

Studies on Imaginal Diapause in *Culex pipiens* Complex in Japan

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Abstract Follicular development and feeding activity were examined in unfed females of 3 strains (Sapporo, Nagasaki and Kagoshima) of *Culex pipiens pallens* and one strain (Amami) of *Cx. p. quinquefasciatus*, which were reared to adulthood under photoperiods from 8 to 16 hours at 21°C, to examine the geographical variation of the critical daylength for induction of the state of diapause in the females of *Culex pipiens* complex in Japan. The females of the Sapporo strain had well-developed follicles and a very high feeding rate when the photoperiod was longer than 13 hours. With shorter photoperiods, the females had small undeveloped follicles and a low feeding rate; thus they were in a state of diapause. In both Nagasaki and Kagoshima strains, the diapausing females appeared at a daylength of 12 and 11 hours, respectively. Thus, the critical daylength varies with the strains, being longer in northern strains than in southern strains. However, *Cx. p. quinquefasciatus* females were not in a state of diapause even under a short photoperiod of 10 hours. In addition, in 3 strains of *Cx. p. pallens* the diapausing females were produced under a 10-hour photoperiod at 21°C. After they were exposed to reactivation conditions (photoperiod of 16 hours and 25°C), follicular development, feeding rate and gonotrophic dissociation were examined in the females to elucidate the geographical variation of the diapause of *Cx. p. pallens*. Most of the females of the Sapporo strain had small follicles, and their feeding rate was low for 1 to 3 days after exposure to the reactivation conditions, but on day 5, the follicles began to develop with an increase of feeding rate, and females became gonotrophic. Gonotrophic dissociation was usually observed in the females with low feeding activity for 3

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days after exposure to reactivation conditions. On day 5, it became very low with an increase in feeding activity. The females of Nagasaki and Kagoshima strains reactivated more quickly than those of the Sapporo strains. The Kagoshima strain was reactivated the most quickly among the 3 strains. Thus, it is clear that the depth of diapause varies with the strain, being deeper in northern strains than in southern strains. On the other hand, fed females collected in autumn in the field showed a low rate of gonotrophic dissociation and the 1st follicles were in stages IIa and IIb. Individuals with such large follicles were not found in a population of overwintering females. These findings confirm that the gonotrophic dissociation does not play an important role in the overwintering ecology of *Cx. p. pallens* in Japan, that is, the females of this mosquito usually hibernate in the state of diapause without feeding on animal blood.

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Key Words : *Culex pipiens pallens*, *Culex pipiens quinquefasciatus*, *Culex pipiens* complex, diapause, gonoactivity

Introduction

Oda²⁾³⁾⁵⁾ reported that in Nagasaki, southern Japan, the overwintering population of the mosquito of *Culex pipiens pallens*, a member of *Culex pipiens* complex is mostly composed of diapausing females with undeveloped follicles, which do not take animal blood meal, the state of diapause of which is induced by short daylength in autumn. On the other hand, in northern Europe the mosquito of *Cx. p. pipiens*, another member of *Culex pipiens* complex are widely distributed, and they also overwinter in a state of diapause similar to that of *Cx. p. pallens* in Japan. However, the critical daylength for induction of the diapausing females is longer in *Cx. p. pipiens* than in *Cx. p. pallens*. Moreover, imaginal diapause is also deeper in the former than in the latter. That is, these results show that there is geographical variation in depth of diapause as well as in critical daylength between *Cx. p. pipiens* and *Cx. p. pallens*.⁶⁾⁷⁾¹⁰⁾ Such geographical variation of diapause in *Culex pipiens* complex seems to be important in view of a better understanding of the geographical speciation of this mosquito species. This study examined the geographical variation of diapause in several strains of the *Culex pipiens* complex in Japan by examining follicular development, feeding activity and gonotrophic dissociation, a phenomenon in which females take a blood meal, but their ovaries do not develop to maturity.

