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Studies on the Follicular Development and Feeding Activity of the Females of *Culex tritaeniorhynchus* with Special Reference to Those in Autumn* **

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Abstract

Day length is a principal factor controlling the feeding activity and the follicular development of the females of *Culex tritaeniorhynchus*. Under a longer photoperiod than about 13 : 30 hours the females hold gonoactivity, while under a shorter one there were found various effects on their physiological activity. These are the decrease in the feeding rate ; the retardation in the follicular development ; the occurrence of gonotrophic dissociation ; and the failure in maturing follicles. A great number of unfed nulliparous females which emerged as adults in and after September, and some of nulliparous or parous females which underwent gonotrophic dissociation after feeding in those days were confirmed to enter hibernation.

Introduction

Ecological studies of *Culex tritaeniorhynchus*, a most important vector of Japanese encephalitis, were started in 1965 in the Nagasaki area, in relation to the epidemiology of the disease in the Department of Medical Zoology, Nagasaki University School of Medicine (Omori et al. , 1965 ; Wada et al. , 1967). The results of preliminary experiments showed that in spring there appeared a great number of over-wintered females being attracted to dry ice, although the temperature is very low, and that the number of females decreased very suddenly in September when the temperature was still high enough for the mosquito to breed. These facts suggested that the day length must be the principal factor to control the gonoactivity of the females of this mosquito.

Oda (1968) showed that the gonoactivity of *C. pipiens pallens* females is well reflected

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by the state of follicular development. The same was also roughly demonstrated with *C. tritaeniorhynchus* by preliminary experiments. On the other hand , the induction of diapause in many insects including mosquitoes is closely related to the photoperiod and temperature (Beck, 1968 ; Danilevskii, 1961). Studies on the follicular development and feeding activity of *C. tritaeniorhynchus* were therefore carried out under various artificial and natural conditions of photoperiods and temperatures from 1965 to 1968. Based on the results obtained, the mode of entering hibernation in the Nagasaki area was considered.

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Experimental place, material and method

Place: Experiments were carried out at Nagasaki during from 1965 to 1968.

Material: The material used in the experiments was *Culex tritaeniorhynchus* Giles, 1901, the most important vector mosquito of Japanese encephalitis virus in Japan.

Biological day length: The biological day length (Astronomical day length in hours plus morning and evening twilight hours) is considered effective for the biological activities of the females of *Culex tritaeniorhynchus*. The morning or evening twilight is defined as the time required for the upper limb of the sun to traverse an arc between the horizon and a point lying 7° 21′ 40′′ below the horizon. Each twilight period in Tokyo varies with the season from 32′ in late September to 38.5′ in late June (Rika-nenpyo, 1968 issued from the Tokyo Astronomical Observatory). The biological day length in Nagasaki is obtained by adding twilight hours presented by T.A.O. in Tokyo to the astronomical day length in Nagasaki as shown in Table 12.

Maintenance of laboratory colony of Culex tritaeniorhynchus: The laboratory colony was originated from a group of fed females collected at animal sheds in the Nagasaki area in April, 1965. It has been successfully maintained under the rearing conditions of a daily photoperiod (biological day length) of 14 to 15.5 hours and a constant temperature at 27°C and relative humidity of about 60 to 85%. In the rearing room in which the colony was maintained, the natural light coming through the frosted glass windows was utilized, while, when the biological day length became shorter than 14 hours in September through early April, electric lamps were lighted to make up the shortage far before dawn by raising the illumination from darkness to about 600 Lux.

Copulation took place rather easily mainly in the evening twilight hours when several hundreds to thousands of females and males cohabited from just after emergence in a large cage of 80 cm in diameter and 170 cm in length. The fertilization rate reached about 60% in three days and about 90 % in seven days. The adults were reared with 1 % sugar solution. The feeding of females was allowed mostly on a chicken, rarely on a mouse or a man in a cage of 30×30×30 cm. The larvae were reared with the equally mixed powder of Ebios (brewer's yeast) and mouse pellets.

Rearing conditions and equipments: Indoor insectary, biotron, and outdoor insectary under various rearing conditions were used for obtaining materials to examine the follicular development and feeding activity of the females.

(1) Rearing conditions in the indoor insectaries were as presented in Table 7. The mosquitoes reared were from the laboratory colony. The daily photoperiod (biological day length) was repeated with a constant length of light and dark periods. Relative humidity varied from 60 to 80 %.

(2) Rearing conditions in the biotron were as follows. The illumination, temperature and humidity were automatically regulated to change with each daily cycle and change gradually by 5 days following the changes in the natural astronomical conditions encountered in early August through the end of September as given in Table 12 and partly in Table 10.

Practically, because of the difficulty in changing the conditions every hour and every day, they were made to change in the following way. A temperature cam was formed by connecting eight points for the mean temperatures at eight time-points of a day for a successive-5-days of a certain month of the five years from 1963 to 1967.

The cam was worked for five days and then substituted by the next one which was made in the same way for the next successive-5-days.

The relative humidity was also controlled so as to repeat the natural daily cycle for a successive-5-days, by the same method as in the case of controlling the temperature. The relative humidity varied with the daily cycle nearly conversely with the temperature according to the time-point of a day, ranging from 62 to 90%, usually from 65 to 85%, during August to early September, but it became a little lower thereafter towards the end of September.

The illumination program was made to change with the daily cycle and to change gradually each 5 days in the same way as in the case of the change in temperature. The daily cycle was made as follows :

(i) About thirty minutes during which illumination was raised gradually from darkness to a plateau or a maximum illumination of 2000 Lux (equivalent to a morning twilight length) ; (ii) a period during which a plateau illumination of 2000 Lux was kept continuous (equivalent to the astronomical day length in a successive-5-days) ; (iii) about thirty minutes during which illumination was dropped gradually from the plateau to darkness(equivalent to evening twilight hours).

(3) The rearing conditions in the outdoor insectary were all subjected to the natural astronomical conditions which were given in Table 12.

Length of follicles : The follicles were measured on a micrometer scale, a unit of which was 10.5μ.

Results of experiments

I. Development of follicles before and after feeding and ovariole changes following oviposition in the female mosquito under the conditions of 14 hours photoperiod and 27°C

The females were reared as adults from the first instar larvae and continuously thereafter under the conditions of daily rhythms of 14 hours of light with about 800 Lux and 10 hours of darkness, and a constant temperature at 27°C. The rearing conditions were near to those in late August in Nagasaki, because the biological day length on those

Table 1. Description of developmental stages of follicles in gonoactive females of *Culex triaeniorhynchus*. (The changes in length and stage of follicles with the lapse of time are shown in Table 2.)

Stage	Before Taking Blood
No1	The follicle of just emerged females is included within the germarium (it is called as the first follicle in this paper) and is nearly of the same length as the second follicle when the first one just reaches N stage.
No2	The first follicle is recognizable from the germarium (or the second follicle in this paper) by the constriction.
N	The first follicle has eight undifferentiated cells and completed an epithelium.
Ia	One oocyte and seven nurse cells are differentiated, but the nucleus of the oocyte is not apparently seen.
Ib	The nucleus of the oocyte is clearly visible.
I-II	A few yolk granules appear around the nucleus of the oocyte.
IIa	Yolk granules are deposited sparsely around the nucleus of the oocyte.
	About 3 days after emergence many females preferably take a blood meal when the first follicles are N to Ia in stage. The follicles range from N to Ib or rarely to I-II stages on the following day and more rarely to IIa with the lapse of time, remaining, however, in these conditions unless the females take a blood meal. Consequently, these stages, usually, however, N to Ib ones can be said to be the first quiescent phase of this mosquito.
	The third follicles i. e., the constrictions on the second follicles appear very rarely, slightly, and usually during the first follicles being respectively in N, Ia to Ib, and I-II to IIa stages.
	After Taking Blood
I-II	The same as above.
IIa	The same as above.
IIb	The oocyte occupies 1/4 to 1/2 of the first follicle. Yolk granules are deposited fairly densely all over the oocyte and the nucleus becomes hardly visible.
IIIa	The oocyte occupies 1/2 to 3/4 of the frist follicle.
IIIb	The oocyte occupies 3/4 to 4/5 of the first follicle.
IV	The oocyte occupies nearly all parts of the first follicle, pushing up the nurse cells upwards. The follicle takes a long and oval shape.
Va	The first follicle gains its maximum size and begins to produce a micropilar apparatus and a chorion.
Vb	The first follicles now are full grown eggs with a completed micropilar apparatus and a chorion.
	Oviposition seems to occur immediately after ovulation. At the time of oviposition, the second follicles are in No2 to Ib but mostly in N to Ib stages and therefore the second quiescent phase can be said also to be in N to Ib stages.

days was 14 : 12 hours and the mean temperature of late August for the past seven years from 1962 to 1968 was 27.3°C.

