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Ovariole and Age Changes in *Anopheles sinensis* Wiedemann, with Special Reference to the Relation to Temperature and Season

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Abstract

Ovariole and age changes were examined with the females of *Anopheles sinensis* reared or captured under different conditions of temperature and season during from May, 1964 to March, 1966. There were found two forms of quiescent phases in follicles of unfed females: The first occurred in nulliparous females and remained in stage I; the second did in parous ones and remained in stage II. Unfertilized females could not lay eggs. Fertilized females could oviposit when they took full blood meal only once. At 27°C or in summer in nature, it took about 2 days before the feeding of females. The first gonotrophic cycle took 4 days and the second and subsequent ones did each 3 days. The females usually took the next blood meal within 24 hours after oviposition. In late autumn, the current follicles of unfed parous females varied greatly in size and stage, similar conditions being observed in those just emerged from hibernation. The follicles of hibernated unfed nulliparous females were unexpectedly in stage II and some were degenerated.

Introduction

Since the establishment of the method of determining the age of mosquitoes from changes in the ovariole during the gonotrophic cycle by Russian workers (Detinova, 1962) and several other pioneers, it has been applied to many species of mosquitoes by many authors of different countries. The age grouping method is very useful for the understanding of the population dynamics of a mosquito species. It is also useful to determine the longevity of vectors of mosquito-borne diseases

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in relation to their natural infection with the parasites and to evaluate the effectiveness of anti-mosquito measures especially of residual insecticides.

As an experimental material for the age determination, the author took up a mosquito species, *Anopheles sinensis* Wiedemann, 1828. It is because that the age determination with the mosquito has never been tried in Japan, though it is important vector of *Setaria cervi* and *S. marshalli* of cattle and is experimentally possible vector of human filariasis and Japanese encephalitis and was important vector of malaria in the past in Japan.

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Material and Method

The material used in this experiment is an anopheline mosquito, *Anopheles sinensis* Wiedemann, 1828, which is widely distributed all over Japan and is one breeding mainly in paddy fields, creeks, ponds, etc.

Wild caught older age larvae and pupae were reared in a room at 27°C±1°C and 75−90% relative humidity or in an outdoor insectary under natural temperature conditions in different seasons. Adults were allowed to emerge from pupae in a cage of 30×30×30cm and were reared usually by 2% sugar solution. If necessary they were reared by 20% or 50% sugar solution. When about 60−70 individuals of each of adult females and males were cohabitated in the cage under the 12.5 to 14.5 hours photoperiod, fertility of about 30−40% were gained within several days. The feeding of females were made on the arm of the author inserting it into the cage or pushing it from the outside of the cage on the point where females in the cage were on rest.

The dissection and pulling out of tracheoles from ovaries were made under the stereomicroscope and the examination and measurement of follicles were made under the microscope. In this paper the length of follicles is given by micrometer scale the unit of which measures 9.804μ.

The fundamental examinations for the ovariole and age changes in the female mosquitoes were carried out with those reared from wild caught older larvae and pupae in the room at 27°C. The examinations for the changes in nature were conducted with the females collected in nature or reared from wild caught larvae and pupae under the then natural air conditions, in summer, autumn, or on February and March.

Results of Experiments

I. Phisiological and calendar ages in the female of *Anopheles sinensis* reared in the laboratory at 27°C

To obtain the basic knowledge on the

ovariole and age changes of *Anopheles sinensis*, experiments were carried out in a rearing room of constant temperature at 27°C and 75—90% relative humidity. The states of the development of the first follicles of nulliparous (or O-parous) unfed females, those of the 1st and 2nd follicles of O-parous fed ones, and of 2nd and 3rd follicles of 1-parous ones were examined with the lapse of time after emergence or feeding, and the results are given in Tables 2, 3, and 4. The results of blood taking pattern of nulliparous and parous females in relation to the elapsed time after emergence or oviposition are shown in Table 5. The result of examinations for changes in the shape of the posterior part of follicular tubes of oviposited females is shown in Table 6. The relation between feeding and oviposition and that between fertilization and oviposition in the female were

