

Follicular development in *Culex tritaeniorhynchus* females showing gonotrophic dissociation

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Abstract: In *Culex tritaeniorhynchus*, examinations were made on follicular development in fed females showing gonotrophic dissociation, which were collected in animal houses in autumn, and fed females with this phenomenon, which were reared as adults in laboratory conditions of short photoperiod. The former females had well-developed follicles of stage I to III, but the latter females had usually small-sized follicles of stage of N and size of 50μ , which were about similar in size and stage to follicles in unfed females with low feeding activity. Such mosquitoes with small-sized follicles were not found in unfed nulliparous females collected in animal houses in autumn. From these facts, it is concluded that females with low feeding activity, which would feed on animals in experimental conditions of short day-length, would not be attracted to animals in the field in autumn.

INTRODUCTION

In *Culex tritaeniorhynchus*, the most important vector of Japanese encephalitis, some females are known to show gonotrophic dissociation, a phenomenon in which females take a blood meal, but their ovaries do not develop to maturity (Harada, et al., 1968, 1971; Kawai, 1969; Oda and Wada, 1973)

Oda and Wada (1973) had examined the incidence rates of gonotrophic dissociation in fed females of this mosquito under various conditions, in order to estimate the possibility for Japanese encephalitis virus to overwinter in the present vector and the significance of this phenomenon for overwintering ecology.

These results showed that the incidence rate of gonotrophic dissociation in fed

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females collected in the field in autumn was lower than in fed females reared under experimental conditions of short photoperiod. As a reason for this, we suggested that females with low feeding activity, which would feed on animals in laboratory conditions of short day-length, would not be attracted to animals in the field.

An attempt was made to confirm this suggestion by examining developmental states of follicles of unfed females, and fed females which showed gonotrophic dissociation, in relation to feeding activity and the incidence of gonotrophic dissociation both in the laboratory and in the field.

MATERIALS AND METHODS

Mosquitoes of *Culex tritaeniorhynchus* (Nagasaki strain) were used in this experiment. Feeding activity and the incidence rate of gonotrophic dissociation were observed. Then follicular development was examined in fed females showing gonotrophic dissociation as well as in unfed females. Females dissected here were reared to adults from the 1st instar larvae under experimental conditions of short photoperiod of 13 hours and low temperature of 21 C. Besides these females, there were examined also females which were bred from the 1st instar larvae until various developmental stages (each larval stage, pupal stage and newly emerged adults) under long photoperiod of 16 hours and high temperature of 27 C, and then under conditions of short photoperiod of 10 hours and low temperature of 21 C.

In addition to these observations, developmental states of follicles were also examined in fed nulliparous females which were collected in pigsties in September and showed gonotrophic dissociation.

Follicular development was observed under binocular stereoscope and microscope. The mosquitoes were allowed to feed on mice. In the present paper, follicular stages are described after Kawai (1969).

RESULTS

1. *Follicular development in fed females showing gonotrophic dissociation and unfed ones which were reared as adults from the 1st instar larvae at short photoperiod (13 hours) and low temperature (21 C).*

A large number of mosquitoes were reared as adults from the 1st instar larvae in the laboratory with short photoperiod (13 hours) and low temperature (21 C), to obtain many females with gonotrophic dissociation. These females engorged at a low rate when they were permitted to feed on a mouse. Fed females were kept for 10 days and thereafter they were dissected.

In this experiment, it was found that most of the fed females demonstrated gono-

Table 1. Developmental states of follicles in unfed females or fed females showing gonotrophic dissociation, which were reared to adults from the 1st instar larvae under experimental conditions with short photoperiod (13 hours) and low temperature (21 C).

Physiological states of females	No. females dissected	Follicular size**				Follicular stage
		4-	5-	6-	7-	
Unfed	11	6	4	1		N-Ib
With GD*	67	35	26	4	2	N-Ib

* GD: Gonotrophic dissociation

** One unit=10 μ

trophic dissociation, and those showing this phenomenon usually had less-developed follicles of 50 μ or less in size and of N to I in stage. Moreover, such follicles were about similar in size and stage to the first follicles in unfed females (Table 1).

2. *Feeding activity, gonotrophic dissociation and follicular development in females transferred in each immature stage or immediately after emerging from long photoperiod (16 hours) and high temperature (27 C) into short photoperiod (10 hours) and low temperature (21 C).*

Females used here were transferred in each immature stage or immediately after emergence from conditions of long photoperiod of 16 hours and high temperature of 27 C into conditions of short photoperiod of 13 hours and low temperature of 21 C. Developmental states of follicles were examined in unfed females or fed females indicating gonotrophic dissociation in parallel with observations on feeding activity and the incidence rate of gonotrophic dissociation (Table 2).

This table shows that follicular size of unfed females and feeding activity decreased gradually, but the gonotrophic dissociation rate increased, when the period of exposure to short photoperiod and low temperature in developmental stages was longer. Also, in the present experiment, it appeared that follicular development of females with gonotrophic dissociation was about similar to that of unfed females.

