Studies on the Follicular Development and Overwintering of the House Mosquito, *Culex pipiens pallens* in Nagasaki Area*

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Abstract

The females of the house mosquito, *Culex pipiens pallens*, are gonoactive in summer and have common or middle sized follicles of N to Ib in stage, while in September when temperatures fall rapidly and photoperiod becomes shorter by high speed, there occur females with large follicles and in some cases those with I-II or IIa stage ones. In late September and mainly in October, under slightly more lower temperatures, there occur females with small follicles. The females with large follicles seem to continue feeding and egg-laying till the end of autumn or even the beginning of winter and seem to die off in winter months. The females with small follicles being gonoinactive enter overwintering places such as caves which become to have middle sized follicles in January and February. The females are now gonoactive and all of them move out from the shelter with the further progress in follicular development by the end of March.

Introduction

The experiments the results of which were reported here were projected to make clear the mode of overwintering of the house mosquito, *Culex pipiens pallens* Coquillett, in Nagasaki area. For the purpose, the follicular development was

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examined with the females collected by human-baited traps in summer and also with those reared as adult under various conditions of temperature and photoperiod modeled after the natural ones encountered in nature in summer.

Taking the data thus obtained in summer, 1967 as a criterion, comparisons and considerations on the follicular development were made with the data obtained in autumn through next spring. During the period collections were made of the egg-rafts laid by females in nature in jars with water mixed with rice straw infusion, of the females coming to bite animals and of the females resting in caves. Besides the above, the follicular development was also examined with the females which were reared as adults under the condition of temperature and photoperiod modeled after mid-September of 1967, and under various conditions of natural outdoor temperature and photoperiod in September and October in 1967 and 1968.

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Place, Material, and Method

The places where the experimental materials are collected were in Nagasaki City and surrounding farm villages. The material was the house mosquito, *Culex pipiens pallens*. Experimental studies were made in the laboratory and outdoor insectaria of the Department of Medical Zoology, Nagasaki University School of Medicine located in Nagasaki City. The outdoor insectarium was used for rearing and keeping larval and adult mospuitoes under natural air conditions. In the laboratory we have seven indoor insectaria and four biotrons. These were used for controlling temperature and illumination for testing the effect of them on the follicular development.

The indoor insectarium is an apparatus which is equipped to keep a constant temperature and to get alternate light and darkness.

The biotron is an apparatus which operates automatically the daily cycles of temperature and illumination modeled after the observed natural daily cycles. For that purpose, the temperature is controlled by a program cam which is shaped as to follow a daily cycle copied by 8 temperature points from the observed natural cycle. The illumination program is set as to lead the following diagram: (1) 30 minutes during which illumination rises gradually from darkness to a plateau. (2) A given period in hours during which a plateau illumination i.e. a maximum and continuous intensity of illumination is being kept. The duration in hours of the plateau illumination is determined according to the astronomical day length at Nagasaki of those days the effect of which is to be examined. The intensity of plateau illumination is determined voluntarily. (3) 30 minutes during which illuminntion drops gradually from the plateau to darkness.

The experiment shown in Fig. 1 was

made in an insectarium under a condition of a constant temperature at 25°C and alternate light of about 200 Lux for 15.2 hours and darkness. The condition was roughly modeled after the natural condition in early July, 1967.

The experiment shown in Fig. 2 was made using a biotron under the conditions of a mean temperature of 28.4°C with a daily cycle and a daily photoperiod of 14.7 hours with a plateau illumination of 2000 Lux for 13.7 hours a day. The conditions are modeled after the natural daily cycles observed on an average in early August in the past 5 years.

The experiment shown in Fig. 4 was made using a biotron under the condition of a mean temperature of 23.2°C with a daily cycle and a daily photoperiod of 13.4 hours with a plateau illumination of 2000 Lux for 12.4 hours a day. The conditions are modeled after the natural daily cycles observed on about middle part of September, 1967.

Larvae were reared with an equally mixed powder of Ebios (Brewer's yeast) and mouse pellet. Adults were kept with a 2% sugar solution in a cage of 30 × 30 × 30 cm in which they easily copulated.

Results of experiments

I. Follicular development in pallens female at 25°C and under summer conditions

(1) Description for the development of the first follicle.

The development of the first follicle before and after taking blood meal in females of *Culex pipiens pallens* was observed under the conditions of a constant temperature at 25°C and 12 hours photoperiod. In the following the outline of the result of observations will be described. The size of the follicle was measured by only its length which is shown in micrometer scale. A micrometer scale : 9.725μ.

Before taking blood meal

Stage	Description
No.1	The follicle of newly emerged females is not apparent and included within germarium which is 4.0–5.1 in length in micrometer scale (10×10) averaging 4.6 for ten females and fairly longer than the germarium (about 3.6) at the time of the separation of the first follicle.
No.2	Twelve hours after emergence the first follicle becomes first recognizable by the constriction marked on the pear-shaped germarium. The newly recognized follicle is 3.0–5.0 averaging about 3.8 in length. Twenty four hours after, cell division and formation of epitherium of follicle advance, the length of follicle being about 4.7 in length. Thirty six hours after, in about 20% females observed the follicle begins to reach N stage with 8 cells and nearly completed epitherium.

The length of the follicle increases only a little.

N–I By adout 48 hours after emergence, in about 50% females the first follicle reaches N stage with eight undifferentiated cells and completed epitherium; in about 10% females the follicle remains in N_{o-2} stage; while, in about 40% females the follicle reaches I stage in which the oocyte or egg cell can be seen in some females. The avearage lengths are in the first, second and third groups, 7.6, 5.2, and 8.0 respectively. In the last group the germarium is about 3.6.

I After 48 hours from emergence, females with I stage follicle increase in number with the lapse of time but the nucleus of the oocyte is not always clearly visible. Yolk granules are not entirely deposited. The length of the follicle is about 8.0 or a little larger in an average and not necessarily increase with the lapse of time. The states of the follicle remains unless females take blood meal and this stage can be said as a quiescent phase under 25°C and summer environmental conditions. It is convenient to divide the I stage into two i. e. Ia snd Ib.

Ia An oocyte is probably being differenciated from nurse cells but the nucleus of the oocyte is usually invisible.

Ib The nucleus of the oocyte is clearly visible.

I-II and IIa In autumn in some females, though they are unfed, the first follicle advance to stage I-II or IIa, the details of which will be stated in chapter II.