1. *Development of follicles before and after feeding and after oviposition.*

On the basis of the result of observations on the follicular development of the female mosquito reared under the above mentioned conditions, developmental stages of follicles are divided and described as shown in Table 1.

Details of the results of examinations for the increase in length and advance in stage of the follicles before and after feeding on mice and after oviposition carried out with the females kept under a 14-hour photoperiod and 27°C are tabulated in Table 2.

The first follicles of females just after emergence as adults appear to be yet germaria themselves but in this paper they are referred to as the first follicles which are about 4.0 in the mean length ranging from 2.6 to 5.4 on the micrometer scale (one unit is 10.5μ).

The variation in length and stage of follicles becomes larger with the lapse of time after emergence not only within the follicles of a female but also within individual females, and necessarily the whole follicles of 4 to 15 females examined at each time after emergence were classified according to their stages, and the percentage distribution of follicles falling in each stage was obtained, while, the mean length of follicles thus classified was obtained with only a limited number of them falling in each stage.

Table 2 shows that the first follicles reach N to Ia stages by about 72 hours and N to Ib stages by about 96 hours after emergence, although a few of them advance to I-II or even to IIa stage in a longer time. The stages N to Ib are considered the first quiescent phase of follicles in this female mosquito. This seems to have an intimate relation to the fact that many females preferably take the blood meal 3 to 4 days after emergence, as showh in Fig. 1. About the time when the first follicles are in the first quiescent phase, there appear the third follicles or the constrictions on the second ones in some ovarioles.

After feeding, the first follicles rapidly gain in length and advance in stage. In the course of the development they are subjected to a great variation in length and stage but by the end of three days after feeding, about 40% of them reach Vb stage and on the next day they all become mature eggs and seem to be deposited socn. After feeding, the second follicles reach No2 to Ib or mostly N to Ib and the third ones appear in all ovarioles.

The states of follicular development just prior to oviposition seem similar to those at the time just after oviposition and most of the second follicles remain in stages N to Ib or in the second quiescent phase unless the females take the blood meal the second time. The third follicles, though in a small number, advance 24 hours after oviposition to No2 producing the fourth follicles. Many females preferably take the blood meal about 24 hours after oviposition as shown in Table 4.

The fact that the first quiescent phase is nearly the same as the second one in the states of follicular development at the time of taking blood by many females seems to suggest that the period required from feeding to maturation of eggs is nearly the same in each gonotrophic cycle.

2. *Comparison of feeding and oviposition habits between fertilized and unfertilized females.*

A feeding experiment was carried out with the females which had been kept with nearly the same number of males for seven days after emergence. On that day females were allowed to feed on a man. Forty-three females, 38 engorged and 5 unfed, were examined for sperms in the spermathecae with the result shown in Table 3 which shows that unfertilized females take the blood meal as readily as fertilized ones do. The prefeeding period, i. e., the period from emergence to feeding was roughly observed with some of the fertilized and unfertilized females in the course of the successive rearing of the laboratory colony of this mosquito. As a result, no definite difference in the period was observed between them. In the periods from feeding to oviposition and from oviposition to feedig, there seems to exist no difference between them as assumed from Table 4. As above, there seems to exist no definite difference in feeding and oviposition habits between the fertilized and unfertilized females of this mosquito. The unfertilized females are nearly the same as the fertilized ones in the number of eggs and the oviposition rate of fed females.

3. *Prefeeding period.*

A group of females reared as adults at a 14-hour photoperiod and 27°C were exposed to mice at intervals of 12 hours from just after emergence. Sixty-four or 87.7% out of

Table 2. Follicular development of the females before and after feeding and after oviposition, showing the mean length (and percentage) of follicles falling in each developmental stage in each group of females with the lapse of time.

Hours	Stage / No. ♀♀	No1	No2	N	Ia	Ib	1-II	IIa	IIb	IIIa
		1st follicles								
Hrs after emergence										
0	10	4.0(100.0)								
6	10	4.4(83.5)	3.0(16.5)							
12	10	4.2(65.0)	2.9(35.0)							
18	10	4.1(18.8)	2.9(81.2)							
24	10	5.0(10.5)	4.2(84.0)	4.5(5.5)						
36	15		4.6(51.8)	4.6(48.2)						
48	15		6.1(37.6)	6.4(62.4)						
72	15			8.5(80.0)	7.5(20.0)					
96	15			9.2(66.7)	8.6(24.6)	7.6(8.1)	7.7(0.6)			
108	15			8.4(41.9)	9.1(29.5)	8.2(28.6)				
120	15			7.8(10.7)	9.0(31.7)	8.9(54.0)	9.2(3.7)			
144	10			7.8(6.5)	6.4(49.8)	7.9(38.5)	8.0(5.2)			
168	10			7.4(0.7)	7.7(20.8)	7.8(70.4)	7.3(8.0)	9.6(0.1)		
Hrs after feeding										
6	6			6.7(5.0)	8.1(6.3)	8.4(48.3)	8.5(20.8)	9.7(19.5)		
12	7			7.3(4.2)	8.1(7.0)	9.5(21.0)	9.7(4.4)	10.6(49.0)	14.0(14.3)	
24	7						8.2(14.6)	10.5(4.9)	12.0(40.1)	15.4(26.0)
36	5									11.8(1.2)
48	5									
72	5									
96	5									
		2nd follicles								
		No1	No2	N	Ia	Ib	I-II	IIa		
Hrs after ovip.										
24	5		4.8(17.0)	6.4(79.0)	6.3(4.0)					
48	5		6.2(5.0)	7.0(43.0)	6.0(40.0)	8.8(12.0)				
72	6			5.9(24.2)	6.5(35.8)	6.8(35.8)	7.5(4.2)			
96	5		5.5(4.5)		6.4(42.5)	6.1(26.8)	8.8(2.5)	9.2(23.7)		

Remarks : (1) The females were reared as adults from the first instar larvae and continuously thereafter under the conditions of a 14-hour photoperiod and a constant temperature at 27°C.
(2) Many females preferably took the blood meal on mice from 72 to 96 hours after emergence, and about 24 hours after oviposition. (cf. Fig. 1 and Table 4)

73 females became engorged with blood. The time distribution of feeding rates and cumulative feeding rates of the fed 64 females are illustrated in Fig. 1 which shows that some females began to feed 54 hours after emergence but many females, about 73 % out of the 64 took the blood meal in 3 days after emergence and in 4 days i. e. from 54 to 96 hours after emergence about 95 % of the females took the meal. Thus, the prefeeding period in this mosquito is about 3 days after emergence. The period is longer by about one day than those in *Anopheles sinensis* (Taketomi, 1967) and *Culex pipiens pallens* (Oda, 1968). The reason may be mainly due to the longer period being required for the first follicles to reach the

Table 2. (continued)

				2nd follicles					3rd f.
IIIb	IV	Va	Vb	No1	No2	N	Ia	Ib	No1
				1.6(16.5)					
				1.8(35.0)					
				1.8(81.2)					
				2.6(89.5)					
				2.7(100.0)					
				3.0(100.0)					
				4.1(100.0)					
				4.5(100.0)					
				4.2(96.7)	2.4(3.3)				1.7(3.3)
				4.4(100.0)					
				4.5(81.0)	3.5(19.0)				1.8(19.0)
				4.7(67.5)	3.3(32.5)				1.6(32.5)
				4.7(30.0)	3.4(70.0)				2.2(70.0)
				4.7(12.1)	3.8(87.9)				1.9(87.9)
17.1(14.4)					3.5(92.9)	3.8(7.1)			2.0(100.0)
25.7(98.8)					4.1(100.0)				2.3(100.0)
31.5(10.0)	37.8(10.0)	53.2(80.0)			4.5(30.0)	4.9(70.0)			2.9(100.0)
		52.1(60.0)	48.9(40.0)		3.8(10.0)	4.3(46.0)	6.0(24.0)	5.6(20.0)	3.7(100.0)
			50.5(100.0)		4.9(4.0)	5.1(16.0)	5.0(40.0)	5.4(40.0)	3.5(100.0)
				3rd follicles					4th f.
				No1	No2				No1
				4.1(96.8)	2.7(3.2)				1.9(3.2)
				4.2(78.0)	2.4(22.0)				2.0(22.0)
				4.1(67.5)	2.5(32.5)				1.9(32.5)
				4.1(57.7)	2.6(42.3)				2.1(42.3)

(3) Examination for stages was made with all follicles of an ovary of each female, while measurement of length was made with a limited number of follicles fallimg in each stage.
(4) No1 stage follicle refers to the germarium in this paper (for details see Table 1).
(5) The length of follicles is shown on micrometer scale, one unit of which is 10.5μ.

first quiescent phase in this mosquito.

