Effect of Photoperiod on Follicular Development of Females of *Culex* Mosquitoes

Tsutomu ODA[1], Akio MORI[2], Yuki ESHITA[3], Keikichi UCHIDA[4], Kenji KUROKAWA[5], Osamu SUENAGA[6], and Mariko MINE[7]

Abstract The *Culex pipiens pallens* mosquito overwinters in diapausing females which have undeveloped follicles and do not feed on animals blood. To determine the state of such diapause, follicular development was examined using the 1st follicles of unfed females in the *Culex* mosquitoes, *Culex bitaeniorhynchus*, *Cx. halifaxii*, *Cx. mimetics*, *Cx. p. quinquefasciatus*, and *Cx. vagans* reared from 4th instar larvae to adulthood under long or short photoperiods. Under a long photoperiod, the females had well-developed follicles, and under a short photoperiod they had undeveloped follicles in 4 mosquito species besides *Cx. p. quinquefasciantus*. In the field, diapausing females of *Cx. hayashii* and *Cx. mimetics* were collected. This suggests that these mosquitoes overwinter in the diapausing females with undeveloped follicles and that do not feed on blood. In *Cx. p. quinquefasciatus*, a few females with undeveloped follicles appeared under the short photoperiod, but this diapause will be extremely light.

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Introduction

The mosquito of *Culex pipiens pallens* overwinters in diapausing females which have the 1st follicles in an undeveloped state and do not feed on animal

1 Department of General Education, The School of Allied Medical Sciences, Nagasaki University
2 Department of Medical Zoology, Nagasaki University School of Medicine
3 Department of Parasitology, Kurume University School of Medicine
4 Department of Bioloy, Premedical Course, Juntendo University
5 Department of Bacteriology, Nagasaki University School of Medicine
6 Reference Center, Institute of Tropical Medicine, Nagasaki University
7 Scientific Data Center of A-Bomb Disaster, Nagasaki University School of Medicine

blood. This diapause state is induced by a short photoperiod[2)3)5)]. The occurrence of such adult diapause has been confirmed with geographically different strains of *Cx. p. pallens* in Japan and strains of *Cx. p. pipiens* in Hamburg, Germany and in Helsinki, Finland[6)8)12)13)]. In addition, *Cx. tritaeniorhynchus* which is an important vector of Japanese encephalitis has also been confirmed to overwinter in a diapause state[9)]. Diapause of such type is assumed to occur in the other mosquitoes of the genus *Culex* in Japan.

Follicular development was examined in *Culex* mosquitoes to make clear the occurrence of the diapause state and the possibility of overwintering of Japanese encephalitis virus in the mosquito body.

Materials and Methods

The *Culex* mosquito larvae of 5 species, *Culex bitaeniorhynchus, Culex halifaxii, Culex minetics, Culex pipiens quinquefasciatus*, and *Culex vagans* were collected in the field. Table 1 shows the outline of the collection. The females of *Culex mimetic* and *Culex hayashii* were collected in caves in Aino, Nagasaki prefecture in early January, 1974. The collected larvae were reared to an early stage of the 4th instar in room temperature conditions at 18-21℃, and natural day-length. The larvae of the 4th instar were transferred to each experimental condition of photoperiod from 8 to 16 hours in illumination at a constant temperature of 21℃ and thereafter bred to adulthood. To rear larvae of *Cx. halifaxii*, young larvae of *Cx. p. pallens* were given to them as their food every day. Larvae of other species were reared on food of an equally mixed powder of Ebios (brewers yeast) and mouse pellet. Adults were kept on a 2% sugar solution in a cage of 20×20×30cm in

Table 1. Species of *Culex* mosquito larvae used for the experiment

Species	Collection site	Date of collection	Stage of larvae collected
Culex bitaeniorhynchus	Nagasaki City, Nagasaki	May, 1972	III, IV
Culex halifaxii	Nagasaki City, Nagasaki	May, 1972	VI
Culex mimetics	Nagasaki City, Nagasaki	July, 1972	III, IV
Culex pipiens quinquefasciatus	Naha City, Okinawa	July, 1982	III, IV
Culex vagans	Nagasaki City, Nagasaki	May, 1973	III, IV

III or IV: The 3rd or 4th larvae.

which adults of *Cx. q. quinquefasciatus* copulated, but the other species did not. The unfed females were killed 10 days after emergence and thereafter preserved in a freezer at minus 18℃ for 10 days or more. The females collected in a cave were also preserved similarly.