Feeding of newly emerged females

A few females begin to take blood meal even 24 hours after emergence but many of them do so from about 48 hours after emergence.

After taking blood meal

IIa About 9 hours after the meal in a very few females yolk granules are sparsely or fairly deposited around the nucleus of the egg cell. The follicle is about 9.2 in length.

IIb About 12 hours after the meal, the egg cell occupies $\frac{1}{5}$–$\frac{1}{3}$ of the follicle which gains 9.0–12.0 in length. Yolk granules are deposited fairly densely all over the egg cell and the nucleus becomes scarcely visible towards 18 hours after the meal.

IIc By the time between 18 and 24 hours after the meal, in some females the egg cell seems to reach IIc stage and to occupy $\frac{1}{2}$ of the follicle.

IIIa About 24 hours after the meal, in about a half of females obseved the egg cell remains to occupy obout $\frac{1}{2}$ of the follicle which is 16.0–17.0 in length, while in the others the cell does about $\frac{3}{4}$ of the follicle of being 19.0–21.0 in length.

IIIb Adout 36 hours after the meal, the egg cell takes up about $\frac{4}{5}$ of the follicle of being about 31.0 in length.

IV About 48 hours affer the meal, in about ten percent females observed the egg cell occupies nearly all part of the follicle pushing up the nurse cells upwards and takes very long oval shape measuring about 54.0 in length. However, in 45 percent females the egg cell remains in IIIb stage and in the remaining ones the egg cell does still in IIIa.

Va About 60 hours after the meal, in half of females observed the egg cell gains its maximum size of 66.0–71.0 in length, and occupies the whole of the follicle and begins to produce the micropilar apparatus.

Vb About 72 hours after the meal, micropilar apparatus and chorion are completed.

By about this time the second follicle reaches N–I stage separating from the germarium and is about 5.5–6.1 in length becoming a little larger (6.0–8.0) in several days.

Oviposition In a very few females eggs are oviposited 3 days after the meal, while in most females 4 or more days after the meal.

The results of above observations made at 25°C are summarized as follows. The first follicle of newly emerged females is not differentiated but included within germarium. The follicle begins to reach the first quiescent phase about 48 hours after emergence. About the time it gains 6–8 in length in micrometer scale and becomes N–I stage without having yolk granules. The second follicle begins to reach the second quiescent phase about 72 hours after the blood meal when the first follicle develops to mature egg. The second quiescent phase is nearly identical to the first one in size and stage.

(2) *Follicular development in females reared as adult from the first instar larvae under the condition of a constant temperature of 25°C and 15.2 hours photoperiod with 200 Lux (An alternate light and darkness).*

The condition of 25°C and 15.2 hrs was modeled after the natural air condition encountered in early July of 1967 in Nagasaki area. Females were reared as adults under the condition from the first instar larvae hatched out for 12 hours from the eggs originated from laboratory colony. The females were kept continuously under the same condition, out of

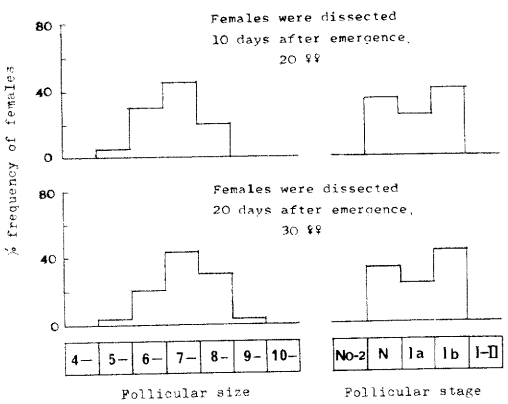

Fig. 1. The percentage frequency distribution of *pallens* females with follicles of different size and stage. The females were reared from the first instar larvae under the condition of a constant temperature at 25°C and a daily photoperiod of 15.2 hours with 200 Lux. (The follicular size of a female is given by the mean length of 5 random samples)

which 20 and 30 females were dissected 10 days and 20 days after emergence with the results shown in Fig. 1.

Fig. 1 shows that in both groups the females mostly had the follicles of from 6 to 8 in size excepting a few which had 5 or 9 sized ones. The follicles were all N to Ib in stage. The determination of N, Ia, and Ib stages was not necessarily very easy and even on the 20 days after emergence, in some females the follicle appeared to be still in N stage and in some others in Ia stage. Consequently, it seems that in *pallens* females the observed follicles being in N, Ia, and Ib stages should be regarded equally as being in the first quiescent phase.

(3) *Follicular development in females reared as adult from the first instar larvae using biotron under the conditions of a mean temperature of 28.4°C with a daily cycle and a daily photoperiod of 14.7 hours with a plateau illumination of 2000 Lux for 13.7 hours a day.*

The conditions are modeled after the natural daily cycles of temperature and illumination observed on an average in early August in the past 5 years. The females were kept continuously at the same conditions and 52 and 30 of them were dissected 7 days and 14 days after emergence respectively. The results are illustrated in Fig. 2.

Fig. 2 shows that the follicular sizes were respectively from 6 to 9 in the first group and from 7 to 9 in the second, mostly, however, being 7–8. The stages were from N to Ib in the former and Ia and Ib in the latter. The above seems to show that the follicles fairly advance

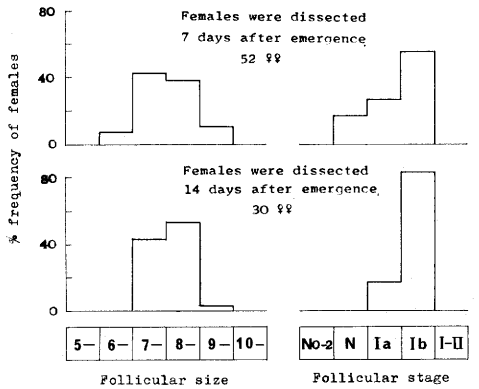

Fig. 2. The percentage frequency distribution of *pallens* females with follicles of different size and stage. The females were reared from the first instar larvae using biotron under the conditions of a mean temperature of 28.4°C with a daily cycle and a daily photoperiod of 14.7 hours with a plateau illumination of 2000 Lux for 13.7 hours. The conditions are modeled after the natural daily cycles observed in early August. (The follicular size of a female is given by the mean length of 5 random samples)

with the lapse of time in size and stage under high temperature and long day conditions such as those taken in this experiment. In only a few females the follicle becomes 9 in length but in none of them yolk granules are deposited in the egg cell.

