Studies on Gonoactivity and Overwintering of the Mosquito, *Anopheles sinensis* Wiedermann in Nagasaki Area

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Abstract In the Nagasaki area, the females of *Anopheles sinensis* emerging before late September are gonoactive with well-developed follicles while the females emerging later are generally in the diapausing state without feeding on animal blood and with less-developed follicles. Gonoactive females will continue feeding activity and oviposition and die off in winter. In nature, few females show gonotrophic dissociation. Therefore, this phenomena does not seem to play an important role in overwintering ecology of this mosquito. Thus, the overwintering population is mostly composed of diapausing females. In January the diapausing females will begin to reactivate from the state of diapause and feed on animal blood with the increase in size of follicles. The diapausing females are considered to be induced by short daylengths in autumn. The overwintering pattern of this mosquito species is considered to be similar to that in *Culex pipiens pallens* and *Culex tritaeniorhynchus*.

Bull. Sch. Allied Med. Sci., Nagasaki Univ. 2 : 25−35, 1988

Key words : *Anopheles sinensis*, imaginal diapause, overwintering, gonoactivity, follicle

Introduction

In Japan, the mosquitoes of *Culex tritaeniorhynchus* and *Culex pipiens pallens* are commonly found. The former is known as the main vector of Japanese encephalitis, the latter as an important vector of dogfilaria[9)12)], *Dirofilaria immitis*, which infects also humans. The females of those culicine

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mosquitoes usually hibernate in the diapausing state without feeding on animal blood and with undeveloped follicles. Diapausing state is induced by short daylengths[4)5)7)]. This indicates that diapausing females take carbohydrates, such as plant juice and develop fat body before entering hibernation[2)]. Thus, the pathogens of disease can rarely overwinter in the mosquito bady. The mosquito *Anopheles sinesis* is also widely distributed in Japan. This is the important vector of *Setaria digitata* and *S. marshalli* of cattle and was an important vector of malaria in the past in Japan[8)]. This mosquito also overwinters in adult stage. Komoto[3)] suggested that the females of *An. sinesis* hibernate in a special physiological state of the gonotrophic dissociation, a phenomenon that females take on animal blood, but the ovaries do not develop to mature eggs, producing fat body as shown in *An. l. maculipennis* in Europe[10)]. However, Wada *et al.*[12)] implied that females of this species which also overwinter show diapause, but the actual situation and the overwintering pattern are unknown. This subject is very important for a better understanding of the mechanism of the imaginal diapause in the mosquito as well as the possibility of pathogens in this mosquito body. The present study was conducted to make clear the overwintering pattern in *An. sinensis* and to compare the results with those in *Cx. p. pallens* and *Cx. tritaeniorhynchus* by examining gonoactivity, which is represented by follicular development, feeding activity and gonotrophic dissociation rates in the females of *An. sinensis.*

Materials and Methods

The females of *Anopheles sinensis* which had been bred from the 1st instar larva of the laboratory colony of the 15th generation of Nagasaki strain were used for follicular development, feeding activity and gonotrophic dissociation rate. The larvae were reared with mixed powder of Ebios (brewer's yeast) and mouse pellets in equal amounts, and the followed adults were kept in a cage (20×20×30cm) with 2% sugar solution at an outdoor insectarium and at indoor insectaria with constant temperatures and alternate light and darkness. The developmental state of follicles was examined by using a binocular stereoscope and microscope. The stages of the follicles were as described previously in *Cx. p. pallens.* The females were allowed to feed on mouse for a night to observe feeding rate. Parous rates were examined with trachioles in an overy and follicular development and number of dilatations in another ovary of unfed females collected in two cowsheds, at Kaizucho, Isahaya City, from September, 1974 to April, 1975.

Moreover, the fed females collected in same cowsheds from February to October, 1974 were individually kept in a small glass tube (1 cm × 5 cm) for 7 to 10 days in outdoor conditions in the campus of Nagasaki University, School of Medicine, to determine incidence of gonotrophic dissociation in the field.