Table 1. Development of the first follicle before and after taking blood in *Anopheles sinensis*

Stage	Description
Before taking blood	
No	The follicle of newly emerged females has eight undifferentiated cells and measures about 4.0 in micrometer scale or 40 μ. Large epitherial cells nearly surround the follicle excepting in a portion joining to the germarium.
N	About 6 hours after emergence, in about 20% follicles of a female, the follicular epitherium appears to complete. It is completed in all follicles in 12 hours after emergence. Some follicles now separate from germarium and go into next stage.
I	By about 18 hours after emergence, the egg cell appears to differentiate from 7 nurse cells, and most of follicles seem to enter stage I. The follicle gains about 7.7 or 75μ, by 36 to 48 hours after emergence. This stage is the initial quiescent phase and lasts untill the female takes blood meal.
Feeding of newly emerged females	A few females begin to take blood meal 12 hours after emergence but many of them do so about 2 days after emergence.
After taking blood	
IIa	About 6 hours after the meal, in about 13% follicles of a female, yolk granules begin to appear very sparsely all over the egg cell, though slightly more densely around the nucleus.
IIb	About 11 hours after the meal, the egg cell takes up about 1/3 of the follicle. Yolk granules are deposited more densely all over the cell.
IIc	About 14 to 20 hours after the meal, the egg cell takes up 1/3 to 1/2 of the follicle. Yolk granules are so densely deposited that the nucleus becomes invisible.
IIIa	About 24 hours after the meal, the egg cell filled with yolk granules becomes to occupy 1/2 to 3/5 of the follicle.
IIIb	About 36 hours after the meal, the egg cell occupies 3/5 to 4/5 of the follicle.
IV	About 48 hours after the meal, the egg cell occupies 4/5 or more of the follicle taking very long oval shape. Nurse cells are pushed up above the egg cell.
Va	About 60 hours after the meal most follicles seem to gain their maximum length and to produce the float.
Vb	About 72 hours after the meal, most float is completed and the chorion covers the whole egg. In about 80 to 90% of females, the eggs are ovulated and 46.2 to 49.4 or 450 to 480μ in length.
Oviposition	Ninety-six hours after the meal, in most females eggs are oviposited.

examined and the results are placed on Tables 7 and 8. From Tables 2, 3, 4, and 5, a comparative table of calendar and physiological ages of the female at 27°C was made in Table 9.

Before going further, the states of the development of the first follicle of the nulliparous female will be summarized on the basis of the results of laboratory experiments carried out at 27°C (Table 1).

In Table 2, the states of the development of first follicles in female reared at 27°C only with sugar solutions are presented. When newly emerged females are reared on 2% sugar solution, the first follicles seem to enter the stage I by about 18 hours after emergence and to reach the initial quiescent phase by about 48 hours. Here, the follicles in the initial quiescent phase are usually observed in unfed nulliparous females becoming very active in feeding on man in the laboratary (cf. Table 5.1) or observed in those coming to bite cattle in nature (cf. Table 11). When females are reared on 20% or 50% sugar solution, the follicles increase in length nearly the same way as in 2% solution by 12 hours, while, thereafter, rapid growth is observed in the cases of the former two solutions. The figures in Table 2 show that the strong sugar solutions appear to advance the development by about 12 to 24 hours and the first follicles reach the quiescent phase about 36 hours or less after the feeding. After 48 hours, the first follicles of the females reared continuously on weak sugar solution still grow by only a little, while that of the females reared on strong solutions continue to grow and more rapidly in the case of the strongest solution. Here, it is to be noted that whatever large the first follicles may grow they remain in stage I.