(4) *Follicular development in pallens females collected by human-baited traps in summer, 1967.*

Female mosquitoes were collected for dissection by human-baited traps at several places near Nagasaki City in 1967. The object was to examine how is the states of follicular development in wild females in summer and to compare the states with those obtained in the laboratory reared females. The number of nulliparous and parous females collected and dissected are shown by place in

Table 1. Number of nulliparous and parous females collected by human-baited traps in summer of 1967

Date, 1967	Place	Host	No. ♀♀ dissected	
			Nulliparous	Parous
Jul. M	Ogino	Man	14	4
Jul. L	Uku	Man	10	8
Ang. F	Uku	Man	43	40
Ang. L	Hongoti	Man	5	1

F, M, and L show first, middle, and late part of a month.

Table 1, and the results of examinations for follicular development are illustrated in Fig. 3.

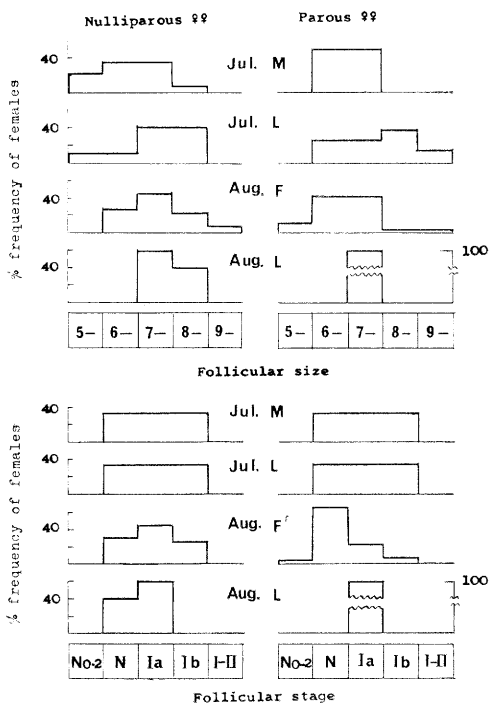

Fig. 3. The percentage frequency distribution of *pallens* females with follicles of different size and stage. The females were collected by human-baited traps in summer, 1967. (The follicular size of a female is given by the mean length of 10 random samples; as for F, M, and L, see Table 1)

Fig. 3 shows that the first and the current follicles of nulliparous and parous females are mostly 6 to 8 in length and N to Ib in stage. The follicles of 5 and 9 in length and No-2 in stage were rarely found and those larger than 9 and those with yolk granules were never found.

(5) *Summary on the follicular development of females in summer.*

The *pallens* females mostly had follicles of from 6 to 8 in size and of N to Ib in stage, irrespective as to whether they were reared as adults from larvae under experimental conditions modeled after the natural air conditions observed in early July and early August, or they were collected in nature when they came to bite man in July and August. The females with follicles smaller than 5 or larger than 9 were very few in number and none of them were found having follicles with yolk granules. The above facts show that the follicles of from 6 to 8 in size and N to Ib in stage are normal type in this mosquito in summer.

(6) *Discussion on the effect of short photoperiod under high temperature conditions.*

The experiment, from the result of

which the description on the follicular development was made, was carried out under the conditions of a constant temperature of 25°C and a daily photoperiod of 12 hours with 200 Lux. The temperature of 25°C usually encounter in July when the astronomical and physiological day lengths ars about 14 and 15 hours. The astronomical day length of 12 hours is usually observed in late March or in late September. Therefore, the experiment must be said to have carried out under the condition of a short photoperiod and a high temperature.

When compared the result obtained under the condition of 25°C and 12 hours with those under conditions of 25°C and 15.2 hours (Fig. 1), 28.4°C and 14.7 hours (Fig. 2), and in nature in early July and early August (Fig. 3), the state of follicular development in the first case can be said to coincide as a general rule well with those in the others. However, in the first case the variation in follicular size in unfed females was extended towards smaller side; and the variation in follicular development in fed females seemed to have been made larger. This seems that the temperature of 25°C is a critical temperature to produce summer type follicles and to render the day length to have little effect on the development of the follicle. At above 25°C, short photoperiod may have no effect on the development of the follicle, while at below 25°C, the shorter photoperiod must have greater effect on the follicular development.

II. Follicular development of the pallens females in autumn, winter, and early spring

Peculiar features in the follicular development are observed in autumn. The features are represented by great variations in follicular size and stage. There occur, besides normal ones, such follicles smaller than 5 in length, those larger than 9, and those in stages I-II or IIa, as scarcely seen in summer. To identify the uncommon sized follicles from common ones, hereinafter the follicles smaller than 5 are tentatively referred to as small ones; those of 6 to 8 as common or summer type ones; and those larger than 9 as large ones.

(1) *Follicular development of the females reared in a biotron under the temperature and illumition conditions modeled after those in mid-September, 1967.*

The first instar larvae originated from laboratory colony were reared in a biotron under the conditions of a mean temperature of 23.2°C with a daily cycle and a daily photoperiod of 13.4 hours with a plateau illumination of 2000 Lux for 12.4 hours a day. The conditions were modeled after the natural cycles of temperature and illumination observed in mid-September, 1967. The adult females thus reared were examined for the follicular size and stage with the results shown in Fig. 4.

Fig. 4 shows that there occurred a great variation in follicular size and also in stage in females when they were reared from younger instar larvae under the conditions modeled after those in mid-September. In summer, as stated

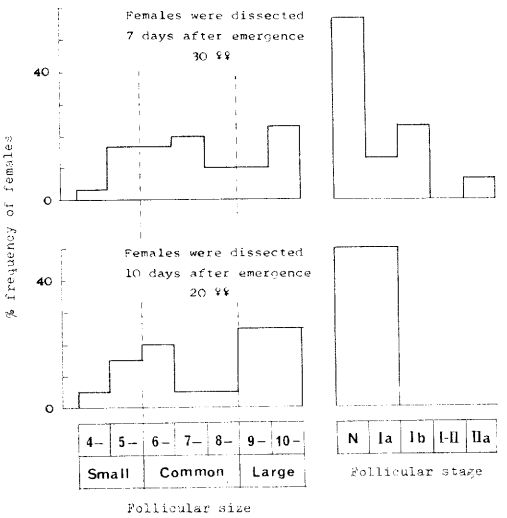

Fig. 4. The percentage frequency distribution of *pallens* females with follicles of different size and stage. The females were reared from the first instar larvae in a biotron under the condition of a mean temperature of 23.2°C with a daily cycle and a daily photoperiod of 13.4 hours with a plateau illumination of 2000 Lux for 12.4 hours. The conditions are modeled after the natural daily cycles of temperature and illumination observed at mid-September. (The follicular size of a female is given by the mean length of 5 random samples)

above, the first follicles being in quiescent phase were mostly from 6 to 8 in size and N to Ib in stage, and those smaller than 5 or larger than 9 were rarely found, and those of I-II or IIa in stage were never found, while in this case there are found a fair number of females with small or large follicles and, interesting to say, a few number of females with IIa stage follicles. This seems to be a peculiar manifestation of physiological change in females induced by the climatic conditions in early autumn.