Results

1. *Gonoactivity of the females reared under outdoor conditions*

Follicular development, feeding activity and gonotrophic dissociation rate were examined with the females which had been reared from the 1st instar larva in outdoor conditions from September to October, 1974 (Table 1).

Table 1. Follicular development, feeding activity and gonotrophic dissociation in the females of *Anopheles sinensis* reared from the 1st instar larvae outdoors, 1974.

Time of emergence*	No. with the 1st follicles of the indicated size**							Follicular stage	Feeding			Gonotrophic dissociation		
	6-	7-	8-	9-	10-	11-	Total		No. exposed	No. fed	%	No. fed***	No. showing	%
Sep. E	1	1	3	0	1		6	Ib	11	4	37.0	3	0	0.0
Sep. M			1	1	1	7	10	Ib-IIa	16	6	37.5	6	0	0.0
Sep. L	6	8					14	N-Ia	20	0	0.0			
Oct. E	9	1					10	N-Ib	20	0	0.0			
Oct. M		1					1	Ib	100	0	0.0			

* E, M and L show the early, middle and late part of the month respectively.

** One unit = 10 μm

*** The fed females were dissected 7 to 10 days after feeding

Table 1 shows that the females which emerged in early and mid-September had generally well-developed follicles of about 80 μm and were in the stage of Ib to IIa, but their follicles were less-developed, about 70 μm or less, and were in the stage of N to Ia in most of the females that emerged in late September. The feeding rates also dropped with emergence time in parallel with the trend of the change in follicular development ; that is, feeding activity decreased as follicular size decreased. Gonotrophic dissociation did not occur in these females, because most of the females that emerged in late September did not feed on the mouse.

2. *Gonoactivity of the females reared in experimental conditions with different photoperiods and the temperature of 21℃*

As described above, the females that emerged after late September did not feed on animales and had small follicles (Table 1). The changes in follicular size and feeding activity is assumed to have been controlled by short photoperiods in autumn, on the basis of the results of *Cx. p. pallens* and *Cx. tritaeniorhynchus*. To confirm this assumption and the presence of gonotrophic dissociation in experimental conditions, follicular development, feeding activity and gonotrophic dissociation were examined with females reared from the 1st instar larva in the laboratory with photoperiods ranging from 8 to 16 hours and constant temperature of 21℃ (Table 2).

Table 2. Follicular development, feeding activity and gonotrophic dissociation in the females of *Anopheles sinensis* reared from the 1st instar larvae under various photoperiods and temperature of 21℃.

Photo-period (hr: min)	Days after emergence to dissection or feeding	No. with the 1st follicles of the indicated size*											Follicular stage	Feeding			Gonotrophic dissociation		
		5-	6-	7-	8-	9-	10-	11-	12-	13-	14-	Total		No. exposed	No. fed	%	No. fed**	No. showing	%
16 : 00	7			1	0	7	2					10	Ia, Ib				26	2	7.7
15 : 00	5-18				1	1	5	2	1			10	Ia-I-II	3	1	33.3			
14 : 00	10						4	1				5	Ib-IIa	40	30	75.0	19	0	0.0
13 : 00	25				1	5	4					10	Ib-IIa	250	82	32.8	30	1	3.3
12 : 00	10		7	10	0	0	1	2				20	N-IIa	63	15	23.8	13	11	84.6
11 : 00	10	1	5		4							10	N	50	4	8.0	3	3	100.0
9 : 00	10-20		6	3	1							10	N	25	3	12.0			
8 : 00	9	2	11	3								16	N-Ib						