The first follicles of unfed females were inspected and measured in an isotonic saline solution under a stereoscopic microscope. The developmental stages of the follicles were classified on the basis of the definition made earlier by Oda and Wada (1972)[3]. As for *Cx. vagans*, a mouse was given to the females to feed on one night 10 days after emergence. The blood fed females were reared under the same conditions, 10 days after blood feeding of a mouse. They were also dissected under a binocular microscope to confirm the presence of eggs (follicles of the 5th stage).

Results

Table 2 shows the developmental state of the 1st follicles of unfed females of 4 species of *Culex* mosquitoes, which were reared from the 4th instar larvae in the experimental conditions of long or short photoperiod at 21℃. The females of 4 mosquito species had well-developed follicles of 60 μm or more and in the stage of I a and I b under a long photoperiod (L : D =16:8). In the short photoperiod (L : D =8:16), the follicles were undeveloped being 50 μm or less in the size and in the stage of N in most females of *Cx. bitaenior-*

Table 2. Follicular development of *Culex* mosquito females reared from the wild-caught larvae of the 4th instar

Species	Photoperiod (L : D)**	No. females with follicles of indicated size*											Total	Follicular stage
		4-	5-	6-	7-	8-	9-	10-	11-	12-	13-	14-		
Culex bitaeniorhynchus	16 : 8		1	1	3								5	Ib
	8 : 16	1	10	2	3								16	N-Ia
Culex halifaxii	16 : 8								1	3	1	2	7	Ib-IIb
	8 : 16		3	4	2								9	N-Ib
Culex mimetics	16 : 8				3	2	1						6	N-Ib
	8 : 16	5	10	3	2								20	N-Ia
Culex pipiens quinquefasciatus	16 : 8			1	3	5	1						10	N-Ib
	8 : 16		2	2	3	3	4						14	N-Ib

* One unit = 10 μm

** hours

hynchus and *Cx. minetics*. That is, they produced diapausing females as shown in *Cx. p. pallens*. In *Cx. halifaxii*, the follicles were very large, being 110 μm or more and well-developed in the stage of I b to II b in a long photoperiod. However, under a short photoperiod, most of the females had the follicles which were 60 to 70 μm and at the stage of N to I b. These follicles were also undeveloped for this species, though they were larger than the follicles in the diapausing females of other species, because there is a large difference in follicular size in the females bred between a long photoperiod and a short photoperiod. This may be related to the fact that this mosquito is predacious.

The females of the 3 species besides *Cx. p. quinquefasciatus* had undeveloped follicles when they were bred under a short photoperiod. By contrast, females of *Cx. p. quinquefasciatus* lack the ability to show the state of diapause[1)14)]. However, a few females had undeveloped follicles under the short photoperiod in this experiment.

Many larvae of *Cx. vagans* were collected in the field. The larvae of the 4th instar were bred to adulthood in the experimental conditions with photoperiods from 11 to 16 hours of illumination at a temperature of 21℃. The gonoactivity of females was examined. (Table 3)

The findings indicated that when the photoperiod was longer than 13 hours, the females had well-developed follicles, showing high feeding activity and low rate of gonotorophic dissociation, a phenomenon that fed females did not produce eggs (follicles of the 5th stage), and when the protoperiod was shorter than 12 hours, most of the females had undeveloped follicles, and a

Table 3. Follicular development and gonoactivity of *Culex vagans* females reared from the wild-caught larvae of the 4th instar at 21℃ and various photoperiods

Photoperiod (L : D)	No. females* with the follicles of indicated size**							Total	Follicular stage	Feeding			GD***		
	4-	5-	6-	7-	8-	9-	10-			No. exposed	No. fed	%	No. fed	No. GD	%
16 : 8			1	5	1			7	N-Ib	-	-	-	-	-	-
13 : 11		2	2	3	1	2		10	N-Ib	23	12	52.2	11	0	0.0
12 : 12	7	2	1	1				11	N	32	2	6.3	2	1	50.0
11 : 13	4	1						5	N	5	0	0	-	-	-

* Ten days after emergence

** One unit = 10 μm

*** GD: Gonotrophic dissociation

very low blood feeding rate. The gonotrophic dissociation usually occurred under a short photoperiod of 12 hours. However, the number of females showing gonotrophic dissociation was very small, because the feeding rate was very low. Furthermore, the critical photoperiod for inducing the diapausing state was 12.5 hours in *Cx. vagans*.

The overwintering females of *Cx. hayashii* and *Cx. mimetics* were collected in small numbers, in early January, 1974. All of them had undeveloped follicles of 50 μ or less. (Table 4) Accordingly, it is estimated that except for *Cx. p. quinquefasciatus*, 4 species of *Culex* mosquitoes enter the state of diapause under short photoperiods.