Table 3 shows the states of development of the first and second follicles of fed nulliparous females. The first follic-

Table 2. Development of follicles in females reared at 27°C only with sugar solution from after the emergence

% of sugar solution	Length of follicle	Elapsed time (hrs) after emergence							
		0	6	12	24	36	48	72	96
2 %	Mean[1)]	4.04	4.63	5.49	6.18	6.91	7.70	8.39	8.19
	Range	3.5−4.8	4.0−5.8	3.8−7.6	5.1−7.2	6.2−8.6	6.2−9.3	6.9−10.4	7.2−8.9
20%	Mean[2)]	−	4.82	5.67	6.92	7.93	8.94	10.70	11.30
	Range	−	3.8−6.0	4.0−7.8	5.2−8.4	6.5−9.1	7.8−11.3	8.8−12.8	9.2−13.2
50%	Mean[3)]	−	4.78	5.51	7.05	7.86	8.80	11.50	12.00
	Range	−	4.1−6.1	3.8−7.6	5.2−8.5	6.3−8.8	7.8−11.5	9.2−13.3	10.3−13.8
Stage of follicle		No	No-N	N	I	I	I	I	I

Remarks: Females were emerged within one hour at 27°C from wild caught pupae and older larvae.
Length: 1 micrometer scale = 9.804μ.
Mean length was obtained with each ten follicles of 30, 15, and 5 females respectively in 1), 2), and 3).

les grow very slowly during first 12 hours, quickly during 12 to 36 hours, and more quickly during 36 to 48 hours. On 48 hours after the feeding some follicles are found approaching to the maximum length. On 72 hours they reach stage Vb or perfect eggs and are ovulated in most females though oviposited in a very few females. On 96 hours, eggs are laid in most females, while in a few females they still remain in the calyx.

The second follicles appear on 24 hours after the feeding at the first time as follicles of the stage No-N which nearly equivalent to that of the first follicles of the females 6 hours after emergence. The second follicles grow very quickly and reach the stage IIa-IIb or the second quiescent phase by the day of oviposition of the first set of eggs. The advance of the second follicles to stage II during the first gonotrophic cycle results in to shorten by one day the duration of the second cycle as seen from Table 4.

It was clearly and repeatedly proved that a full blood meal is enough to mature the eggs of this mosquito species.

Table 4 shows that the second batch of eggs is ovipostied 3 days after the 2nd blood meal and that the 3rd follicles appear on 24 hours after the meal growing very quickly and reaching stage IIa-IIb by the day of the 2nd oviposition. Before going to compare the physiological and calendar ages, it is needed to learn the feeding pattern of nulliparous and parous females (Table 5); the shrinking pattern of the posterior part of follicular tubes following the oviposition (Table 6); and the relation of oviposition to fertili-

Table 3. Development of follicles in the first gonotrophic cycle at 27°C

No. of follicle	States of development		Elapsed time after taking blood meal (No. of females observed)									
			6 (30)	12 (30)	24 (30)	36 (30)	48 (30)	72 (20)	96 (20)	120 (15)	144 (15)	192 (10)
1st follicle	Length of follicle	Mean	8.15	9.52	17.36	23.30	43.78	49.42	46.17			
		Range	6.0–11.0	7.5–12.0	14.0–20.5	18.5–28.0	37.7–49.7	42.8–56.6	42.8–51.4			
	Amount of yolk		0–1/3	1/3–1/2	1/2–3/5	3/5–4/5	4/5<	Full	–			
	Stage of follicle		I–IIa	IIb–IIc	IIc–IIIa	IIIa–IIIb	IV–Va	Vb	Oviposit			
2nd follicle	Length of follicle	Mean			4.50	5.17	6.11	8.23	9.28	9.38	9.22	9.73
		Range			4.0–5.0	4.5–6.0	4.5–7.2	7.0–11.5	7.8–10.3	8.5–11.3	8.0–11.2	9.0–12.0
	Amount of yolk				0	0	0	0–1/3	1/3	1/3	1/3	1/3
	Stage of follicle				N_0–N	N	I	I–IIa	IIa–IIb	IIa–IIb	IIb	IIb

Remarks: Both sexes emerged within one hour at 27°C from wild caught pupae and older larvae were reared on 2% sugar solution for 24 hours and starved for next 24 hours and then females were allowed to feed for one hour on a man.
Length: 1 micrometer scale = 9.804 μ.
Mean length was obtained with each ten follicles of the indicated number of females in each observed time.

zation (Tables 7, 8,).