* One unit=10 μm

** The fed females were dissected 7 to 10 days after feeding.

Table 2 shows that females had well-developed follicles and that feeding rates were high in about 30% when the photoperiod was longer than 13 hours, but females had small follicles of about 70 μm and feeding activity became lower when the photoperiod was shorter than 12 hours. On the other hand gonotrophic dissociation occurred commonly in the females with low feeding activity and small follicles which emerged in photoperiods shorter than 12 hours. Five females showing gonotrophic dissociation had less-developed follicles, 60-110 μm in long and in the stage of N-I. These findings indicate that this phenomenon also occurs in the females with low feeding activity and less-developed follicles, and that the gonoactivity is controlled by photoperiod. Taketomi[11] wrote that unfed nulliparous females in cowsheds generally had the first follicles of 80 μm or more and in stage I to II. Judging from Taketomi's data and ours in Table 2, tentatively we defined the unfed nulliparous females with well-developed follicles of 80 μm or more and in stage I as gonoactive females and those with follicles of about 70 μm and in N to I stage as diapausing females. The diapausing females contained a large fat body, and also those showing gonotrophic dissociation appeared to have some fat body in experiments as indicated in Table 2.

3. *Follicular development of the unfed females collected in cowsheds*

Table 3 shows the results on follicular development in unfed nulliparous or parous females collected in cowsheds which were a part of the materials shown in Table 5. The nulliparous females had generally well-developed follicles in the size of 80-180 μm and in stage Ib to IIb.

Table 3. Follicular development of unfed females of *Anopheles sinensis* with were collected in cowsheds in autumn, to spring, 1974 to 1975.

Month of collection	No. nulliparous females with the 1st follicles of the indicated size*										Total	Follicular stage	No. parous females** with the 1st follicles of the indicated size*									Total	Follicular stage
	6-	7-	8-	9-	10-	11-	12-	13-	14-	15-18			7-	8-	9-	10-	11-	12-	13-	14-	15-18		
Sep.	1	2	11	4	1		1				20	Ib-IIa			1	4	8	6	8		1	28	IIa-IIb
Oct.	2	5	21	4	3		2				37	Ib-IIa		2	1	1	5	16	3	1	2	31	Ib-IIa
Nov.																							
Dec.	0	0	0	0	0	0	0	0	0	0				0	0	0	0	0	0	0	0		
Jan.	0	0	0	0	0	0	0	0	0	0				0	0	0	0	0	0	0	0		
Feb.		2	6	15	23	23	15	12	17	4	82	Ib-IIb		0	0	0	0	0	0	0	0	0	Ib-IIb
Mar.			1	1	1	2	3	1	5	1	15	Ib-IIa		1		2	2	1	2		8	16	Ib-IIb
Apr.					1	1	2	4			8	IIa-IIb				1	1	1	1			4	Ib-IIb

* One unit=10 μm

** All females had only dilatations

Table 4. *Seasonal changes in the rate of gonotrophic dissociation in the fed females of Anopheles sinensis* which were caught at cowsheds and pigsties and thereafter kept outdoors.

Date of collection	Days from collection to dissection	No. dissected	No. with mature eggs	No. showing gonotrophic dissociation	% of gonotrophic dissociation
Feb. 14	10	11	11	0	0.0
Mar. 14-23	10	41	41	0	0.0
Apr. 17	7	8	8	0	0.0
May. 2, 15	7	52	52	0	0.0
Jun. 4	7	25	25	0	0.0
Jul. 14	7	65	60	5	7.7
Aug. 22, 18	7	44	44	0	0.0
Sep. 6-26	10	268	265	3	1.1
Oct. 1-22	10	115	93	22	19.1

The follicles were more advanced in nulliparous females collected in spring than in those collected in autumn. In autumn and in early spring, such a difference in follicules size was not found in parous females. This result conforms to Taketomi's finding[11]. In autumn these females with well-developed follicles are considered to be gonoactive and to have emerged before middle September from the results shown in Table 1.

Table 5. Age-distribution of unfed females of *Anopheles sinensis* which were collected in cowsheds in autumn to spring, 1974-1975.