Materials and Methods

Three strains of *Cx. p. pallens*, which were collected in the cities of Sapporo, Nagasaki and Kagoshima, and then established as laboratory colonies at the insectarium with a photoperiod of 16 hours at 25°C as well as one strain of *Cx. p. quinquefasciatus* collected in Naze, Amami Island, were studied. Table 1 shows the outline of these strains. Follicular development, feeding activity and incidence of gonotrophic dissociation were examined with females of *Cx. p. pallens* and *Cx. p. quinquefasciatus* that were reared from 1st instar larva in the laboratory with various photoperiods ranging from 8 to 16 hours at a temperature of 21°C. They were exposed to a mouse for one night to observe feeding activity 7 to 10 days after emergence. In addition, the females of *Cx. p. pallens* (3 strains) that had emerged in the experimental conditions of a short photoperiod of 10 hours at 21°C were reactivated by exposure to a long photoperiod of 16 hours at 25°C, to determine the depth of diapause. Follicular development, feeding activity and gonotrophic dissociation rate were examined in such females which were reared under reactivation conditions. The food (2% sugar solution) for adults was removed 12 hours before feeding on mouse. Developmental states of follicles were observed under a stereoscope. The stages of the follicles were as described previously.³⁾

The larvae used for experiments were bred with an equally mixed powder of Ebios (brewer's yeast) and mouse pellet, and the emerged adults were kept in a cage (20×20×30 cm) with 2% sugar solution at the insectarium.

Table 1. Outline of strains of *Culex pipiens* complex used in this study.

Strain	Place of collection	Latitude of place of collection	Generation used in this experiment
<i>Culex pipiens pallens</i>			
Sapporo	Sapporo City, Hokkaido	43° N	F 30
Nagasaki	Nagasaki City, Nagasaki	32° N	F 25
Kagoshima	Kagoshima City, Kagoshima	31° N	F 23
<i>Culex pipiens quinquefasciatus</i>			
Amami	Naze City, Amami Island	28° N	F 30

Results

1. *Follicular development and feeding activity of females of 3 strains of Cx. p. pallens reared from the 1st instar larva in the experimental conditions (photoperiods) ranging from 8 to 16 hours and 21°C*

In 3 strains of *Cx. p. pallens*, follicular development and feeding activity were examined with females reared from the 1st instar larva under a photoperiod from 8 to 16 hours at 21°C (Table 2 and Fig. 1).

Table 2 and Fig. 1 show that the gonoactivity, represented by the follicular size and stage and feeding rate, tended to decrease with shortening photoperiod in 3 strains. In the Sapporo strain, the females had well-developed follicles and fed on the mouse actively when the photoperiod was longer than 13 hours, and females had mostly undeveloped follicles, and did not feed on the mice actively, when the photoperiod was shorter than 13 hours. Here we will tentatively call the females that had undeveloped follicles of about 50 μ or less

