ha	13	13	13	100.0
HJ	Ha	Ja	3	3	1	33.3
HN	<i>pipiens</i> × <i>pallens</i>		16	16	11	68.8
	Ha Na	Ha Na				

* Initial number of females before feeding was 100 in all cases.

** Initial number of females before oviposition were No. of females feeding in each strain.

1) Significant (p < 0.01) by χ^2 -test between Na and OH, JH, HJ, HN, respectively.

2) Significant (p < 0.01) by χ^2 -test between OK and OH, JH, HJ, HN, respectively.

3) Significant (p < 0.001) by χ^2 -test between Ha and OH, JH, HJ, HN, respectively.

pipiens in Europe is known to have a taste for avian blood¹²⁾. Our results were consistent with these findings. In this study, the blood-sucking rate in each hybrid was not so high as that in *Cx. p. quinquefasciatus* but not so low as that in *Cx. p. pipiens*, being roughly intermediate between them. Thus, the hybrids may not have a marked taste for human blood. A previous study on the diapause of hybrids between the same lines showed a shallow diapausing state under a short day condition⁸⁾. This finding together with our results suggests that the physioecological characteristics of the hybrids are intermediate between the characteristics of *Cx. p. pipiens* and those of *Cx. p. quinquefasciatus*. However, the fertilization rate in hybrids between the same lines at high temperature(30 °C) was high, being similar to that in *Cx. p. quinquefasciatus*, and did not show a decrease as is observed in *Cx. p. pallens*⁸⁾. Therefore, the physioecological characteristics of the hybrids are not always intermediate and may considerably vary. This may be mainly due to the lines of *Cx. p. pipiens* and *Cx. p. quinquefasciatus* used in the experiment. Gillett (1971) reported that *Culex pipiens* (*Cx. p. pipiens*) will play a part of transmission of West-Nile-Virus¹²⁾. Based on the results of this experiment, even if *Cx. p. pipiens* with West-Nile-Virus produced in Europe invades Japan, these mosquitoes may not immediately attack humans but may rather feed on avian blood.

REFERENCES CITED

1) Sasa M, Shirasaka A, Kurihara T: 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, 1966.
 2) Laven H: Speciation and evolution in *Culex pipiens*. in Wright TW, Pal R (eds.) Insect vectors of disease, Elsevier Publishing Company, New York, 1967: 251-275.
 3) Tanaka K, Mizusawa K, Saugstad ES: 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, 16: 1987, 1979.
 4) Oda T: Studies on overwintering of mosquitoes. Akaieka Newslet, 15: 1-15, 1992.
 5) Oda T, Uchida K, Mori A, Mine M, Eshita Y, Kurokawa K, Kato K, Tahara H: 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, 1996.
 6) Oda T, Eshita Y, Uchida K, Mine M, Kurokawa K, Ogawa Y, Kato K, Tahara H: Reproductive activity and survival of *Culex pipiens pallens* and *Culex quinquefasciatus* (Diptera: Culicidae) in Japan at high temperature. J Med Entomol, 39: 185-190, 2002.
 7) Kurokawa K, Kato K, Yoshi M, Oda T, Uchida K, Eshita Y, Tahara H, Mine M, Ogawa Y: Insemination rates of hybrids between *Culex pipiens pipiens* and *Culex pipiens quinquefasciatus* or *Culex pipiens pallens* (Diptera: Culicidae) at light temperature. J Am Mosq Control Assoc, 20: 121-124, 2004.
 8) Kurokawa K, Yoshii M, Oda T, Kato K, Uchida K, Eshita Y, Tahara H, Mine M: Effect of photoperiod on blood feeding activity of female hybrids between *Culex pipiens pipiens* and *Culex pipiens quinquefasciatus* or *Culex pipiens pallens* (Diptera: Culicidae). Bull Nagasaki Univ Sch Hlth Sci, 17: 15-17, 2004.
 9) Mori A, Oda T, Zaito M, Ueda M, Kurokawa K: Studies on the developing period of larval stage of the *Culex pipiens* complex in Japan. Trop Med, 30: 155-161, 1988.
 10) Wada Y: Epidemiology of bancroftian filariasis in Nagate and Abumize village, Nagasaki Prefecture, especially in relation to vector mosquitoes. Endem Dis Bull Nagasaki, 8: 45-53, 1966.
 11) Mattingly, P F: The biology of mosquito-borne disease. in The Science biology series 1, George Allen and Unwin Ltd, 1969:164.
 12) Gillett, J D: Mosquitoes. Weidenfeld and Nicolson Ltd, 1971:188.