4. ***Periods required for gonotrophic cycles.***

Females reared as adults from the first instar larvae and continuously thereafter at a 14-hour photoperiod and 27°C were allowed to feed on a man seven days after emergence. The females which took the first meal were kept individually and observed for oviposition at regular intervals. The female which laid eggs was given the chance of feeding from the nearest observation time till the time when she took the second meal.

The mean periods from feeding to oviposition and from oviposition to the next feeding could be observed with seven 3-parous females and ten 4-parous ones with the results as

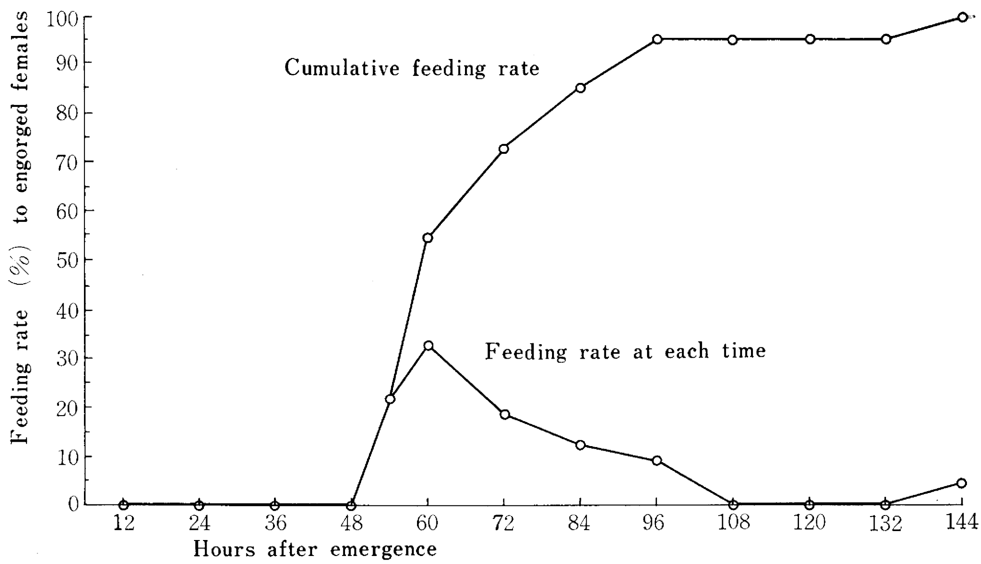

Fig. 1. Feeding pattern of the females with the lapse of time after emergence at a 14-hour photoperiod and 27°C. The total number of engorged females was 64 out of 73 ones exposed to mice at intervals of 12 hours.

Table 3. Feeding activity of fertilized and unfertilized females allowed concurrently to feed on a man seven days after emergence at a 14-hour photoperiod and 27°C.

Fertilized or not	No. (and %) of females		Total
	engorged	unfed	
Fertilized	18(47.4%)	2(40.0%)	20
Unfertilized	20(52.6%)	3(60.0%)	23
Total	38(100.0%)	5(100.0%)	43

shown in Table 4 (A). The fact that the females given in the table were fertilized was proved by the hatching of eggs.

Table 4 (A) shows that the shortest periods from feeding to oviposition are always just or a little over 3 days. The fact shows that the period required for development of follicles from the quiescent phase to maturation of eggs in the ovary of the female is nearly the same in each gonotrophic cycle. Nevertheless, the period is subjected to a variation by individual females and appears to shorten in the mean period with the progress in the gonotrophic cycle. This may be due to the shortening in time of ovulating mature eggs. The females which laid eggs take the second blood meal from just after oviposition. In general, however, the period to feeding after oviposition may be said to be about one day, because the female which laid eggs on one night may feed on the next night in nature. The mean periods for maturation of eggs are between 3 and 4 days, and the period to feeding after oviposition is about one day as stated above, and therefore it is assumed that in nature in summer many females lay eggs at night 4 days after feeding and take the second blood meal on the next night.

For comparison, the similar data obtained with unfertilized females are given in Table 4 (B) which shows that the periods for gonotrophic cycles and to feeding after oviposition are nearly the same in both fertilized and unfertilized females.

5. *Ovariole changes following oviposition.*

The shrinking process of the posterior part

Table 4. Periods in days : hours from feeding (F.) to oviposition (O.) and from oviposition to feednig in the females at a 14-hour photoperiod and 27°C. The feeding was made on a human arm. Observations for oviposition were made at intervals of 2 hours at daytime and 4-6 hours at night. The female which laid eggs was given the chance for feeding from the nearest observation time.

A : Fertilized females

Parity and No. ♀♀	Mean and range	Periods in days : hours required for feeding-oviposition-feeding cycles						
		1st F. to O.	O. to F.	2nd F. to O.	O. to F.	3rd F. to O.	O. to F.	4th F. to O.
3-parous (7♀♀)	Mean	4:11	0:20	3:16	1:09	3:12		
	Range	3:01-8:04	0:02-3:20	3:00-4:16	0:06-3:17	3:02-4:00		
4-parous (10♀♀)	Mean	3:17	1:06	4:00	0:16	3:06	1:00	3:11
	Range	3:01-5:12	0:05-3:14	3:02-5:17	0:11-2:11	3:00-3:13	0:02-1:22	3:06-4:14

B : Unfertilized females

Parity and No. ♀♀	Mean and range	1st F. to O.	O. to F.	2nd F. to O.	O. to F.
2-parous (3♀♀)	Mean	4:10	0:23	3:23	0:20
	Range	3:12-5:08	0:12-1:17	3:07-4:11	

of follicular tubes following oviposition was observed with some females reared at a 14-hour photoperiod and 27°C. The summary result is given in Table 5.

The states of follicular tubes of parous females were examined in nature to estimate the time of feeding after oviposition. In a farm village, 671 females coming to feed on cattle were collected at about 9 p. m. in summer, 1965. Of these, 124 females were parous. On examination, 23 or 18.5 % of the parous ones were found having sac-like and shrinking tubes. This suggests that the females having such tubes probably oviposited the latter part of one night and came to feed on the following night in less than 20 hours after oviposition. The rest of the parous females had completed dilatations. They might have taken the blood meal mostly on the night before or even earlier.

6. *Calendar and physiological ages of gonoactive females at a 14-hour photoperiod and 27°C.*

Summarizing the data obtained from the above experiments, calendar and physiological ages of the female mosquito at a 14-hour photoperiod and 27°C are compiled as shwon in Table 6.

One of the peculiar features in the development of the follicles of this mosquito is that the first follicles of newly emerged females

Table 5. Shrinking process of follicular tubes following oviposition in the females.

hours after oviposition	Shrinking process
10 :	Sac-like in all ovarioles of all females examined.
12 :	Sac-like in most ovarioles and shrinking in some ones in most females. Dilatation appears in a few of those in a few females.
18 :	Sac-like in many ovarioles and shrinking in some ones in a few females. Sac-like in some ovarioles, shrinking in some ones, and dilatation appears in some ones in most females.
20 :	Dilatation appears in most ovarioles and sac-like and shrinking in a few of those in some females. Dilatation is completed in all ovarioles in many females.
24 :	Dilatation is completed in all females.

Table 6. Calendar and physiological ages in the females under the condition of a 14-hour photoperiod and 27°C.

Physiological age or succession in gonotrophic cycle and development stage of follicles: States of follicles	Calendar age (Days after emergence) 0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17
1st foll.	No 1	No 1 ~N	No 2 ~N	Feed. N ~Ia	1 I-II ~IIIb	2 IIIb ~Va	3 Va ~Vb	4 Ovip.										
2nd foll.		No 1	No 1	No 1	No 2 ~N	No 2 ~N	No 2 ~Ib	No 2 ~Ib	Feed. No 2 ~Ib	1 I-II ~IIIb	2 IIIb ~Va	3 Va ~Vb	4 Ovip.					
3rd foll.					No 1	No 1	No 1	No 1	No 1 ~No 2	No 2 ~N	No 2 ~N	No 2 ~Ib	No 2 ~Ib	Feed. No 2 ~Ib	1 I-II ~IIIb	2 IIIb ~Va	3 Va ~Vb	4 Ovip.
4th foll.									No 1	No 1	No 1	No 1	No 1	No 1 ~No 2	No 2 ~N	No 2 ~N	No 2 ~Ib	No 2 ~Ib

Remarks: Feed.: Feeding, Ovip.: Oviposition

are very young and require a long period, usually 3 to 4 days, to reach the first quiescent phase or N to Ib stages. Another one is that the follicles are subjected to a great variation in stage and size in the course of the development not only within follicles of an ovary but also within females. The facts must be taken into a special consideration in the case of the determination of the calendar age of the females of unknown history.