Month of collection	No. females by age 0-P	1-P	2-P	Total No.	Parous rate (%)
Sep.	41	34	0	75	45.3
Oct.	49	31	0	80	38.8
Nov.					
Dec.	0	0	0	0	
Jan.	0	0	0	0	
Feb.	105	0	0	105	0.0
Mar.	21	16	2	39	46.2
Apr.	8	3	0	11	27.2
Total	224	84	2	310	

p : parity

4. *Seasonal changes in gonotrophic dissociation rates in the females collected in cowsheds*

To determine the incidence of gonotrophic dissociation in the field, the gonotrophic dissociation rate was examined in the fed females that had been caught in cowsheds and thereafter reared outdoors for 7 to 10 days. Here, on the term of gonotrophic dissociation, we mean the phenomenon that fed females do not produce mature eggs. Table 4 suggested that most of the fed females produced mature eggs throughout the season, though a small portion of the fed females about 20% showed gonotrophic dissociation in October. Seven females showing gonotrophic dissociation were dissected. All of them were nulliparous and had well-developed follicles of 180 to 280 μm and that were in the stage of IIa to IIIb. Such females had only a few fat bodies. Tables 2 and 4 show the follicular size and stage of females showing gonotrophic dissociation in experimental conditions and of those with gonotrophic dissociation which were collected in cowsheds.

5. *Changes in age distribution in the unfed females collected in cowsheds from September to April.*

To clarify the changes in age-distribution of this species from autumn to spring, 310 unfed females collected in cowsheds were dissected as indicated in Table 5. This table shows that in autumn of September and October the uniparous females were collected at a high rate of about 40%, but in the early spring of February the females were all nulliparous. In March and April the number of parous females increased again. Most of them were 1-parous, a few females 2-parous. Of the 310 mosquitoes dissected only 2 females had the follicular tube of the sac.

6. *Changes in follicular development in diapausing females emerging in late September and which were kept till April outdoors.*

The follicular development was examined with diapausing females emerging in late September and kept outdoors untill April. As shown in Table 6, the follicle began to develop in size and stage in January when the females begin to awake in the diapausing state.

Table 6. Changes in the follicular development in *Anopheles sinensis* females which had been reared as adults in late September and thereafter were kept until early March outdoors.

Date of dis-section	No. with the 1st follicles of the indicated size*							Follicular stage
	5-	6-	7-	8-	9-	10-	Total	
Oct. 5		6	8				14	N
Dec. 29	1	1	2				4	N
Jan. 9, 26			1	2			3	Ia, Ib
Feb. 4, 27		1	1	1			3	Ia, Ib
Mar. 2			1	0	0	1	2	Ib

* One unit=10 μm

Discussion

Komoto[3] reported that the fed females of *An. sinenses* collected in the animal house showed a high gonotrophic dissociation rate in Shimonoseki in October, but that in fact females were rarely collected in animal house. In the present experiment the fed females collected in cowsheds showed a high gonotrophic dissociation rate of about 20% in Octorber, but few such females could be collected. These facts indicate that this species undergoes gonotrophic dissociation in autumn but in nature such females are very few. Thus, this phenomenon does not seem to play an important role in the overwintering ecology in this species.

The present study shows that the fed females collected in cowsheds and that showed the gonotrophic dissociation had generally large follicles in the size range of 140 to 280 μm and in the stage of IIb to IIIb. As females with such large follicles were not found among the unfed females collected in February, those with gonotrophic dissociation probably died in winter. On the other hand, the females showing the phenomenon in experimental short photoperiod condition had follicles 70 to 110 μm long and those were in the stage of N to Ib ; there being a clear difference in follicular development between females with gonotrophic dissociation in experiments and those collected in cowsheds. This may imply that the females with gonotrophic dissociation in the experiment condition would not be attracted to animals in nature. Such has been reported to be true in *Culex tritaeniorhynchus* by Oda *et al.*[6]

Thus, generally, the females with less-developed follicles emerging after late September will hibernate in the diapausing state without taking a blood meal. In January they will reactivate from the state of diapause with advance of follicles and begin to feed on animals. The appearance of diapausing females is cosidered to be induced by the short daylength in autumn.