As seen from Table 5, nulliparous females begin to take blood meal 12 hours after emergence. The feeding activity becomes active and reaches highest on about 48 hours after emergence. On the other hand, parous females feed on man even just after the oviposition and thereafter a considerable number of females feed whenever given a chance, becoming, however, rather active about 16-18 hours after the oviposition. It is thought from

Table 4. Development of follicles in the second gonotrophic cycle at 27°C.

No. of follicle	States of development	Elapsed time after taking blood meal						
		0	6	12	24	36	48	72
2nd follicle	Length of follicle: Mean	9.28	9.38	9.53	16.89	26.36	46.33	49.12
	Length of follicle: Range	8.0−11.2	8.0−11.5	8.0−12.3	13.7−20.2	23.0−29.5	42.8−51.4	42.8−54.8
	Amount of yolk	⅓	⅓−½	½−⅗	⅗−⅘	⅘<	Full	−
	Stage of follicle	Ⅱa−Ⅱb	Ⅱb−Ⅱc	Ⅱc−Ⅲa	Ⅲa−Ⅲb	Ⅳ	Ⅴ	Oviposit
3rd follicle	Length of follicle: Mean				5.15	6.08	7.75	9.69
	Length of follicle: Range				4.2−5.8	5.3−7.0	7.0−8.8	8.0−11.3
	Amount of yolk				0	0	0−⅓	⅓
	Stage of follicle				N	Ⅰ	Ⅰ−Ⅱa	Ⅱa−Ⅱb

Remarks: Females prepared by the same way as those shown in the footnote in Table 2 and allowed to deposit eggs within 2 hours, were allowed to feed for one hour on a man 24 hours after oviposition.

Rearing conditions and the unit of length of follicles are the same as shown in Table 2. Mean length was obtained with each 10 follicles of 10 females in each observed time.

Table 5. Blood taking pattern of females after emergence or oviposition at 27°C

1. The nulliparous female

	Elapsed time (hrs) after emergence						
	0	6	12	24	36	48	72
No. observed	182	180	200	169	198	400	153
No. fed	0	0	52	54	135	347	107
% feeding	0.0	0.0	26.0	32.0	68.2	86.8	70.0

2. The uniparous female

	Elapsed time (hrs) after oviposition												
	0	2	4	6	8	10	12	14	16	18	20	—	24	26
No. observed	28	48	36	36	59	38	28	39	57	50	28		28	20
No. fed	1	5	6	3	8	4	4	7	19	11	3		2	2
% feeding	3.6	10.4	16.7	8.3	13.6	10.5	14.3	17.9	33.3	22.0	10.7		7.1	10.0

Remarks: Females reared as adult within 30 minutes from wild caught pupae and larvae, and those oviposited within 2 hours were allowed to feed on man. They were starved adequately before given a chance to feed.

Table 6. Changes in the shape of the posterior part of follicular tubes with the passage of time after the first oviposition of females at 27°C, showing percentage of the tubes being sac-like, shrinking, with dilatation, and with degenerated follicle.

% of follicular tubes	Elapsed time (hrs) after oviposition							
	0	6	12	24	36	48	72	96
sac-like	95.4	86.8	83.0	65.8	69.5	55.0	56.5	59.5
shrinking	2.6	10.6	13.6	22.2	20.3	16.1	16.5	14.5
with dilatation	0.0	0.5	1.7	9.5	7.9	26.3	23.7	23.0
with degenerated follicle	1.9	2.0	1.7	2.5	2.3	2.5	3.3	2.9

Remarks: At each time 10 females were examined for the number and the shape of follicular tubes. The number of ovarioles in two ovaries of a female varied from 99 to 254 coming to 189.5 in an average for 80 females.

the feeding activity of parous females that, in nature, females which laid eggs during the whole night, though they might do mostly after dusk, may come to feed on the following night.