The females preferably take the blood meal about 3 days after emergence and do so about one day after oviposition. The developmental stages of follicles on the days of the feeding are nearly the same; in other words, the first and the second quiescent phases are nearly the same in stage and size, and consequently gonotrophic cycles are usually repeated at regular intervals in many females, though the period for the cycle appears to be shortened little by little in some females probably by the shortening in the time of ovulating mature eggs.

The calendar and physiological ages obtained experimentally, may generally be used in nature in summer. The calendar age of the females in nature may be obtainable from an equation: $3+n\ (4+1)$ days. Where, a 3-day is the prefeeding period; n is the number of dilatations; 4-day and 1-day are the periods from feeding to oviposition and from oviposition to feeding respectively.

II. Effect of day length and temperature on the development of follicles and the feeding activity of the female mosquito

Dr. Omori, the head of the Department of Medical zoology, suggested in 1964 that hibernation of the female of *Culex tritaeniorhynchus* is probably induced by the shortening of the day length in autumn. Experiments were, therefore, carried out to determine the influence of the daily photoperiod and the temperature upon the development of follicles and the feeding activity of the female mosquito.

The combined conditions of various photoperiods, temperatures and relative humidities under each of which the females were reared to adults from the first instar larvae and continuously thereafter are given in Table 7.

With the females, which had been reared under the various rearing conditions, examinations were carried out for the size in length and the stage of follicles and for their feeding rate concurrently or independently on some days after emergence. The results of the examinations are tabulated in Table 8.

Table 7. Combined rearing conditions of the photoperiod (biological day length), temperature and humidity in the indoor insectaries (Experiment A to I).

Exp.	Illumination		Temperature		Humidity
	hrs:min	Lux	Mean	Constant	%
A	10:00	2000	23.2		60-80
B	11:00	160		21.0	60-80
C	11:00	150		23.0	70-80
D	11:00	800		27.0	70-80
E	14:00	160		21.0	60-80
F	14:00	800		27.0	70-80
G	15:50	2000	28.5		60-80
H	16:00	160		21.0	60-80
I	16:00	150		25.0	60-80

Remarks: (1) In A, maximum and minimum temperatures in each day were 25.9 and 21.1°C. In G, those were 31.0 and 25.7°C.
(2) As for the detailed accounts for the regulation of rearing conditions in the indoor insectaries, see the paragraph about the experimental place, material, and method.

The data given in Table 8 are classified into five supergroups by the mode of manifestation of the influence caused by the different combinations of rearing conditions.

Supergroup I: The supergroup includes groups A.1.0, B.1.1-2, and B.2.1-4 which were reared under the short photoperiod and low temperature. Under the short photoperiod the follicles remain in smaller sizes and younger stages than those in the quiescent phase being 3 to 5 in size and No2 to N in stage. The females with such small and young follicles entirely reject taking the blood meal showing them to be in the diapause state.

Supergroup II: It includes Exps. E, F, G, H, and I. The females were reared under long photoperiods and temperatures of 21 to 28.5°C. Under these long photoperiod conditions the follicles of the females developed and reached the quiescent phase and the females took the blood meal irrespective of the rearing temperatures. The feeding rates were, however, rather higher in higher temperatures than in lower ones.

Supergroup III: It includes groups B. 2. 5-6. The females were reared during the period covering the immature stages and 9 days after emergence under a short photoperiod and 21°C, but thereafter under a 24-hour photoperiod and the same temperature. The follicles of the females reached the quiescent phase and their feeding rates were rather higher. The result shows that the gonoinactive state of females can be changed to the gonoactive state by the exposure of adult females to the long day condition for

Table 8. Developmental states of follicles and feeding rate of the females reared as adults from the first instar larvae and continuously thereafter under the combined conditions of various photoperiods and temperatures in the indoor insectaries

Condition: photoperiod (hrs:min), temperature	Exp., Batch, & Group number	Days from emerg. to dissection or feeding	No. of ♀♀ with the 1st follicles of the indicated mean length									Developmental stages of		Feeding rate on chicken			As for the fate of 1st foll. of fed ♀♀, see Table
			3-	4-	5-	6-	7-	8-	9-	10-	Total	the 1st follicles	the 2nd follicles	No. ♀♀ exposed	No. ♀♀ fed	Rate (%)	
10:00, 23.2°C	A.1.0	3-6	18	4							22	No1～Ia	No1	350	0	0.0	
11:00, 21°C	B.1.1	11												500	0	0.0	
11:00, 21°C	B.1.2	15		6	1						7	No2～N	No1	400	0	0.0	
11:00, 21°C	B.2.1	5		8							8	No2～N	No1	310	0	0.0	
11:00, 21°C	B.2.2	8		5							5	No2～N	No1	290	0	0.0	
11:00 21°C	B 2.3	22	1	7	2						10	No2～Ia	No1	20	0	0.0	
11:00, 21°C	B.2.4	48	2	12	1	1					16	No2～N	No1				
(1), 21°C	B 2.5	22						1	2		3	N～I-II	No1	191	59	30.9	
(2), 21°C	B.2.6	48						1	6	1	8	N～IIa	No1	86	64	74.4	
11:00, 21°C	B.3.1	7												500	0	0.0	
11:00, 21°C	B.3.2	11	An additional 3-hour-illumination was given for the females on one day before the feeding.											300	0	0.0	
11:00, 21°C	B 3.3	12	The same was given each on two days.											250	1	0.4	Table 9
11:00, 21°C	B.3.4	25	The same was given each on four days.											157	28	17.8	Table 9
11:00, 21°C	B.3.5	27	The same was given each on four days.											110	28	25.5	Table 9

Remarks: (1) Females were reared at an 11-hr photoperiod and 21°C for first 9 days and then a 24-hr photoperiod and 21°C for 13 days.

(2) Females were reared at an 11-hr photoperiod and 21°C for 9 days and a 24-hr photoperiod and 21°C for next 39 days.

Table 8. (Continued)

Condition: photoperiod (hrs:min), temperature	Exp., Batch, & Group number	Days from emerg. to dissection or feeding	No. of ♀♀ with the 1st follicles of the indicated mean length									Developmental stages of		Feeding rate on chicken			As for the fate of 1st foll. of fed ♀♀, see Table
			3-	4-	5-	6-	7-	8-	9-	10-	Total	the 1st follicles	the 2nd follicles	No. ♀♀ exposed	No. ♀♀ fed	Rate	
11:00, 23°C	C. 1. 1	4	An additional 3-hour-illumination was given on one day.											300	6	2. 0	
11:00, 23°C	C. 1. 2	18		2	1	(The same, on two days.)					3	N	No1	100	6	6. 0	
11:00, 27°C	D. 1. 1	6												467	17	3. 6	Table 9
11:00, 27°C	D. 1. 2	11	16	11	3						30	No2～Ia	No1	430	19	4. 4	Table 9
14:00, 21°C	E. 1. 1.	3-7												500	6	1. 2	
14:00, 21°C	E. 1. 2	15-21												260	74	28. 5	
14:00, 21°C	E. 1. 3	16-22												169	11	6. 5	
14:00, 21°C	E. 2. 0	5-9				6	8	10	3		27	N～IIa	No1～No2	322	261	81. 1	Table 9
14:00, 27°C	F. 1. 1	5-8				6	22	30	20	2	80	N～IIa	No1～No2	411	280	68. 1	Table 9
14:00, 27°C	F. 1. 2	11-14												108	47	43. 5	
15:50, 28. 5°C	G. 1. 0	3-16		1	4	14	15	6	1		41	No2～IIa	No1～No2 (*)	629	324	51. 5	
16:00, 21°C	H. 1. 1	5-7												248	105	42. 3	
16:00 21°C	H. 1. 2	5-8				2	6	26	25	1	60	No2～I-II	No1～No2 (*)	457	62	13. 6	Table 9
16:00, 21°C	H. 1. 3	10-13												394	159	40. 4	
16:00, 25°C	I. 1. 0	16			3	8	6	3			20	N～Ib	No1～No2				

(*) : A small number of the second follicles in a few females developed to N stage probably owing to the death of the first follicles just after reaching N or Ia stage.

some days or for 13 days in this experiment.