(2) Follicular size and stage in pallens females collected by animal-baited traps in September through April in 1967 to 1968.

Females were collected when they came to bite animals in from mid-September through early April, 1967 to 1968 by using animal-baited traps at irregular intervals in Nagasaki area. As a bait, man, dog, man+dog, or hen was used. The percentage frequency distributions of the females with different sized follicles and of those with follicles of different stage are illustrated in Figs. 5 and 6, and the age distribution and parous rate of the females collected in each time were presented in Table 2.

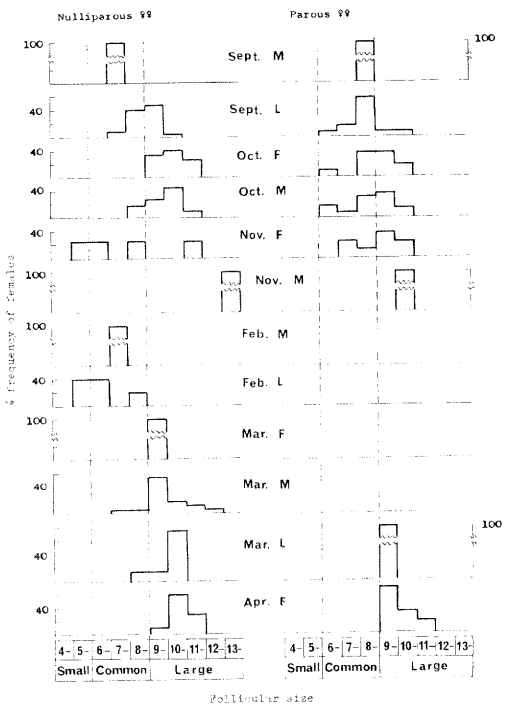

Fig. 5. The percentage frequency distribution of *pallens* females with follicles of different size in autumn-to-spring collections by using animal-baited traps.
(Remarks; The follicular size of a female is given as a mean length of 10 random samples; F, M, and L show the first, middle, and late part of a month)

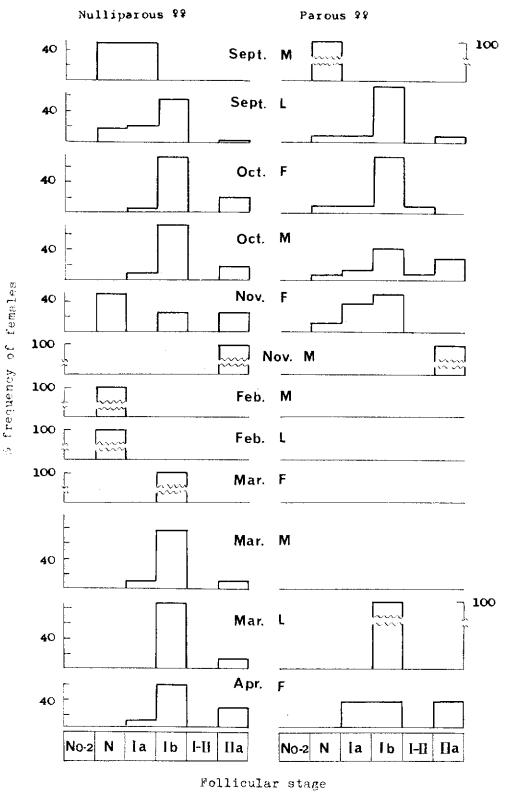

Fig. 6. The percentage frequency distribution of *pallens* females with follicles of different stage in autumn-to-spring collections by using animal-baitdd traps. (As for remarks, see Fig. 5)

Figs. 5 and 6 show that the females coming to bite host animals in autumn, exactly speaking, in from late September through mid-November were found usually having middle or large follicles of N-Ib or IIa stages, irrespective of their being nulliparous or parous. In other words, the females with larger follicles of rather developed stages seemed to be gonoactive and to continue the feeding activity till before the days of the activity being stopped by low temperatures.

Overwintered females came to bite animals on warm days in as early as mid-and late February (as for temperatures on these days, see Table 3). They had common or small follicles of stage N. On March, however, the follicles became larger and in some females they advanced to IIa stage.

(3) The follicular size and stage of pallens females collected in caves in late October through late March, 1967 to 1968 in Nagasaki area.

Observations and collections of *pallens* females were made in six caves in from late October through late March, 1967 to 1968 in Nagasaki area. Caves A, B, C, D, E and F were about 16.0, 21.8, 14.0, 15.8, 3.4, and 3.8m^2 in area respectively. The first four were large, deep and nearly or totally dark at the bottom, while the last two were small, shallow and faintly light. Cave A sheltered a great number of mosquitoes, and B did a fair number of them, and therefore in these caves the number of *pallens* females resting on the wall were counted at certain intervals and about ten percent of them were only collected to examine the age and the follicular development. In the other four caves, mosquitoes were rather small in number and about one-third of observed ones were only collected when necessary for examination. The number of the females observed in caves A and B, and the number and age compo sition of them collected for dissection in the six caves are tabulated in Table 2. The percentage frequency distribution of females with different sized follicles and of those with different stage ones are illustrated in Figs. 7 and 8.