Table 6 shows the states of shrinking of the posterior part of follicular tubes and formation of dilatation after oviposition. The shrinkage in this mosquito species is extraordinary slow and even on the 4th day after oviposition about 60% of the ovarioles observed in ten females are sac-like, 15% are shrinking, and only 23% are with dilatation. It is practically noteworthy that when females come to feed in nature in 12—24 hours after oviposition, most of ovarioles will be found to be sac-like or shrinking.

In Table 7, percentage feeding of nulliparous and parous females and the percentage of females laid eggs are given. The percentage feeding of nulliparous females appears a little lower than that of parous ones, probably owing to the coexistence of unfertilized ones in the former which may be lower in feeding activity than fertilized ones. The percentage of females laid eggs also appears lower in nulliparous ones probably also owing to the coexistence of unfertilized females which, if engorged, can not lay eggs as will be understood from Taple 8.

Table 7. Percentage feeding and oviposition of females in successive gonotrophic cycle

Gonotrophic cycle	No. females observed	No. females engorged (% to the observed)	No. females oviposited (% to the engorged)
1 st	691	458(66.3)	155(33.8)
2 nd	155	130(83.9)	50(38.5)
3 rd	50	31(60.0)	16(51.6)
4 th	16	14(87.5)	0

Remarks: Females of ten batches (691 in total), reared as adult within one hour from wild caught pupae and older larvae were cohabitated with males of nearly the same numbers in cages of 30×30×30cm at 27°C. They were reared for 24 hours on 2% sugar solution and starved for next 24 hours and then females were separated and allowed to feed on man for one hour from 6 : 00 to 8 : 00 PM

Out of 458 engorged nulliparous females shown in Table 8, 198 ones died before oviposition; 115 laid eggs; remaining 105 not laid eggs. All these females were examined for fertilization when they died. Similar examinations were carried out with the engorged females in each of successive gonotrophic cycles. As a result, it was clearly demonstrated that irrespective of having been fertilized or not the females take blood meal, although unfertilized ones appear a little lower in percentage feeding, and the follicles in them develop quite in the same way, but that unfertilized ones can not absolutely deposit their eggs.

The results shown in Table 8 and of other experiments showed that some 30–40% of females were fertilized when each 60–70 mosquitoes of both sexes were reared in a cage of 30×30×30cm, for about 2 days at 27°C under the 12.5 to 14.5 hours photoperiod.

From the data on the physiological age determined at 27°C and the results of observations on the feeding activity and etc., the relation between the physiological and calendar ages are illustrated in Table 9.

Table 9 shows that newly emerged nulliparous females take blood meal most actively on the 3rd day of the calendar age (C-age), when the 1st follicles are in stage I or the first quiescent phase.

Table 8. Relation between fertilization and oviposition in engorged females shown in Table 7.

Gonotrophic cycle	No. females engorged on man	No. females died before oviposition			No. females oviposited			No. females not oviposited and died		
		Sperm (−)	Sperm (+)	Total	Sperm (−)	Sperm (+)*	Total	Sperm (−)	Sperm (+)	Total
1st	458	154	44	198	0	155	155	102	3	105
2nd	130	0	74	74	0	50	50	0	6	6
3rd	31	0	15	15	0	16	16	0	0	0
4th	14	0	14	14	0	0	0	0	0	0

* The existence of sperm was ascertained after the death of the females.

Table 9. A model of calendar and physiological ages of the female at 27°C.