Supergroup IV: It includes groups B. 3. 1-5 and C. 1. 1-2. The females were reared under a short photoperiod plus additional illuminations (for only adults) and low temperatures. In B. 3. 1 additional illumination was not added, in B. 3. 2 the same was added on one day, while in B. 3. 3 on 2 days and in B. 3. 4-5 on 4 days. The feeding rates were zero in the first two groups, only 0.4 % in the third group, while 17.8 and 25.5 % in the last two groups, showing that the feeding activity becomes higher little by little with the increase in light hours.

Supergroup V: It includes groups D. 1. 1-2. The females were reared under a short photoperiod and high temperature. The follicles remained small in size and young in stage at least in the females examined, but the females of these groups took the blood meal, though in only 3.6 and 4.4 %. This suggests that high temperatures perhaps 27 °C and above may have some effect on the feeding activity of the females, details of which will be discussed later.

The particulars on the development of the first and second follicles of females which took the blood meal are given in Table 9.

All the females given in Table 9 are divided into three groups according to the maturation of the first follicles: (1) the females had a considerable or the usual number of mature eggs, (2) the females had a few or a small number of mature eggs, and (3) the females had no mature eggs. In the first group the remaining first follicles were all degenerated and the second follicles were found well developed to reach the quiescent phase. In the

Table 9. Development of the first and second follicles in the females fed on a chicken (continued from Table 8).

Exp., Batch, and Group number	Condition: photoperiod (hrs:min), temperature	No. of ♀♀ fed	No. ♀♀ dissected	♀♀ with mature eggs (1st follicles)*: No. of ♀♀	♀♀ with mature eggs (1st follicles)*: Mean No. (range) of eggs/ovary	♀♀ with a few or no mature eggs (1st follicles): No. of ♀♀	♀♀ with a few or no mature eggs (1st follicles): Eggs per ovary	♀♀ with a few or no mature eggs (1st follicles): Stages of remaining follicles	♀♀ with a few or no mature eggs: (2nd follicles)
B. 3. 3	11:00, 21°C (An additional 3-hour-illumination was given for the females each on different two days before the feeding)	1	1	0	-	1	0	Ib~IIb	No2
B. 3. 4	11:00, 21°C (The same was given each on four days)	28	8	3	ca. 80	5: 1♀	5	degenerated	N
						1♀	15	degenerated	N, Ia
						1♀	20	degenerated	Ia, Ib, I-II
						2♀♀	0	Ia~I-II	No1, No2
B. 3. 5	11:00, 21°C (The same was given each on four days)	28	2	0	-	2	0	I-II~IIa	No1, No2
D. 1. 1	11:00, 27°C	17	11	2	69(57-81)	9: 5♀♀	0	N	No1, No2
						2♀♀	0	N~Ia	No1
						1♀♀	0	N~Ib	No1, No2
						1♀♀	0	Ib~IIIa	No2
D. 1. 2	11:00, 27°C	19	8	6	79(60-90)	2: 1♀	0	No2~N	No1, No2
						1♀	0	N	No1, No2
E. 2. 0	14:00, 21°C	261	2	2	ca. 90	0			
F. 1. 1	14:00, 27°C	280	14	14	95(46-146)	0			
H. 1. 2	16:00, 21°C	62	5	5	81(47-113)	0			

* The remaining ones of the first follicles were all degenerated.
The second follicles reached the quiescent phase being No2 to I-II or mostly N to Ib stages.

second group the remaining first follicles were all degenerated and the second ones were in the quiescent phase. In the third group, the first follicles were mostly in the quiescent phase, though greatly varied in development with follicles and females. In the extreme cases, one female in B. 3.3 had advanced follicles of Ib to IIb stages, two females in B. 3.5 had those of I-II~IIa, and one in D. 1. 1 had those ranging from Ib to IIIa. However, the second follicles remained small in size and young in stage in all females of the third group.

The fact that the second follicles develop and reach the quiescent phase seems to show the maturation of eggs en masse or maturation of some eggs and the death of the remainder. On the contrary, the fact that the second follicles remain in a small and young developmental state seems to show that the first follicles after reaching the quiescent phase or a little more advanced stages do not farther develop by the occurrence of gonotrophic dissociation.

From the above observations, it is concluded on the unusual development of follicles as follows. Additional illuminations added to the daily short photoporiod seem to cause the follicles of some females to develop to some extent inducing the females to take the blood meal. After feeding, in some females the first follicles develop to mature eggs en masse and in some others only a small number of them complete the maturation. In these cases the remaining first follicles degenerate and the second follicles advance to the quiescent phase suggesting the possibility in such females of taking the next blood meal. On the contrary, in some fed females, the first follicles remain in the quiescent phase and the second ones in a small and young developmental state, showing that the females entered into the state of diapause by the occurrence of gonotrophic dissociation. High temperature conditions above 27°C combined with short day length seem to cause similar abnormality in follicular development in a few females. These unusual developments of follicles must take place in nature in autumn when the day length and temperature are getting shorter and lower.

III. Comparison of follicular development and feeding activity of the females reared to adults from larvae in the biotron or in the outdoor insectary, and those collected as adults in nature

1. *Follicular development and feeding activity of the females reared to adults in the biotron under the rearing conditions modeled after those in nature in August to September.*

Detailed accounts for regulating temperature, humidity and day length in the biotron were described in the paragraph about the experimental place, material and method. Under the conditions (Table 10) in the biotron modeled after those (Table 12) in nature in early August to late September, the first instar larvae originated from the laboratory colony were reared to adults. With the females reared as above, the follicular development and feeding activity on chickens were examined with the results shown in Table 11. The females which emerged from early August to mid-September had the follicles nearly similar in size and stage to those of the females reared under a 14-hour photoperiod and a constant temperature of 27°C (Table 2). The feeding rates were also nearly the same. The results show that the females are gonoactive if they emerge as adults before

Table 10. Changes in the rearing conditions by 5 days in the biotron produced after the natural ones encountered in August to September at Nagasaki, showing the photoperiods (biological day length) and mean temperatures at the time of the hatching of larvae (A), the emergence of adults (B), and the dissection of females (C).

Exp. & Batch No.	Month, part (subpart)			Photoperiod (hrs: min)			Temperature (°C)		
	(A)	(B)	(C)	(A)	(B)	(C)	(A)	(B)	(C)
J-1	Aug. E(2)	Aug. M(1)	Aug. L(1)	14:44	14:35	14:15	28.4	28.4	27.5
J-2	Aug. M(1)	Aug. L(1)	Aug. L(2)	14:35	14:15	14:04	28.4	27.5	27.5
J-3	Aug. M(2)	Aug. L(2)	Sep. E(2)	14:25	14:04	13:43	28.4	27.5	26.5
J-4	Aug. L(2)	Sep. E(1)	Sep. M(1)	14:04	13:54	13:33	27.5	27.2	24.5
J-5	Sep. E(1)	Sep. M(1)	Sep. L(1)	13:54	13:33	13:13	27.2	24.5	22.1

Remarks : (1) As for the method of regulating the photoperiod, temperature and relative humidity, see the paragraph about the experimental place, material and method.
(2) The relative humidity varied with daily cycle nearly conversely with the temperature according to the time of a day, ranging from 62 to 90%, usually from 65 to 85%, during August to early September, but it became a little lower thereafter towards the end of September.
(3) E, M, and L mean the early, middle, and late parts of a month, which are divided into two subparts (1) and (2).

Table 11. Developmental states of follicles and the feeding rate of the females reared as adults from the first instar larvae and continuously thereafter in the biotron under the conditions given in Table 10.

Exp. and Batch No.	Month in biotron		Days from emerg. to dissection	No. of ♀♀ with the 1st follicles of the indicated mean length							Developmental stages of		Feeding rate on chicken 9 days after emergence		
	at hatching	at emergence		5-	6-	7-	8-	9-	10-	Total	the 1st follicles	the 2nd follicles	No. ♀♀ exposed	No. ♀♀ fed	Rate (%)
J-1	Aug. E (2)	Aug. M (1)	9-13	3	10	16	8	1		38	N∼IIa	No1∼No2	461	240	52.1
J-2	Aug. M (1)	Aug. L (1)	9-10	1	3	10	6			20	N∼I-II	No1∼No2	522	174	33.3
J-3	Aug. M (2)	Aug. L (2)	9-13		3	10	12	4	1	30	N∼IIa	No1	531	286	53.9
J-4	Aug. L (2)	Sep. E (1)	12-13			3	7			10	Ia∼Ib	No1∼No2	321	200	62.3
J-5	Sep. E (1)	Sep. M (1)	12-15	1	3	3	2	1		10	N∼Ib	No1	213	72	33.8

Remark : As for the subdivision of a month, see footnote in Table 10.

mid-September ; i. e., before the days when the biological day length becomes about 13 : 30 hours.