Discussion

The present study showed that the diapausing females with undeveloped follicles appear in experimental or natural conditions under a short photoperiod in 5 species of *Cx. bitaeniorhynchus, Cx. halifaxii, Cx. hayashii, Cx. mimetics* and *Cx. vagans*. The same phenomena has been reported in *Cx. p. pallens* and *Cx. tritaeniorhynchus*[2)7)]. Therefore, the 5 species of *Culex* mosquitoes are also assumed to overwinter in unfed and nulliparous females in a diapausing state with undeveloped follicles.

In *Cx. vagans*, gonotrophic dissociation usually occurred with the low feeding rate, when the photoperiod was short in 12 hours. The same findings have also been reported in *Cx. p. pipiens, Cx. p. pallens*, and *Cx. tritaeniorhynchus*[4)7)9)13)]. In all experiments, the number of females showing gonotropric dissociation was very small, because the feeding rate was very low.

Table 4. Follicular development of *Culex* mosquito females collected in the cave in early January, 1974.

Species	No. females with follicles of indicated size *		Total no. fimales	Follicular stage
	4-	5-		
Culex hayashii	1	1	2	N
Culex mimetics		3	3	N

* One unit = 10 μm

Accordingly, gonotrophic dissociation may not play an important role in the overwintering ecology of *Cx. vagans*. We considered the possibility of Japanese encephalitis virus overwintering in the body of *Cx. vagans* to be very low.

Also the *Anopheles sinensis* mosquito overwinters in a diapausing state as does *Cx. p. pallens*[2)13)14)]. On the other hand, *Armigeres subalbatus* larvae overwinter in a diapausing state[11)]. These females had well-developed follicles of stage I a to II a when they were bred from the 1st instar larvae as adults in a short photoperiod[10)]. Therefore, the appearance of undeveloped follicles in *Culex* and *Anopheles* mosquitoes is considered to be specific for the mosquitoes with a physiological character of the imaginal diapause. This physiological mechanism concerning the formation of undeveloped follicles is not clear and will be an interesting problem in the view point of the physiology of diapause.

The *Cx. p. quinquefasciatus* mosquito does not undergo diapause experimentally[1)13)]. Reisen et al[15)], reported that many females of this species which were collected in resting shelters in fall and winter in the field had ovaries with follicles in a diapause state. The females also seem to have undeveloped follicles. Their findings are in agreement with ours in the point that diapausing females appeared, though the incidence of occurrence of the females is different. This implies that occurrence of diapausing females varies with the strain. The diapause of the females with undeveloped follicles in Japan, is considered to be extremely light because most females feed on animals actively even under short photoperiods[14)]. The findings obtained data shown by Reisen et al.(1986)[15)] and ours are very interesting when considering the geographical speciations of the *Culex pipiens* complex in the view point of diapause.

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イエカ類の濾胞の発育に及ぼす日長の影響

小田　力[1]，森　章夫[2]，江下　優樹[3]，内田　桂吉[4]，
黒川　憲次[5]，末永　敏[6]，三根真理子[7]

1　長崎大学医療技術短期大学部一般教育
2　長崎大学医学部医動物学教室
3　久留米大学医学部寄生虫学講座
4　順天堂大学医学進学課程生物学教室
5　長崎大学医学部細菌学教室
6　長崎大学熱帯医学研究所資料室
7　長崎大学医学部付属原爆被災学術資料センター

要　旨　アカイエカやコガタアカイエカでは吸血欲を欠き，未成熟濾胞を持った，いわゆる休眠雌が越冬する．休眠状態は短日条件により誘起される．他のイエカ類でも休眠雌が越冬に入ると推測される．そこで，長崎産のカラツイエカ，ミナミハマダラウスカ，トラフカクイカ，スジアシイエカと沖縄産ネッタイイエカの4令幼虫を21℃の長日または短日条件下で飼育羽化させて雌の第1濾胞の発育状態を調べた．長日条件下では，いずれの種類もよく発達した濾胞を持ったが，短日条件下では前4種のほとんどの雌は未成熟濾胞を保有した．野外ではミナミハマダラウスカとコガタクロウスカの休眠雌が採集された．したがって，これらのイエカも休眠状態に入って越冬するものと思われる．また，ネッタイイエカでも短日条件下で未成熟濾胞保有雌が，少数ではあるが，出現した．しかし，ネッタイイエカは短日条件下でもよく吸血するので，未成熟濾胞の雌は休眠状態にあるとしても，極めて浅いものと思われる．

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