Physiological age or succession in gonotrophic cycle and developmental stage of follicles	Calendar age: Emergence	2nd day	3rd day	4th day	5th day	6th day	7th day	8th day	9th day	10th day	11th day	12th day	13ty dah	14th day	15th day
1st follicle			Feed.	2nd day	3rd day	4th day	5th day								
			I	II c—III a	IV—V a	V b	Ovip.								
2nd follicle								Feed.	2nd day	3rd day	4th day				
				N	I	I—II a	II a—II b	II a—II b	III a—III b	V	Ovip.				
3rd follicle												Feed.	2nd day	3rd day	4th day
									N	I—II a	II a—II b	II a—II b	III a—III b	V	Ovip.
4th follicle															
													N	I—II a	II a—II b

Remarks: This table is based on the results shown in Tables 1,2,3,4, and 5.
Feed: Feeding. Ovip.: Oviposition.
Females which oviposited within 2 hours during 5:00–8:30 PM were allowed to feed on man for an hour, 24 hours after oviposition.

The first follicles grow up after feeding to mature eggs in 4 days and are (now eggs) deposited on the 5th day of the first gonotrophic cycle or the 7th day of C-age, when the 2nd follicles reach IIa-IIb or the second quiescent phase. Following the first oviposition, the nearest peak of feeding activity comes on the next day. The second follicles grow up to mature eggs in 3 days and are deposited on the 4th day of the second gonotrophic cycle or the 11th day of C-age. By this time the 3rd follicles reach IIa-IIb. The same developmental process as in the second gonotrophic cycle is repeated in the subsequent gonotrophic ones. Here, it is to be noted that, in this mosquito species, the 1st gonotrophic cycle requires 4 days, while, the 2nd and subsequent ones do only 3 days owing to the advance in development of the next follicles to stage IIa-IIb by the day of the current follicles (or eggs) being deposited.

II Age distribution and ovariole changes in wild caught females in summer

Catches of mosquitoes in cow-sheds were carried out in Matsushima village, Nagasaki Prefecture in 1964 before and after the residual spray (Table 10). The purpose of the experiment was to examine the effect of the residual spray on the population density and age distribution of cow-shed frequenting mosquitoes, namely, *Anopheles sinensis* and *Culex tritaeniorhynchus*. The village was consisted of three sub-villages, Setobatake (30 houses), Hyugashi (55 houses), and Ota (22 houses). All houses of each sub-village were sprayed by residual imagicides, 0.5% Diazinon Emulsion, 0.5% Nankor E., and 0.5% Baytex E. respectively on July 27 and 28 of the year. Although we are thinking to report the whole results of the above experiment on the other occasion, here general outline of the results will be summarized. The residual imagicides were found to have only a little and temporal effect

Table 10. Percentage age distribution of females captured in cow-sheds at Matsushima during from July to September, 1964.

Date of capture	Females examined	Percentage of females of different parity									
		0-parous	1-parous	2-parous	3-parous	4-parous	5-parous	6-parous	7-parous	?(*) parous	1-7 parous
Jul. 5-7	212	50.9	25.0						0.5	23.6	49.1
Jul. 16-17	55	25.5	65.5	3.6		1.8				3.6	74.5
Jul. 25-28	84	54.8	32.1	6.0	2.4					4.8	45.2
Jul. 27, 28. Residual spray: 0.5% Diazinon E, 0.5% Nankor E, 0.5% Baytex E.											
Aug. 6-7	31	19.4	32.3	25.8	6.5	6.5	3.2	3.2		3.2	80.6
Aug. 18-19	71	18.3	42.3	16.9	16.9	1.4	1.4		2.8	0.0	81.7
Aug. 28-29	64	17.2	25.0	29.7	9.4	9.4	6.3			3.1	82.8
Sept. 9-11	216	20.4	41.7	16.2	4.2	0.9	0.9			15.7	79.6
Sept. 26-28	34	5.9	29.4	44.1	5.9	2.9	8.8			2.9	94.1
Total	767	31.8	35.5	12.5	4.3	1.7	1.4	0.1	0.4	12.3	68.2

* The age of the females could not be determined because they had all sac-like follicular tubes.

just after the spray to reduce the population density but little effect to reduce the density of multiparous females of the both species.