2. *Follicular development and feeding activity of the females reared tc adults in the outdoor insectary especially in and after September.*

Similar experiments were carried out under the rearing conditions shown in Table 12 in the outdoor insectary. The detailed figures on the astronomical and biological day length, mean air temperature and mean relative humidity by ten days at Nagasaki in 1965 to 1969 are presented in Table 12. The larvae which hatched in and after early November from eggs originated from the laboratory colony could not complete their larval development as shown in Table 13. The larvae could develop to adults in a rather low rate

Table 12. Day length, mean air temperature and relative humidity by five days at Nagasaki, 1965-1969.

Month and Part		Day length (hrs : min)		Mean air temperature (°C)					Mean relative humidity(%)				
		astro-nomical	biolo-gical	1965	1966	1967	1968	1969	1965	1966	1967	1968	1969
Jan.	E	10:06	11:16	5.6	7.3	4.4	7.7	4.5	62	68	70	66	71
	M	10:14	11:24	5.3	7.3	3.8	6.9	6.3	69	71	71	64	73
	L	10:28	11:36	7.1	4.7	8.0	5.3	12.1	72	67	73	64	83
Feb.	E	10:44	11:52	5.5	7.8	7.3	2.8	5.9	73	71	72	61	69
	M	11:02	12:08	9.0	9.3	4.0	3.5	10.7	67	71	63	69	82
	L	11:22	12:27	6.6	9.3	8.7	4.8	5.5	56	76	71	70	62
Mar.	E	11:37	12:42	7.1	14.2	7.9	7.6	7.3	56	78	68	65	64
	M	11:57	13:01	0.9	12.0	11.8	10.0	8.6	64	66	77	65	73
	L	12:18	13:23	9.4	10.5	11.5	12.3	11.0	64	64	74	70	72
Apr.	E	12:39	13:44	11.5	13.6	14.6	15.1	12.7	62	74	77	65	58
	M	12:59	14:05	13.4	13.3	14.5	14.7	15.7	68	70	78	63	73
	L	13:18	14:26	15.5	18.2	17.7	15.8	18.0	74	82	68	68	80
May	E	13:35	14:45	17.0	17.7	18.3	17.7	18.9	74	72	79	73	70
	M	13:50	15:02	19.6	19.5	19.0	18.5	19.6	68	68	72	74	80
	L	14:03	15:17	19.6	19.1	22.2	18.9	18.9	76	74	75	78	71
Jun.	E	14:14	15:30	20.4	20.1	23.0	20.7	19.9	72	76	68	73	73
	M	14:18	15:34	20.9	21.4	23.4	21.2	21.4	84	85	70	82	78
	L	14:20	15:37	22.9	24.6	24.2	21.9	22.8	86	83	81	86	83
Jul.	E	14:15	15:31	24.6	22.8	24.4	21.9	23.0	90	82	88	88	91
	M	14:07	15:22	27.3	26.8	27.7	26.3	26.0	83	83	85	84	79
	L	13:56	15:08	28.0	28.6	28.8	26.7	28.0	82	79	72	80	77
Aug.	E	13:40	14:50	27.5	29.7	28.0	26.9		74	78	77	79	
	M	13:23	14:31	28.0	29.5	28.8	27.5		78	75	79	76	
	L	13:05	14:12	26.9	28.1	28.7	27.0		78	77	78	76	
Sep.	E	12:44	13:50	23.5	28.3	29.0	25.8		73	73	74	67	
	M	12:25	13:30	21.6	22.9	23.1	24.8		76	69	62	76	
	L	12:04	13:08	21.4	20.4	22.8	22.4		64	77	62	80	
Oct.	E	11:44	12:48	19.0	20.4	20.3	22.4		66	74	61	79	
	M	11:25	12:30	17.3	19.2	19.3	18.0		65	70	63	59	
	L	11:05	12:11	17.6	17.1	17.4	16.3		75	70	68	62	
Nov.	E	10:46	11:53	18.3	15.2	16.5	15.5		73	75	74	74	
	M	10:30	11:38	15.1	14.7	13.1	11.3		73	71	65	67	
	L	10:17	11:27	12.3	9.8	13.2	14.6		69	67	71	77	
Dec.	E	10:08	11:18	10.5	7.9	6.9	14.9		70	68	69	83	
	M	10:01	11:13	8.4	9.7	7.5	10.1		74	70	68	74	
	L	10:01	11:13	6.9	5.6	4.3	9.3		68	70	76	67	

from the end of October to early November only when they were reared before mid-October (K-6 in Tables 13 and 14).

In Table 14, the results of examinations for the developmental states of follicles and the feeding rates of the females which emerged in September through early November were presented, together with those of the females which emerged in May through August for comparison. In May the first follicles

Table 13. Development of immature stage mosquitoes reared from the first instar larvae under natural outdoor conditions (given in Table 12) in the outdoor insectary.

Exp. & Batch No.	Year	Date of hatching	Pupation rate (%)	Starting date of emergence	Emergence rate (%) from pupae
K-1	1966	Aug. 10	88.8	Aug. 16	87.7
K-2	1966	Aug. 25	91.0	Aug. 31	90.3
K-10	1967	Aug. 26	72.9	Sep. 1	
K-11	1967	Sep. 3	79.1	Sep. 10	
K-3	1966	Sep. 9	74.9	Srp. 16	91.8
K-4	1966	Sep. 15	74.1	Sep. 21	89.9
K-5	1966	Sep. 26	80.6	Oct. 9	84.7
K-12	1967	Sep. 30	58.3	Oct. 11	
K-6	1966	Oct. 12	69.3	Oct. 31	37.4
K-7	1966	Nov. 2	Ail died by Dec. 19 in 4th instar.		
K-8	1966	Nov. 28	All died by Dec. 5 in 1st instar.		
K-9	1966	Dec. 23	All died by Dec. 27 in 1st instar.		

of the females reared from the larval stage under the natural outdoor conditions became very large and advanced to N~IIa in stage. In June through late August, the follicles were mostly middle-sized but some were larger in size and N~IIa or even IIb in stage. The feeding rates in May through August were high, showing that the females which emerged in this period were obviously gonotrophically active. In early to mid-September small follicles (3 to 5 in length) began to appear though the feeding rate was still high, while in mid-September the rate abruptly decreased very much. In and after late September the follicles were mostly small and No2~N in stage and the feeding rates were zero or very low. The above shows that females of the mosquito begin to become gonoinactive about mid-September. The factor which induces the females to the gonoinactive state is the day length, as already stated in the previous paragraph, and now the critical time of becoming inactive for the females is thought to be in mid-September when the biological day length is about 13 : 30 hours. The shortening in day length seems to have also a great influence on the maturation of eggs as shown in the next Table.

Table 15 shows the states of follicular development of fed females on chickens among the females given in Table 14. It is of interest that there were some females which failed after feeding to mature the normal number of eggs. Among such females there were two types. The first type is the females which could mature no egg or only a small number of eggs. The remaining first follicles were degenerated and the second follicles in quiescent phase or slightly advanced stages, as seen in K-1 and K-11 in Table 15 and also in B. 3. 4 in Table 9. The females of this type were found even in summer but seemed to become larger in number under the conditions of the shortening day length in and after September. The second type is the female in which the first follicles remained in quiescent phase or in a little advanced stages of IIa (cf. Table 15) or even IIIa (cf. Table 9 and 17), and the second follicles in the very young stages of No1 to No2. The females

Table 14. Developmental states of follicles and feeding rate of the females reared from the first instar larvae or wild-caught old larvae under the natural outdoor conditions (given in Table 12) in the outdoor insectary.