3. *Follicular development of fed nulliparous females showing gonotrophic dissociation, which were collected at animal houses in late September and then reared at outdoors.*

In order to know developmental states of follicles in fed females with experience of gonotrophic dissociation in nature, fed females which had been caught in animal houses were kept for 10 days, and then their follicles were examined only for nulliparous females showing this phenomenon (Table 2).

In this observation, it was found that the females with gonotrophic dissociation had well-developed follicles of Ib or higher.

Table 2. Gonoactivity in females which were transferred at each immature stage or just after emergence from conditions of long photoperiod (16 hours) and high temperature (27 C) into conditions of short photoperiod (10 hours) and low temperature (21 C).

Developmental stages exposed to specific photoperiod and temperature		No. females dissected	Follicular size*									Follicular stage			Feeding rate			Gonotrophic dissociation rate			
			4-	5-	6-	7-	8-	9-	10-	11-	12-	N	I	II a	No. unfed	No. fed	%	No. fed	No. GD**	%	
16 hours, 27 C	1 2 3 4 P A	15 (1)	12 (1)	3									15 (1)			100	1	1.0	1	1	100.0
	2 3 4 P A	5 (2)	2 (2)	2	1								5 (2)			100	2	2.0	2	2	100.0
1 2	3 4 P A	15 (0)	9	6									15			100	0	0.0	—	—	—
1 2 3	4 P A	15 (2)	15 (1)		(1)								15 (1)	(1)		100	4	4.0	2	2	100.0
1 2 3 4	P A	15 (4)	2 (2)	3 (1)	6 (1)	4							15 (2)	(2)		100	10	10.0	10	6	60.0
1 2 3 4 P	A	14 (8)	3 (1)	2 (4)	3 (1)	3 (1)	2 (1)	1 (1)		1			8	6 (6)	(2)	100	55	55.0	52	10	19.2
1 2 3 4 P A		15 (0)		1	5	6	2	1					15			100	81	81.0	30	0	0.0

Figures without parenthesis show the numbers of unfed females dissected, and figures within parenthesis indicate the numbers of fed females showing gonotrophic dissociation.

* One unit = 10 μ

** GD: Gonotrophic dissociation

Table 3. Developmental states of follicles in fed nulliparous females showing gonotrophic dissociation, which were collected in pigsties in September and then kept at outdoors.

No. females dissected	Follicular stage				
	N	I	I - II	II	III
7	0	2	1	2	2

DISCUSSION

Oda and Wada (1973) reported that in *Culex tritaeniorhynchus*, the incidence rate of gonotrophic dissociation in fed females collected in the field in autumn was lower than in fed ones reared under experimental conditions with short photoperiod. A possible explanation for this was that females with low feeding activity, which would feed on animals under experimental conditions with short day-length, would not be attracted

to animals in the field.

The present experiment clearly shows that females with gonotrophic dissociation, which emerged and took a blood meal in the laboratory conditions of short day-length, usually had small-sized follicles of 50 μ in size and N in stage. On the other hand, unfed nulliparous females attracted to animals in the field in autumn have well-developed follicles of 60 μ and Ib or higher (Kawai, 1969). Accordingly, it is concluded that females with low feeding activity, which would engorge under laboratory conditions of short photoperiod, would hardly come to bite animals in nature. These findings well support our suggestion mentioned above.

Kawai (1969) wrote that fed nulliparous females caught in animal houses in autumn showed gonotrophic dissociation and their current follicles were well-developed, being of stage I to III. Our data on follicular development of such females is also similar to Kawai's results. From the present data, these females are estimated to have been briefly exposed to short day-length in autumn, though the period is not clear.

As for the overwintering pattern of *Culex tritaeniorhynchus*, we stated that gonotrophic dissociation in this mosquito species does not play important role for the overwintering ecology. This mosquito usually overwinters in unfed nulliparous state, and the possibility for Japanese encephalitis virus to overwinter in the mosquito body is also very low, as far as Kyushu is concerned (Oda and Wada, 1973; Oda et al., 1978). Our view given above is strongly supported by the present results.

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コガタアカイエカの栄養生殖分離雌の濾胞の発育状態

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前報で秋の短日条件下で採集されたコガタアカイエカの吸血雌は低率において栄養生殖分離を起こすが, 短日実験条件下で羽化した吸血活性の低い雌が吸血すると, そのほとんどが本現象を

起こすことを報告した。これは両者の吸血活性の程度が異なり、後者のような吸血活性の低いものは秋に動物に吸血に来ないためと想像された。この点を確かめるために、秋に動物舎で採集された吸血雌で栄養生殖分離を起こしたものと短日の実験条件下で羽化して吸血した雌の中で本現象を示した雌について濾胞の発育状態を調査した。前者の濾胞はⅠ期あるいはそれ以上に発育していたが、後者の濾胞は一般には吸血活性の低い未吸血雌のそれとほぼ同じで、 50μ 位の小型で、N期の未成熟であった。このような小型濾胞を持つ未吸血未経産雌は秋に動物に吸血に来る雌の中には含まれていない。したがって、我々が予想したように、実験環境の短日条件下で羽化した吸血活性の低い雌は秋自然界では動物に吸血に来ないことがわかる。