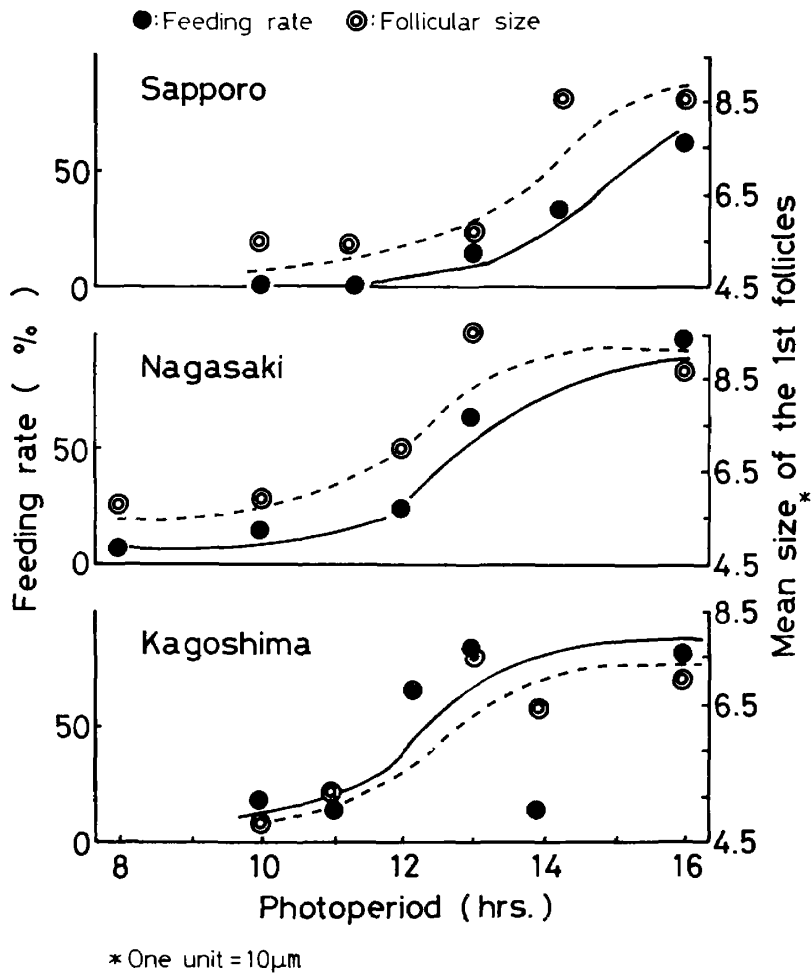


Fig. 1. Follicular size and feeding rate of females of 3 strains of *Culex pipiens pallens* reared from the 1st instar larva and kept in photoperiods from 8 to 16 hours at 21°C.

Table 2. Follicular development and feeding rate of females of 3 strains of *Culex pipiens pallens* which were reared from the 1st instar larva and kept in experimental conditions with various photoperiods at 21°C.

Strain	Potoperiod (hrs: min)	Follicular size*											Total	Follicular stage	Feeding					
		4	5	6	7	8	9	10	10	9	8	7			6	5	4	No. exposed	No. fed	%
Sapporo	10:00			8	2											10	N	100	0	0.0
	11:00	1		9												10	N	100	0	0.0
	13:00			15	3	2										20	N-Ia	99	2	2.0
	14:00				4	5	10	1								30	Ib	97	28	28.3
Nagasaki	16:00					2	5	3								10	Ib	50	27	54.0
	8:00	1	12	7												20	N-Ia	92	4	4.3
	10:00	2	21	17												40	N	122	13	10.7
	12:00	1	5	11	7	4	2									30	N-Ib	73	16	21.7
Kagoshima	13:00					3	6	13	11							33	Ia-IIb	97	61	62.9
	16:00					9	13	2								24	Ib	155	154	99.4
	10:00	5	5													10	N	20	2	10.0
	11:00	3	6	1												10	N	98	6	6.1
Kagoshima	12:00																	58	40	69.0
	13:00					1	10	6	3							20	Ib	98	91	92.9
	14:00				3	8	15	4								30	Ib	99	14	14.1
	16:00				3	6	1									10	Ib	35	30	85.7

* One unit = 10 μm

in and at the N stage, and did not take blood meal, as diapausing females. Therefore, the critical daylength for inducing diapause in the females of the Sapporo strain is about 13 hours. In both strains of Nagasaki and Kagoshima diapausing females appeared at the daylength of 12 and 11 hours, respectively, which can be regarded as the critical daylength for the Nagasaki and Kagoshima strains. Judging from these results, the critical daylength for inducing diapausing females of *Cx. p. pallens* varies with the geographical location of the strain and is longer in northern areas than in southern areas. In these experiments, the incidence of gonotrophic dissociation, a phenomena in which the female takes blood meal but do not develop mature eggs (follicles of the 5th stage), was examined in Sapporo and Kagoshima strains. Gonotrophic dissociation was not seen in blood-fed females in both strains. The Nagasaki strain showed this phenomena in a very low rate in the previous experiments.⁴⁾