Exp. and Batch No.	Year	Month of emergence	Days from emerg. to dissection or feeding	No. of ♀♀ with the 1st follicles of the indicated mean length											Developmental stages of		Feeding rate on chicken			As for the fate of 1st foll. of fed ♀♀, see Table
				3-	4-	5-	6-	7-	8-	9-	10-	11-	12-	Total	the 1st follicles	the 2nd follicles	No. ♀♀ exposed	No. ♀♀ fed	Rate (%)	
K-13	1968	May M	7-9							2	3		2	7	N~IIa	No1				
K-14	1968	Jun. E	7-8				3	12	8	3				26	N~IIb	No1~No2				
K-15	1968	Jun. M	6-8					4	9	1	1	1	1	17	N~IIa	No1~No2				
K-1	1966	Aug. M	4														239	157	65.7	Table 15
K-2	1966	Aug. L-Sep. E	4-8														557	436	78.3	Table 15
K-10	1967	Sep. E	9-19			1	10	8	3					22	N~Ib	No1~No2	213	76	35.7	Table 15
K-16	1968	Sep. E	11-12		2	1	3	1						7	N~I-II	No1~No2				
K-11	1967	Sep. E-M	3-11		1	3	8	8						20	N~Ib	No1~No2	344	245	71.2	Table 15
K-3	1966	Sep. M	6-8														295	1	0.3	
K-4	1966	Sep. L-Oct. E	4-5														513	0	0.0	
K-5	1966	Oct. E-M	7-11	11	2									13	No2~N	No1	350	0	0.0	
K-12	1967	Oct. M	24-25	1	4									5	No2~N	No1	167	11	6.6	Table 15
K-6	1966	Nov. E	10-14	1	19	3		1						24	No2~N	No1				

Remark : The first three batches, K-13, -14, -15, were reared from wild-caught old larvae, while the others were reared from the first instar larvae of the laboratory colony.

Table 15. Development of the first and second follicles in the females fed on a chicken (continued from Table 14).

Exp. and Batch No.	Year	Month of emergence	No. of ♀♀ fed	No. ♀♀ dissected	♀♀ with mature eggs (1st follicles)*		♀♀ with a few or no mature eggs			
							(1st follicles)			(2nd follicles)
					No. of ♀♀	Mean No. (range) of eggs/ovary	No. of ♀♀	Eggs per ovary	Stages of remaining follicles	
K-1	1966	Aug. M	157	36	34	111(34-169)	2 { 1♀ 1♀	0 0	degenerated N	N～Ib N
K-2	1966	Aug. L -Sep. E	436	32	31	92(35-138)	1	0	Ia～IIa	No1
K-10	1967	Sep. E	76	15	14	82(40-150)	1	0	N	No1, No2
K-11	1967	Sep.E-M	245	6	5	96(65-117)	1	21	degenerated	N～Ib
K-12	1967	Oct. M	11	6	0	—	6 { 4♀♀ 2♀♀	0 0	No2～N Ib	No1 No1

* Remaining ones of the 1st follicles were degenerated. The 2nd follicles reached the quiescent phase being No2 to I-II or mostly N to Ib stages.

of this type are those which underwent gonotrophic dissociation. The factor which induces the gonotrophic dissociation is principally the shortening of the day length. With the advance of the shortening in the day length such females seemed to increase in number.

3. *Follicular development of unfed and fed females collected as adults in nature.*

Examinations for the follicular development of wild caught unfed and fed females were carried out in 1966 to 1968 in the Nagasaki and Saga areas with the results shown in Table 16. Unfed females were collected by dry ice traps and in cow- and pig-sheds. Fed ones were caught just after feeding in animal-sheds. The first follicles of wild caught unfed females in May through August being in gonoactive states are middle-sized, ranging from 5 to 10, usually from 6 to 9 excepting those of the females collected in May as will be stated later, while after September the follicles become rather larger and are mostly N to Ib, rarely extending to IIa in stage.

In the parous females the developmental states of current and next follicles were nearly the same throughout the period of the experiment, with some rare exceptions.

In May, the nulliparous females collected in nature might be composed of those late in coming out from hibernation and those newly emerged as adults in this month. The follicles of the former may be large and advanced in stage and those of the latter may be subjected to a great variation in size and stage because the day length is getting longer but temperatures are rather low and very variable. Therefore, the mosquitoes in their immature stages are subjected to a great variation in the environmental conditions under which they bred. This may be the reason for a great variation in size and stage of follicles in females caught in this month.

Fed females which were collected just after feeding in cow- and pig-sheds in nature in September through October, were examined for the states of follicular development with the results shown in Table 17. Among fed nulliparous females there were some females which could mature eggs in normal number even in early October, and some others in which the first follicles remained in the quiescent phase and the second follicles in very

Table 16. Developmental states of follicles of wild caught unfed females by dry ice traps and in cow- and pig-sheds in Nagasaki and Saga, 1966 to 1968.

Month	Nulliparous females														Parous females										
	No. of ♀♀ with the 1st follicles of the indicated mean length												Developmental atages of		No. of ♀♀ with the 1st follicles of the indicated mean length									Developmental stages of	
	5-	6-	7-	8-	9-	10-	11-	12-	13-	14-	15-	Total	1st foll.	2nd foll.	5-	6-	7-	8-	9-	10-	11-	12-	Total	1st foll.	2nd foll.
Mar.		1	1	1								3	Ia～Ib	No1～No2			2	3	1				6	Ia～IIa	No1～No2
Apr.	1	3	5	8	8							25	No2～IIa	No1	1	1	1	5	5				13	No2～Ib	No1
May	1	5	11	13	12	12	3	1	1		1	60	N～IIIa	No1～No2	1	2	1	2		2	1		9	No2～IIb	No1
Jun.		2	14	19	7	2						44	N～I-II	No1～No2		1	6	3				1	11	Ia～IIb	No1～No2
Jul.	2	14	36	27	10							89	N～IIa	No1～No2			5	8	1				14	N～I-II	No1
Aug.	6	6	28	22	5							67	N～I-II	No1～No2		2	4	6	4				16	N～I-II	No1～No2
Sep.		1	14	24	13	8						60	N～IIa	No1～No2		1	4	4	2				11	N～IIa	No1～No2
Oct.			1	1	1	1	1					5	Ia～IIa	No1		1	2	1					4	Ib	No1
Nov.												0								1			1	Ib～IIb	No1

young stages as the result of the gonotrophic dissociation. The number of females which underwent gonotrophic dissociation appeared to increase with the lapse of time. The parous females, at least in these experiments, were all subjected to gonotrophic dissociation, although it was uncertain whether this was always true or not because of the scanty number of cases observed. The above suggests that when the day length becomes shorter in September, some fed nulliparous females and many fed uniparous ones are subjected to gonotrophic dissociation probably increasing in number with the lapse of time, and that some others, however, can repeat their gonotrophic cycles till the end of life probably without entering hibernation.

4. *Comparison of developmental states of follicles of females reared tc adults tn the biotron or outdoor insectary, and those collected as adults in nature.*

In Table 18 a comparison of the developmental states of follicles of females reared to adults from larvae in the biotron oı outdoor insectary with those of females collected in nature as adults, shows that the states are found to be nearly the same throughout the gonoactive season for this mosquito from March through August, while in and after September the situation becomes different. The follicles in the former group become smaller in size and younger in stage, while in the latter they are as usual or in rather advanced states. This may be due to the fact that the nulliparous females which have small and young follicles are gonoinactive and seems to enter hibernation without taking a blood meal, while some others which have the usual or slightly more advanced follicles retain their gonoactivity till the end of life in late autumn.

Table 17. Development of the current and the next follicles in fed nulliparous (and parous) females caught in nature at cow- and pig-sheds and kept outdoors.