In Nagasaki area, as stated by Shimogama and Takatsuki (1967), the number of females in caves suddenly increased

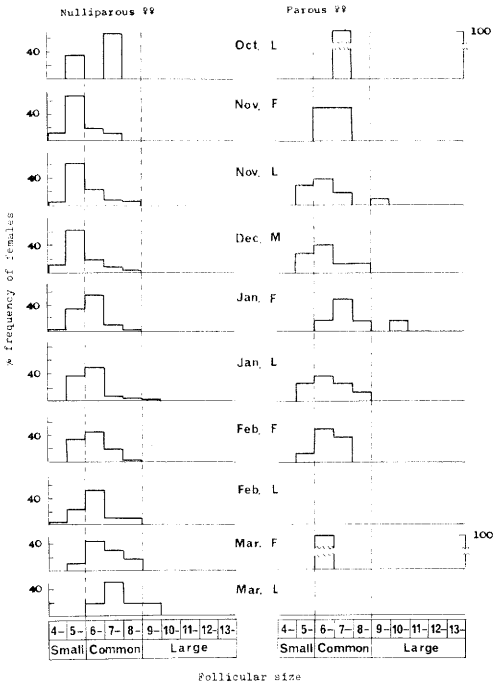

Fig. 7. The percentage frequency distribution of *pallens* females collected in caves with follicles of different size. (As for remarks, see Fig. 5)

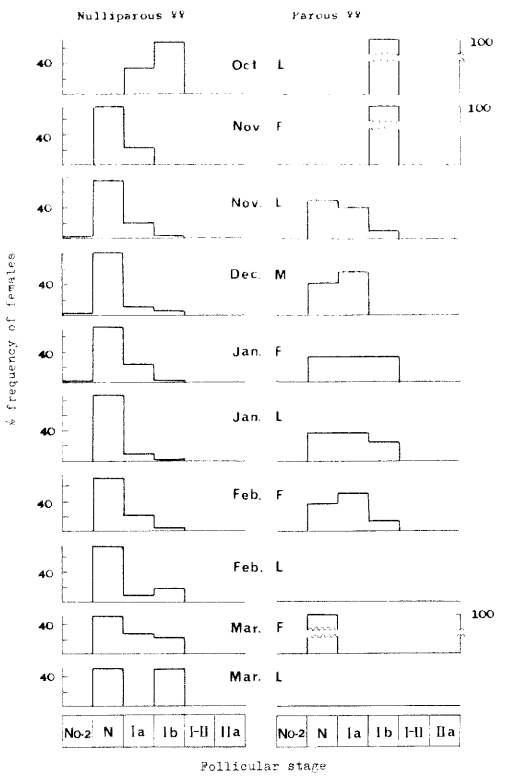

Fig. 8. The percentage frequency distribution of *pallens* females with different stage follicles. The examination was made with the same females shown in Fig. 7.

from November reaching maximum in December and January, while the number abruptly decreased in February or in some caves in March, as seen from Table 2. It is very much noteworthy fact that the overwintering nulliparous females, as seen from Figs. 7 and 8, had very small follicles mostly of N stage, although the parous females had a little larger and mostly common sized follicles of stages N to Ib. The fact seems to suggest that such females as those collected in autumn by animal-baited traps which had large and developed (IIa) follicles do not utilize the caves as an overwintering place but continue gonotrophic activity till their end of life.

From January the follicles of nulliparous females began to gain their size very little by little reaching middle size by early March. In parallel with the gaining size, the stage of follicles advanced though very much slightly, while the current follicles in the parous females collected in late January and early February were nearly the same in size and stage as before. It is again very much notable fact that in caves no such females were collected as those collected by animal-baited traps in and after mid-March which had large sized and developed follicles. The fact seems to suggest that such females go out from the caves with the progress in follicular development.

(4) Seasonal change in the number of egg-rafts laid in nature.

Egg-rafts laid in two jars with water mixed with rice straw infusion were collected every day and totaled by part of a month. The jars were placed outdoors near our laboratory. The results obtained in mid-September through early April were given in Table 2. The mean air temperature and astronomical day length in 1967 and 1968 are given in Table 3.

The egg-rafts decreased in number from after mid-November but continued to be laid to early December till shortly after the end of biting activity of females. Seven and one out of the females which came to lay eggs respectively in

Table 2. Gonoactivity of *pallens* females in cold season which is presumed from the number of egg-rafts laid in nature, and the number and parity of females collected by animal-baited traps and in caves in September through April, 1967 to 68 in Nagasaki area.

Date of collection		No. of egg-rafts laid in two jars	Females collected by animal-baited traps						No. ♀♀ observed in caves A and B, No. ♀♀ collected for dissection in six caves and parity of them						
			Bait	No. ♀♀ by age				Parous rate	No. ♀♀ in		No. ♀♀ dissected	No. ♀♀ by age			Parous rate
				0-p	1-p	2-p	Total		A	B		0-p	1-p	2-p	
Sep.	M	41	Man	2	1	0	3	33.3							
	L	94	Dog	38	12	0	50	24.0							
	F	90	M&D	15	11	0	26	42.3							
Oct.	M	81	Dog	11	13	1	25	56.0							
	L	102							—	20	5	3	2	0	40.0
	F	78	M&D	4	6	2	12	66.7	—	83	23	21	1	1	8.7
Nov.	M	23	Man	1	1	0	2	50.0							
	L	32							441	173	50	40	8	2	20.0
	F	2													
Dec.	M	0	Hen	0	0	0	0		332	167	78	71	6	1	9.0
	L	0													
	F	0							232	91	88	82	6	0	6.8
Jan.	M	0	Dog	0	0	0	0								
	L	0							201	71	115	107	8	0	7.0
	F	0							73	98	116	108	8	0	6.9
Feb.	M	0	Dog	2	0	0	2	0.0							
	L	0	Dog	5	0	0	5	0.0	47	81	71	71	0	0	0.0
	F	0	Dog	1	0	0	1	0.0	23	33	31	30	1	0	3.2
Mar.	M	0	M&D	17	0	0	17	0.0							
	L	0	Hen	8	1	0	9	11.1	2	1	6	6	0	0	0.0
Apr.	F	47	Man	10	6	0	16	37.5							

Remarks : (1) F, M, and L mean the first, middle, and late part of a month.
(2) M&D mean Man and Dog.

Table 3. Day length and mean air temperature during experimental period. Day length shows that on the 5th, 15th, and 25th of each month.