Taketomi[11] found 3 to 7 parous females contained among unfed females collected in cowsheds in autumn. On the other hand, in our experiments only 2-parous females were collected, and they were few. There were no females of 3 or more in parity. It is not certain why our results on the parity differ from those of Taketomi. One explanation is as follows. The females form many ovarioles with the same number of dilatations in an ovary when they lay normally many eggs, but a small number of follicles will degenerate without showing normal oogenesis. Sometimes such ovarioles will produce more degenerate follicles looking like dilatations, than the normal dilatation in the ovarioles which produced normal oviposition.[1] Taketomi[11] might have chosen the ovarioles with many false dilatations as criteria to determine the physiological age.

The population of mosquitoes in 1967, when Taketomi[11] collected the mosquitoes seems to have been larger than that in 1974, when we did so (Oda, unpublished). Thus, such multiparous females might not have been found among those collected in cowsheds, because the population was so small in our experiment. The change in environmental conditions in the present experiments might be another explanation. For example, the distance between oviposition site and resting place in cowsheds is supposed to have become longer. This may have made it necessary for the female to take a long time for one gonotrophic cycle.

In Taketomi's results, it is clear that many unfed parous females collected in cowsheds had ovariales with the sac like follicular tubes, but our data showed that most females had ovarioles with only dilatations which shrunk from sac conditions, i. e., very few had a sac. Also this contradiction may be related to the changes in environmental conditions indicated above, although the physiological state in 1963 may have been changed by strain by 1972.

We found experimentally that the diapausing females awoke from the diapausing state in January. This well agrees with the results of Wada *et al.*[12] that in January the females were collected in cowsheds. The hibernationg females of *Cx. p. pallens* and *Cx. tritaeniorhynchus* begin to feed on animals in the field in February or in March respectively[4][12]. Therefore, the diapause of *An. sinesis* is considered to be lighter in depth than that of *Cx. p.*

pallens and *Cx. tritaeniorhynchus*, as pointed by Wada *et al.*[12]

As mentioned above, the amount of fat body appears to be slightly different between the females with gonotrophic dissociation in experimental conditions and those collected in the cowsheds, but the volume of fat body was not measured exactly with the two kinds of materials. Whether the volume of fat body varies with the females in relation to gonotrophic dissociation and whether or not a large amount of fat body is actually formed in the females with gonotrophic dissociation in nature, remains to be confirmed.

As mentioned above, the females are thought to overwinter in unfed nulliparous females in a diapausing state. Therefore, there is very little possibility that pathogens such as *Setaria digitata* and *S. marshalli* overwinter in the body of *An. sinensis* at present, or that in the past, malaria hibernated in the body.

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(Received December 23, 1988)

シナハマダラカの生殖活性と越冬に関する研究

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要　旨　種々の条件下でシナハマダラカの濾胞の発育状態と生殖活性の変化を検討して本種の越冬様式を明らかにした。長崎地方では大型濾胞の吸血活動雌が9月中旬頃まで羽化するが，9月下旬以降には小型濾胞の吸血しない休眠雌が羽化する。吸血活性雌は吸血と産卵を繰り返し冬期までに死亡するであろう。栄養生殖分離は吸血にくる雌の個体数が極めて少ない秋に低率におこる。したがってその絶対数は極めて少なく，本現象はこの蚊の越冬生態にとって重要ではない。それ故，9月下旬以降に羽化する休眠雌が一般に越冬し，1月に濾胞が発育し休眠状態から覚醒し吸血活動を開始する。休眠雌の出現は秋の短日により誘起される。本種の越冬様式はアカイエカやコガタアカイエカのそれと基本的には同じであるといえる。

長大医短紀要 2：25－35，1988