2. *Follicular development, feeding activity and gonotrophic dissociation in diapausing females of Cx. p. pallens which were kept in a reactivation condition (16 hours photoperiod and 25°C).*

The females of 3 strains reared from the 1st instar larva to 10 days after emergence experimentally under a short daylength of 10 hours at 21°C, were exposed to a long photoperiod of 16 hours at 25°C, to reactivate them, that is, to recover gonoactivity. Follicular development, feeding activity and incidence of gonotrophic dissociation were examined with the females exposed to reactivation conditions for 7 to 15 days after reactivation. Table 3 and Fig. 2 show the results.

Most of the females of the Sapporo strain had small follicles and a low feeding rate from the 1st to 3rd day after reactivation. Follicles began to develop with the increase of feeding activity on the 5th day, that is, the state of diapause was gradually overcome and the females were reactivated by exposure to a long daylength and high temperature. The rate of gonotrophic dissociation was very high for 3 days after reactivation during which time the feeding rate was very low, and after 5 days, gonotrophic dissociation became low with the increase in feeding activity. In both Nagasaki and Kagoshima strains, reactivation, indicated by development of follicles and increase of feeding rate, began after 3 or 2 days, respectively. Also in these two strains, the gonotrophic dissociation occurred in a low rate in early reactivation time when the feeding activity was low. The speed of reactivation was slower in the northern strain than in the southern strain. In other words, the depth of diapause is deeper in northern strains than in southern strains. These results clearly show that gonotrophic dissociation usually occurs in the females with

a low feeding activity, those which are not completely reactivated and it appears more commonly in a northern strain than in a southern strain under experimental conditions.