Parts of month	Day length in the common year	Mean air temp. °C 1967	1968	Parts of month	Day length in the common year	Mean air temp. °C 1967	1968
F	10 : 06	4.4	7.7	F	14 : 15	24.4	21.9
Jan. M	10 : 14	3.8	6.9	Jul. M	14 : 07	27.7	26.3
L	10 : 28	8.0	5.3	L	13 : 56	28.8	26.7
F	10 : 44	7.3	2.8	F	13 : 40	28.0	26.9
Feb. M	11 : 02	4.0	3.5	Aug. M	13 : 23	28.8	27.5
L	11 : 22	8.7	4.8	L	13 : 05	28.7	26.9
F	11 : 37	7.9	7.6	F	12 : 44	29.0	25.8
Mar. M	11 : 57	11.8	10.0	Sep. M	12 : 25	23.1	24.7
L	12 : 18	11.5	12.3	L	12 : 04	22.8	22.3
F	12 : 39	14.6	15.1	F	11 : 44	20.3	
Apr. M	12 : 59	14.5	14.7	Oct. M	11 : 25	19.3	
L	13 : 18	17.7	15.8	L	11 : 05	17.4	
F	13 : 35	18.3	17.7	F	10 : 46	16.5	
May. M	13 : 50	19.0	18.5	Nov. M	10 : 30	13.1	
L	14 : 03	22.2	18.9	L	10 : 17	13.2	
F	14 : 14	23.0	20.7	F	10 : 08	6.9	
Jun. M	14 : 18	23.4	21.2	Dec. M	10 : 01	7.5	
L	14 : 20	24.2	21.9	L	10 : 01	4.3	

As for F, M, and L: See remarks (1) in Table 2.

November and December were examined for the development of the current follicles. They had mostly large follicles (9.0 to 10.5 by female) of Ia to I-II stages. They were all fertilized but mostly had no fat dody. The fact seems to show that they were gonoactive and would not enter the cave, because in that season the females having such large and rather developed follicles were scarcely found in caves.

In the next spring egg-laying was first observed on and after the lst of April about half a month later than usual probably because of much lower temperatures in February, 1968 as seen in Table 3. The first egg-laying was about 50 days later than the first coming of a female to bite dog on the 13th of Feb ruary.

(5) *Considerations on the gonoactivity of pallens females in autumn and the fate of gonoactive females in autumn and winter.*

As seen from Table 2, in 1967, females were collected by animal-baited traps when they came to bite host animals till mid-November, and some females came to lay eggs till early December. The parous rates of females in the former group in middle and late autumn were very high, fifty or more in percentage, and the rates in females of the latter group in late autumn including two in

early December were found also very high i.e. among eight females dissected after oviposition, 3, 4, and one were uni-, bi-, and tri-parous respectively.

Overwintered females first came to bite dog in the warm afternoon in mid-February, but the parous rate was zero, and thereafter the rates were continuously zero till the end of March when the first uniparous female for the new year was collected. From about the same days, egg-laying was newly started.

On the other hand, parous rates of females collected in caves were rather lower even in late autumn. The rate markedly decreased and bi-parous females disappeared in winter through early spring, excepting one uni-parous female which was collected in early March. The uni-parous female was not likely to have laid eggs in early March but seemed to have overwintered as uni-parous one.

From the above findings, it is concluded that the gonoactive females seem to continue their feeding and oviposition till the end of life in late autumn or in winter, mostly without entering over-wintering places such as caves. On the contrary, nulliparous and gonoinactive females which emerge in late September through early November seem to enter mostly into overwintering places and afterwards begin to go out from the places from early February with the advance in follicular development. Parous females seem to survive scarcely the winter and those collected by animal-baited traps in late March and early April may probably be those which fed and laid eggs after emergence from hibernation.

(6) The states of feeding and follicular development in females reared as adults in mid-October, 1967 under the natural outdoor air conditions.

The experiments the result of which is shown in Table 4 were carried out under the expectation that the follicles of the females reared as adults under the natural conditions of mid-October may become larger in size and developed in stage. Contrary to the expectation, however, the first follicles of females in A.1, A.2, and A.4, and in B.1 and B.3 were mostly small and in 19% females in the former group and in 16% ones in the latter the follicles were middle in size, and almost all were N stage.

The feeding rate was examined with the female population consisting of a great number of females with small follicles and some number of those with middle ones. The results were that in group A, 2.6 percent and in B, 6.9 percent females were only engorged with blood of chickens in 11 days and 22 days respectively inspite of their being exposed to a chicken almost every day during the experiments. Whether the fed females were with small sized follicles or with middle ones before feeding was unknown but it is assumable that the latter might be the case and the females might be still gonoactive from the fact that the second follicles of most of them were middle in size.

From the above, it may be concluded that the females emerge as adults under the temperature condition of about 20°C are mostly gonoinactive and have small sized follicles.

Table 4. Follicular development in *pallens* females reared as adults from wild caught larvae in early and mid-October, 1967 and continuously kept under natural outdoor conditions in Nagasaki area.

Group	Capture date of larvae	Date of emergence as adults	Mean temp. (3)	Date of feeding	Date of egg-laying	Females dissected		
						Date	No.	Subgroup
A	Oct. 5	Oct. 9 (1)	20.0	–	–	Oct. 16	24	A. 1
				–	–	Oct. 24	25	A. 2
				Oct. 30 to Nov. 9 (4)	Nov. 10 to Nov. 27	Nov. 30	4	A. 3
				–	–	Dec. 22	23	A. 4
B	Oct. 11	Oct. 15 (2)	20.0	–	–	Oct. 23	10	B. 1
				Oct. 26 to Nov. 16 (5)	Nov. 5 to Nov. 25	Nov. 30	4	B. 2
				–	–	Dec. 25	115	B. 3

Subgroup	Number of ♀♀ with follicles of different size and stage										
	Follicular size						Follicular stage				
	4	5	6	7	8	9	No-2	N	Ia	Ib	I-II	IIa
A. 1	7	16	0	1				24				
A. 2	5	14	5	1				20	1	4		
A. 3 (6)			1	1	2			4				
A. 4	5	11	5	0	2		1	22				
B. 1		10						10				
B. 2 (6)		1	2	1				3	1			
B. 3	19	76	17	2	1			111	3	1		

Remarks : (1) and (2). The astronomical day length at the dates of emergence of adults were about 11:36 in (1) and 11:25 in (2).
(3). Mean temperature (°C) for 7 days before emergence.
(4). The four and other four out of 307 females only engorged with blood in 11 days during which they were exposed to a chicken almost every day for 18 hours from 4 p.m. to 10 a.m.
(5). The four and another one out of 72 females only engorged in 22 days during which they were exposed to a chicken by the same way as the above case.
(6). With each four engorged females, the second follicles were examined after oviposition for size and stage.