Date of collection	Days from collect. to dissect.	No. of ♀♀ dissected	♀♀ with mature or nearly matured eggs: No. of ♀♀	Mean No. (range) of eggs/ovary	States of remaining current follicles	Stages of next follicles	♀♀ without eggs: No. of ♀♀	Stages of current follicles	Stages of next follicles
Sep. 7 '68	6	4 (1)	3	99(97-101)	degenerated	N	1 (1)	Ib~IIIa (Ib~IIIa)	No1~No2 (No1~No2)
Sep. 11 '68	3	(1)					(1)	(N~Ia)	(No1)
Sep. 14 '67	3	5	5	100(90-112)	degenerated	No2~N			
Sep. 17 '67	3	2					2	Ib~IIa	No1
Sep. 18 '67	3	12	12	88(59-132)	degenerated	No2			
Sep. 28 '68	4	4	1	99	degenerated	N	3 {1, 1, 1}	N~IIIa I-II~IIa N~Ib	No1~No2 No1~No2 No1~No2
		(1)					(1)	(N~Ib)	(No1)
Oct. 5 '68	10	1	1	98	degenerated	N			

Table 18. Comparison of developmental states of follicles of unfed nulliparous females collected in nature as adults (Table 16), and those reared as adults from larvae under natural conditions in the biotron (Table 11) or in the outdoor insectary (Table 14). (The states of follicles of females reared from larvae in the biotron or outdoors are given in parentheses)

Month of collection (emergence)	No. of ♀♀ with the 1st follicles of the indicated mean length: 3-	4-	5-	6-	7-	8-	9-	10-	11-	12-	13-	14-	15-	Total	Developmental stages of the 1st follicles	the 2nd follicles
Mar.				1	1	1								3	Ia~Ib	No1~No2
Apr.			1	3	5	8	8							25	No2~IIa	No1
May			1	5	11	13	12 (2)	12 (3)	3	1 (2)	1		1	60 (7)	N~IIIa (No2~IIa)	No1~No2 (No1)
Jun.			(1)	2 (6)	14 (19)	19 (20)	7 (4)	2 (1)	(1)	(1)				44 (53)	N~I-II (No2~IIb)	No1~No2 (No1~No2)
Jul.			2	14	36	27	10							89	N~IIa	No1~No2
Aug.			6 (4)	6 (16)	28 (36)	22 (26)	5 (5)	(1)						67 (88)	N~I-II (N~IIa)	No1~No2 (No1~No2)
Sep.		(3)	(6)	1 (24)	14 (23)	24 (12)	13 (1)	8						60 (69)	N~IIa (N~I-II)	No1~No2 (No1~No2)
Oct.					1	1	1	1	1					5	Ia~IIa	No1
Nov.	(13)	(25)	(3)		(1)									(42)	(No2~N)	(No1)

5. *Consideration on the mode of entering hibernation in this mosquito.*

In September the biological day length becomes shorter being 13:50, 13:30 and 13:08 hours in the early, middle, and late parts of the month. The shortening in day length induces the reduction of the feeding rate of females, the retardation of follicular development and the increase in the number of females subjected to gonotrophic dissociation after taking a blood meal. The feeding rate of females abruptly decreases very much or reaches zero in and after mid-September. The females with small follicles (3 to 5 in length) begin to appear from early September, gradually increasing in number with the lapse of time. Fed females which undergo gonotrophic dissociation become fairly larger in number at that time than in summer.

In October, most of the females which emerged as adults in this month have small follicles, scarcely take a blood meal, and if they rarely take blood they seem to be subjected mostly to gonotrophic dissociation.

These females, which undergo such influences as above by the shortening of day length, seems to enter hibernation, although there may be some females which can repeat the gonotrophic cycle in spite of the shortening of day length till the end of autumn probably without entering hibernation.

Summary

1. The females which were reared from the larvae under the combined conditions of a 14-hour daily photoperiod (biological day length) and a constant temperature of 27°C modeled after natural conditions in late August in the Nagasaki area, are gonoactive and feed as readily as in nature in summer. The prefeeding period requires about 3 days, being a little longer than in the case of other mosquito species, because of the first follicles are very young at the time of the emergence as adults. The gonotrophic cycles from feeding to oviposition require above 3 to 4 days and are nearly equal for each time because the first and the second quiescent phases are nearly equal, being middle (6-9 in length on the micrometer scale) or large (10 or more) in size, and N to Ib in the stage extending in some cases to IIa or rarely to IIIa. The period from oviposition to feeding is about one day. Thus, the calendar age of this female mosquito is determined by an equation : $3+n(4+1)$ days, where $n=$ number of dilatations.

2. Examinations for the follicular development before and after feeding were carried out with the females reared from the larval stage under the combined conditions of various photoperiods and temperatures in the indoor insectaries, under conditions modeled after natural ones in the biotron, and under natural outdoor conditions. As a result, it was revealed that : The females are gonoactive and high in the feeding rate during the period from late March through mid-September when the biological day length is longer than 13 : 30 hours. Their follicles at the quiescent phase are middle-sized or large in size and N to Ib or slightly more advanced in stage. In early to mid-September the first follicles are mostly middle-sized, but some are of small size (3 to 5 in length). The females with small follicles increase in number rapidly with the shortening of the biological day length below 13 : 30 hours. In and after early October most of the follicles become small. The feeding rate abruptly decreases in and after mid-September when the biological day length is getting shorter and shorter below 13 : 30 hours.

3. In the course of the period when the day length is getting shorter in and after September, usually, the feeding rate of the females decreases rapidly and their first follicles become smaller and younger as stated above. However, some females which probably had middle or rather smaller sized follicles take a blood meal, resulting in failure in many cases in maturing the follicles. After the feeding of such females, there occur (i) a minority of females in which all the first follicles or majority of them degenerate with

a small number of matured eggs, and all the second follicles reach the second quiescent phase; (ii) some females in which the first follicles reach the quiescent phase or a little more advanced stages but the second ones constantly remain in small and young states by the occurrence of gonotrophic dissociation. The number of these females increases with the advance in the shortening in day length; (iii) some parous females which underwent gonotrophic dissociation after feeding following oviposition. Besides these, there are some females which can repeat gonotrophic cycles even till the end of October probably dying before long.

4. The females of this mosquito seem to hibernate from about mid-September under the gradual shortening in the day length in the following states: Many females having small follicles enter hibernation in the nulliparous and unfed states; some females hibernate in the nulliparous state after undergoing gonotrophic dissociation after feeding; some parous females do the same in the state of gonotrophic dissociation after feeding following oviposition.

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コガタアカイエカの濾胞の発育と吸血活動に関する研究，特に秋季における状態について

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摘　　要

日本脳炎の主伝搬者であるコガタアカイエカの，野外における生態の研究結果から，本蚊の雌成虫の生殖活性は，日長によって大きく左右されるであろうことが暗示された．一方，生殖活性は濾胞の発育状態によく反映されることが，予備実験によって明らかとなった．そこで，1965～1968年に，日長と温度を組合せた各種実験条件下及び自然環境下で飼育した雌の，吸血活動と濾胞の発育状態を調査した．その結果は次のように要約される．

1.　生物学的日長（以下，日長と略称する）14時間，温度 27°C（長崎での８月末の条件）で飼育した雌は生殖活性で，野外の夏の雌と同じようによく吸血する．羽化から吸血までの期間は約３日である．第１生殖環，即ち初回の吸血から産卵までは約４日，産卵後吸血までは約１日を要し，この期間は，第２回以後の生殖環においてもほゞ同じである．これは，第１静止期と第２以後の静止期の，濾胞の状態がほゞ等しく，大部分の雌では，大きさは中型で６～９ミクロメータースケール（１スケール＝10.5 μ），発育期はN～Ibであることによる．以上のことから，雌の歴年令は次式によって求められる．３＋n(４＋１)日，ただし，nは産卵回数である．

2.　４月上旬から９月上旬までのように，日長が13時間30分よりも長いと，雌は生殖活性で吸血率は高い．第１静止期の濾胞は大多数が中の大きさでN～Ib 期であり，大型で発育期がより進んだものも少数ある．しかし，９月上・中旬頃から大きさ３～５の小型濾胞が現れ始め，日長が13時間30分以下になると急増して，10月上旬以後はそれが大多数を占め，発育期も多くはNo2～Nにとゞまる．吸血率も９月中旬以後は急に低下する．

3.　日長の短縮が甚しくなる９月およびそれ以降には，中型またはこれに近い小型の濾胞をもった雌に，吸血はするが濾胞が正常に成熟しないという現象がしばしばおこる．そしてその場合の濾胞は，次のいづれかの状態を示す．a）すべての第１濾胞が退化をするか，大多数の第１濾胞が退化して少数の成熟卵が形成される．いづれの場合にも第２濾胞は静止期に達する．b）栄養生殖分離をおこして，第１濾胞は静止期のまゝか僅かに進んだ状態になるが，第２濾胞は小さくて未熟の状態にとゞまる．このような雌の数は，短日化が進むに従って増加する．c）吸血後の栄養生殖分離が，産卵を経験した経産雌の濾胞にもおこる．以上の他に，９・10月の短日下でも生殖活性状態が持続されて産卵と吸血を繰返す，極く少数の雌群があるが，そのことはこの時期にも多経産雌が発見されることから明らかで，これらの雌はおそらく越冬に入る前に死滅するものと思われる．

4.　日長が約13時間30分からはげしく短縮していく９月中旬頃，雌は越冬に入り始めるものと思われる．越冬に入るものとしては，小型濾胞をもった未吸血未経産雌がその大多数を占めるが，吸血後栄養生殖分離をおこした未経産雌，および産卵・吸血後に栄養生殖分離をおこした経産雌も含まれる．