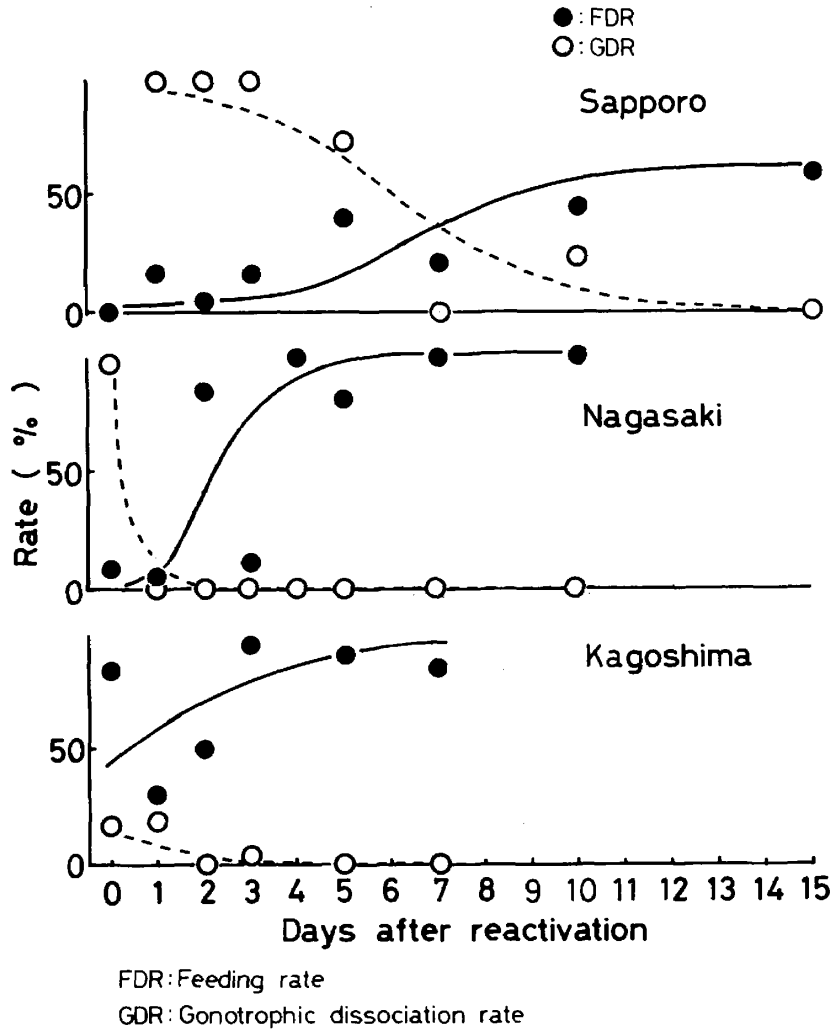


Fig. 2. Feeding rate and gonotrophic dissociation rate in the females of 3 strains of *Culex pipiens pallens* which were reared from the 1st instar larva and then until 7 to 10 days after emergence at under 10 hr photoperiods at 21 °C and kept in reactivation conditions (16 hr photoperiod at 25°C).

Table 3. Follicular development feeding activity and gonotrophic dissociation rate of the females of *Culex pipiens pallens* which were reared from the 1st instar larva until 7 to 10 days after emergence under a 10-hour photoperiod at 21°C and then kept in reactivation conditions (16-hour photoperiod at 25°C).

Strain	Days after reactivation	Follicular size*								Total	Follicular stage			Feeding			GD**	
		4	5	6	7	8	9	10	11		No. exposed	No. fed	%	No. fed	No. GD	%		
Sapporo	0		8	2				10		N	20	0	0.0					
	1										20	3	16.6	3	3	100.0		
	2										20	1	5.0	1	1	100.0		
	3	1	9					10		N	20	3	16.6	3	3	100.0		
	5	1	8	1				10		N	20	8	40.0	8	6	75.0		
	7		5	5				10		N-Ia	20	4	20.0	4	0	0.0		
	10	1	3	3		3		10		N-Ib	20	8	40.0	8	2	25.0		
	15		2	2		4	2	10		N-Ib	20	12	60.0	15	0	0.0		
	0	3	7					10		N	10	1	10.0	1	1	100.0		
	1										20	1	5.0	1	0	0.0		
Nagasaki	2										20	17	85.0	17	0	0.0		
	3	1	3	5		1		10		N-Ia	20	2	10.0	2	0	0.0		
	4										21	21	100.0	21	0	0.0		
	5	1	3	5		1		10		N-Ib	20	16	80.0	15	0	0.0		
	7		1	5		2	2	10		N-Ib	20	20	100.0	19	0	0.0		
	0	3	6	1				10		N	19	16	84.2	16	3	18.8		
	1	1	7	0		1	1	10		N-Ia	20	6	30.0	5	1	20.0		
Kagoshima	2	1	5	3		1		10		N-Ia	20	10	50.0	10	0	0.0		
	3	1	2	3		3	1	10		N-Ib	20	19	95.0	20	1	5.0		
	5		1	2		2	2	11		Ib	20	18	90.0	15	0	0.0		
	7			3		4	3	10		Ib	20	17	85.0	17	0	0.0		

* One unit = 10 μ m

** GD : Gonotrophic dissociation

3. *The developmental state of the 1st follicles in Cx. p. pallens females showing gonotrophic dissociation in the field*

Outdoors in the campus of Nagasaki University School of Medicine, 12 fed females of *Cx. p. pallens* females which were collected by dog bait trap in late October, 1969 and thereafter reared in the laboratory of 20°C for 10 days. Only two of them showed gonotrophic dissociation. Their 1st follicles were well-developed and in the stage of IIa to IIb. This means that females showing this phenomena are rarely found in nature.

4. *Follicular development of Cx. p. pallens females collected in caves*

Follicular development was examined with unfed females collected in caves in late autumn in the cities of Sapporo and Kagoshima. All the females collected in all the caves were nulliparous from counting the number of dilations. The 1st follicles were mostly in stage N and undeveloped with a size of 50 μ or less. Thus, they are considered to have been in the state of diapause (Table 4).