(7) *Follicular development in females reared as adults in late October, 1967 under natural outdoor air conditions.*

Experiments were projected to examine how is the follicular development of females when they were reared as adults from wild caught larvae under slightly

Table 5. Follicular development in *pallens* females reared as adults from wild caught larvae in late October, 1967 and continuously kept under natural outdoor conditions in Nagasaki area.

Date		Mean temp. °C	Day length hr:min.	No. ♀♀ dissected	Number of ♀♀ with follicles of different size and stage													
					Follicular size									Follicular stage				
					4	5	6	7	8	9	10	11	12	N	Ia	Ib	I-II	IIa
Oct.	L	17.4	11:05		Older instar larvae were collected on 23rd; adults emerged on 30th, Oct.													
	F	16.5	10:46															
Nov.	M	13.1	10:30															
	L	13.2	10:17	29	1	20	8							28	1			
	F	6.9	10:08	42		29	11	2						39	2	1		
Dec.	M	7.5	10:01															
	L	4.3	10:01	10		7	2	0	1					8	1	1		
	F	7.7	10:06															
Jan.	M	6.9	10:14	61		15	32	10	3	0	1			47	13	1		
	L	5.3	10:28															
	F	2.8	10:44	40		6	15	12	1	3	3			36	4			
Feb.	M	3.5	11:02															
	L	4.8	11:22	30		1	12	7	6	3	1			17	9	4		
	F	7.6	11:37	40			8	12	13	4	2	1		23	11	5	0	1
Mar.	M	10.0	11:57															
	L	12.3	12:18	38			1	4	9	14	9	1		2	2	33	1	
Apr.	F	15.1	12:39	40				1	9	17	9	4			3	31	0	1

As for F, M, and L: See remarks (1) Table 2.

lower temperatures than that under which the previous experiments were carried out, and to examine how is the development when they were kept under natural outdoor conditions from November through next spring. The results are given in Table 5.

A great number of older instar larvae were collected at natural breeding places on the 23rd of October from which some 1000 adults emerged on the 30th. About 400 females were kept at outdoor insectarium under natural air conditions. Out of them some were each dissected at irregular intervals during from late November through early April.

In November and December the follicles were small and N stage in many females, in January and February they gained size little by little, and finally in March and early April they became large sized ones in many females and moreover in a few of them they developed to I-II or IIa stage.

Here, considerations will be made on the cause and the meaning of the peculiar follicular development observed in *pallens* females in late winter through early spring. Air temperature, though it is the lowest in February, rises suddenly

and rapidly on and after mid-March. On the other hand, day length grows longer by high speed from mid-February through March as seen from Table 3. The sudden rises in temperature and the getting longer by high speed in day length on those days may be the cause of such a peculiar advance in follicular development as seen from the result of this experiment (Table 5). The reason may be also true of the occurrence of large follicles in most females and I-II or IIa stage ones in some females which come to bite animals in March and early April in nature. While, as already stated, in caves, no females with such advanced follicles can not be observed throughout the period from October when females suddenly begin to increase in number through March when all of them leave the caves. This shows that with advance in follicular development the females leave the overwintering places such as caves.

(8) *Follicular development in females reared as adults in September, 1968 under natural outdoor air conditions.*

To make clear the reason of peculiar advance in follicular development in autumn, experiments were carried out in

Table 6. Changes in the frequency distribution of *pallens* females with follicles of differnt size and stage in September, 1968. In groups A and B, females were reared as adults outdoors from wild caught mature larvae; in C, females were reared as adults outdoors from the first instar larvae originated from laboratory colony. The females were kept continuously under natural outdoor conditions till the days of their being dissected.

Group	Date of larval collection	Date of emergence	Temp. and (day length)	Dissection of females	No.	No. ♀♀ with the indicated sized follicle							No. ♀♀ with the indicated stage follicle				
				Date		5	6	7	8	9	10	11	N	Ia	Ib	I-II	IIa
A	Sep. 3	Sep. 5	25.1 (12 : 44)	Sep. 10	5	1	1	1	2				1	4			
				11	9	4	1	2	1	1			5	2	2		
				13	5			3	2					1	4		
				15	20	2	4	10	4				1	3	16		
				19	6	1	1	1	2	1			1	1	4		
				total	45	8	7	17	11	2			8	11	26		
B	Sep. 14	Sep. 18	24.6	Sep. 23	10		1	2	4	2	1		3	2	5		
		17	24.5	23	10			1	3	4	1	1	3	1	5	0	1
		19	24.8	26	10			1	2	6	1			1	9		
		16	24.3 (12 : 20)	27	8	1	1	1	2	2	1		1	2	5		
				total	38	1	2	5	11	14	4	1	7	6	24	0	1
C	Sep. 11	Sep. 28	22.3 (11 : 58)	Oct. 3	14	1	0	0	3	7	3		1	1	9	0	3
				5	9	2	1	0	2	3	1		2	1	5	0	1
				6	10	2	0	1	4	3			1	0	9		
				8	10	1	1	2	2	2	2		2	2	5	0	1
				Total	43	6	2	3	11	15	6		6	4	28	0	5

Remarks on Temp. and day length : Temp. shows a mean temperature (°C) for 7 days before emergence of adults; day length shows an astronomical day length (hr : min.) on the day of emergence.

September, 1968 with the results shown in Table 6.

Table 6 shows that the follicles in females emerged as adults in early September were mostly middle in size and N to Ib in stage. In females emerged in mid-September they were middle and large half-and-half and in a few female they were in IIa stage. In females emerged in late September they were middle and large nearly half-and-half too, while in some females they advanced to IIa stage. The reason may be due to the rapid decreases in temperature and getting shorter of day length by high speed during September.

(9) *Considerations on the states of follicular development in autumn.*

Main points of the results of observations and experiments made on the follicular development in autumn are arranged in Table 7 in the order of the days the females being collected or reared as adults. Females coming to bite animals by mid-September were found having middle sized follicles, while from late September through November the females with large sized follicles increased progressively in number and some were found with IIa stage ones. The peculiar advance in follicular development was demonstrated in the experiment carried out using biotron under the condition modeled after the natural outdoor condition observed in mid-September, 1967. The same phenomenon was also observed in experiments made under natural outdoor conditions in middle and late September, 1968. However, no such developed follicles were observed in

Table 7. The states of development of follicles in females on the days of their being collected or reared as adults in autumn.

First, middle, and late part of a month		Day length (hr:min.)	Mean air temp. (°C)		The states of development of follicles in females			
					came to bite animals in 1967 (cf. Figs. 3, 5 and 6)	reared as adults in biotron under conditions near to mid-Sept., 1967 (cf. Fig.4)	reared as adults from larvae under natural outdoor conditions	
			1967	1968			in 1967 (cf. Table 4)	in 1968 (cf. Table 6)
	F	13 : 40	28.0	26.9	M			
Aug.	M	13 : 23	28.8	27.5				
	L	13 : 05	28.7	26.9	M			
	F	12 : 44	29.0	25.8				M
Sep.	M	12 : 25	23.1	24.7	M	S, M, L, IIa		M, L, IIa
	L	12 : 04	22.8	22.3	M			M, L, IIa
	F	11 : 44	20.3		and		S	
Oct.	M	11 : 25	19.3		L		S	
	L	11 : 05	17.4		and		S	
	F	10 : 46	16.5		IIa			
Nov.	M	10 : 30	13.1					
	L	10 : 17	13.2					

Remarks : S, M, L, and IIa show the females with follicles of small, middle, large size, and IIa stage.

experiments made under the natural outdoor conditions in and after early October, 1967. The above data show that the peculiar advance in follicular development depends on the air condition to which the females are exposed from their larval stage. Such air conditions are represented by the rapid falls in temperature and the rapid getting shorter of day length and seem to take place restrictedly in September.

(*10*) *A consideration on the mode of overwintering of the pallens female.*

In September day length becomes shorter by very much high speed and temperature usually suddenly and rapidly falls in early, middle, or late September depending on the year. At the period when the great change of air conditions occurs, the perculiar advance is observed in follicular development represented by large sized follicles and IIa stage ones. The females with large follicles appear to be gonoactive till the end of their life and continue the feeding and egg-laying till the end of autumn or even the beginning of winter.

On the other hand, in September and mainly in October the breeding of this mosquito becomes fairly active and many females emerge as adults with small follicles. These females, as a rule, enter into overwintering places such as caves. The small follicles become middle in January and February. From mid-February the females begin to leave the caves with advance in follicular development and all of them go out from there by the end of March.

Summary

The first follicles in *pallens* females reared as adult under the conditions of temperatures at and above 25°C and various photoperiod, or in those collected in summer in nature by human-baited traps, were mostly 6 to 8 in length in micrometer scale and N to Ib in stage i.e. middle sized or summer type follicles. Under the rearing temperatures higher than 25°C, short photoperiod seemed to have little effect on the follicular development or on the gonoactivity of the female.

In September there occurred peculiar features in follicular development probably owing to the sudden and rapid falls in temperature and getting shorter of day length by very much high speed at the turning of the climate from summer to autumn. Under the great change in climate, there occurred females with large follicles larger than 9 in length and, in some cases, those with I-II or IIa stage ones. Under slightly more lower temperatures i.e. in late September, there occurred some females with small follicles smaller than 5 in length, and in and after October females all with the small ones.

The females with large follicles seemed to continue the feeding and egg-laying till the end of November or even early December judging from the result of collections of females by animal-baited traps and those of egg-rafts in nature on those days. These females seemed

probably to die off by mid-winter as inferred from the fact that parous females became to be scarcely collected everywhere by the end of winter.

The females with small follicles were gonoinactive and entered overwintering places such as caves. The small follicles gained size little by little reaching middle size in January and February. They further developed to large ones in some females in February and in many females in March. There occurred in March some females with follicles of I-II or IIa stage. The females, with gaining size in follicles, became gonoactive and came to feed on animals from mid-February and more actively in March and shortly thereafter began to lay eggs.

In caves, however, the females with large follicles were never found throughout the period in which the females utilized the cave as overwintering place. The fact showed that in and after February they moved out from the cave with the progress in follicular development. The reactivation of the females in February and especially in March seemed to be caused by the rapid getting longer in day length and rapid rise in temperature in from late February to March.

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アカイエカの濾胞の発育と越冬に関する研究

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摘　　要

25°C, 12時間照明の下で1令幼虫から飼育羽化させた♀群の羽化後並びに吸血後の第1濾胞の発育を経時的に調べて記載した．この際における未吸血♀群の濾胞の発育状態を基準として，各種条件下で飼育羽化させた♀群或るいは自然界で採集した♀群のそれとを比較検討して，本種蚊の越冬様式を知ろうとした．

夏季，吸血に来る♀群；25°C，15.2時間照明或るいは28.4°C，14.7時間照明の下で飼育羽化させた♀群の第1静止期における第1濾胞の大きさは6～8 micrometer scale（1：9,725μ）で，発育期はN～Ib期のものが普通である．上の基礎実験の飼育条件である25°C，12時間照明は，日長の点では短日処理をしたことになるが，濾胞の発育状態はよい一致を示したので温度が25°C以上の場合には短日処理の影響は殆んどないものと考え得る。

9月に入ると日長の短縮の度合が急に大きくなり，気温の急低下が起るが，この時期になると9以上の濾胞を持った多くの♀が出現し，発育期にもⅠ-Ⅱ又はⅡa期迄進むものもみられる．気温の低下，日長の短縮が更に進むと5以下の濾胞を持ったものが多くなる．そこで，6～8の濾胞を中型又は夏型，9以上を大型，5以下を小型，と区別して吟味することにした．

9月中，下旬には大型濾胞を持った♀群が多く出現するが，これらの♀はgonoactiveで秋遅くまで吸血と産卵を繰り返し多くは冬の始め迄に，或るいは冬季中に死滅するように思われる．

小型濾胞を持つ♀は9月中，下旬にも出て来るが，次第に多くなり，10月に羽化するものは殆んどが小型を持つようになる．これらの♀はgonoinactiveで殆んど吸血せず，防空壕などの越年場所へ集まる．冬季中小型濾胞は中型となるが，越年場所では，越年期間を通じて，大型を持った♀は殆んど全く発見されない．所が2月中旬から野外では既に吸血活動が始まり，気温の上昇と日長の伸長が急に速度を増す3月になると次第に吸血に来る♀の数が多くなるが，これらの♀は大型濾胞を持っており，殆んどすべて未経産♀である．これらのことから越年中の♀群は，濾胞が発育するにつれてgonoactiveとなり越年場所を去ること及び経産♀の越年は非常に少ないであろう事が窺われる．