Table 4. Follicular development of *Culex pipiens pallens* females collected in caves in late autumn.

Date of collection	Place of collection	No. females dissected	Size of the 1st follicles*					Follicular stage
			4	5	6	7	8	
Nov. 1969	Sapporo	12	not measured					N
Nov. 1980	Kagoshima	35	2	30	3			N

* One unit = 10 μ m

5. *Gonoactivity of Cx. p. quinquefasciatus females reared from the 1st instar larva in a short daylength of 10 hours at 21°C*

Feeding activity and incidence of gonotrophic dissociation were observed with the females that were bred from 1st instar larva in the condition of a short daylength of 10 hours and 21°C. Ten days after emergence, 100 females were exposed to the mouse. Sixty fed females were reared for 10 days but did not show gonotrophic dissociation. Thus, *Cx. p. quinquefasciatus* females are considered to lack the ability to enter into a state of diapause.

Discussion

The present findings showed that the critical daylength for inducing diapausing females and depth of diapause vary geographically with the strain of *Cx. p. pallens* in Japan, that is, the critical daylength is longer in northern strains than in southern strains, and the depth of diapause is also deeper in northern strain than in southern strain. Even *Cx. p. pallens*, has geographical variation of adult diapause. This is very important for understanding the geographical speciation of this mosquito.

The present findings are in agreement with our previous report⁸⁾ that in the Hamburg strain of *Cx. p. pipiens* gonotrophic dissociation occurs commonly in females with low feeding activity, *i. e.*, those which are not reactivated completely in experimental conditions.

Hosoi¹⁾ found that gonotrophic dissociation occurred commonly in the females of *Cx. p. pallens* emerged in late autumn in Tokyo, and he found a high incidence of this phenomena in the females that were allowed to take human blood meal. From this, he considered that the occurrence of gonotrophic dissociation was due to taking human blood meal. His mosquitoes are assumed to be at about a similar stage of diapause as the Sapporo strain because it occurred commonly in the Sapporo strain in the present experiments, and this may be related to the high rate of gonotrophic dissociation in his experiment.

In our experiments, few females showed gonotrophic dissociation in nature and the follicles of females showing this phenomenon were well-developed and in the stage of IIa-IIb. Those with such large follicles were rarely observed among overwintering females, which had mostly small follicles. Therefore, it is reconfirmed that gonotrophic dissociation does not play an important role in overwintering of *Cx. p. pallens* in Japan, even if it appears in a high rate in experimental conditions.

Finally, we can conclude that the females of *Cx. p. pallens* in Japan usually hibernate in a state of diapause without feeding on blood meal, and the diapause of the females is induced by a short daylength, the critical daylength of which varies geographically with the strain.

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日本産アカイエカ群の成虫休眠に関する研究

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要 旨 21°Cの温度と8~16時間の範囲の日長とを組み合わせた実験条件下で、飼育羽化させた札幌、長崎と鹿児島系のアカイエカの各系統の雌と奄美系のネッタイエカの雌について休眠蚊の出現を誘起させる臨界日長をしらべた。ネッタイエカは短日下でも活発に吸血し成熟卵を形成したので休眠はないと思われる。札幌系のアカイエカでは日長が13時間より短いときには吸血しない小型濾胞の休眠蚊が出現したので、13時間は臨界日長と考えられる。長崎と鹿児島系では休眠蚊は12あるいは11時間日長下で出現したので臨界日長は札幌系より短かいことになる。同じ3系統のアカイエカの休眠蚊を高温長日下に移して覚醒させた。札幌系では移してから5日目頃から休眠状態から覚醒して吸血したが、長崎と鹿児島系ではより早く覚醒した。以上のことから日本産アカイエカでも休眠蚊の出現を誘起させる臨界日長には地理的変異があり、北へいくほど長くなるし、また休眠の深さも地理的に変異し北へいくほど深くなることがわかった。アカイエカ群の系統によって休眠性が違っていることは、この群の地理的分化を考える上に重要なことである。

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