Of the results of the experiment, only the percentage age distribution of *Anopheles sinensis* collected in the three sub-villages is tabulated in Table 10. The number of females examined for age is near to that collected in cow-sheds on each date but not reflects the seasonal abundance of the mosquito, because the number of cow-sheds examined was varied with the date of collection. In fact, the females began to increase in number from mid-June and made a peak on mid- and late July. They decreased in number on August but at early September they made a small second peak and decreased rapidly thereafter.

In the active breeding season or in July the percentages of nulliparous females were very high excepting in mid-July when the percentage decreased because of decrease in breeding number owing to drying up of some rice fields by drought. During August the suppression of breeding number owing to the high temperature reduced the percentage of newly emerged nulliparous females. The percentage increased a little during the second peak on early September. After this, the percentage rapidly decreased by rapid decrease in breeding. In contrast with the above, the percentage of parous females increased when the breeding number decreased.

It is of great interest that there were found the females of up to 7 in parity in this mosquito in nature, in contrast to those of only 3 in parity in laboratory experiment (Table 7) and also those of only 3 in the cases of *Culex pipiens pallens* and *C. tritaeniorhynchus* in nature. The longevity in days or calendar age of *Anopheles sinensis* in nature will be roughly given by the following equation (cf. Table 9):

Longevity = The $[3+4n+\alpha]$th day.

Where, n = parity; α = days from the last oviposition to the day of being captured.

For example, when n = 7, the longevity = The $[31+\alpha]$th day.

In Table 11, the size and stage of first follicles of unfed nulliparous females which were captured when they came to bite cattle in summer in nature are presented. The figures in the table are comparable with those given in Table 2.

Table 11. Size and stage of the first follicles in unfed nulliparous females which came to bite cattle in cow-sheds during from July to August, 1965 (Five follicles were randomly measured with each female).

Mosq. No.	Fertility	Length of follicles		Developmental stage
		Mean	Range	
1	+	9.80	9.0−10.5	I
2	+	7.00	7.0− 7.0	I
3	+	12.04	11.0−13.2	I
4	+	8.46	8.0− 9.0	I
5	+	7.96	7.3− 8.5	I
6	+	9.10	8.5−10.0	I
7	+	9.50	9.0−10.0	I
Mean	+	9.12	7.0−13.2	I

The mean length of the first follicles of the females in summer appears a little larger than that of those reared by 2% sugar solution at 27°C. However, when except the female No. 3 which had very large follicles, the mean for the remainder becomes 8.64 with a range 7.0–10.5 and approaches the mean of those reared at 27°C by 2% sugar solution for 72 hours or by 20% solution for 48 hours. The follicles of the female No. 3 is near to those of the females reared at 27°C by 50% solution for 96 hours. The above suggests that the females in summer in nature may be spending 2-3 or more days taking usually dilute carbohydrate food before they come to bite cattle, such as nectar or fruit juice but occasionally something more nutritious other than blood. However, it is to be noted that whatever large the follicles may be, they remain in stage I without producing any trace of yolk.

The size and stage of the second or the third follicles of unfed 1-parous or 2-parous females collected in nature in summer are 8.83 in the mean length ranging 8.0 to 11.0 and in stage IIa-IIb as seen in Table 12 and are nearly the same as those in females reared at 27°C (cf. Tables 3 and 4).

Table 12. Size and stage of the current follicles of unfed parous females, No. 1 to 8, shown in Table 13 (Five follicles were measured randomly with each female).

Mosq. No.	Fertility	Parity	Length of follicles		Developmental stage
			Mean	Range	
1	+	1	8.92	8.0– 9.3	II a– II b
2	+	2	9.10	9.0– 9.5	II b
3	+	2	8.90	8.0– 9.5	II a– II b
4	+	1	8.50	8.0– 9.0	II a– II b
5	+	1	8.76	8.2– 9.0	II a– II b
6	+	1	9.50	9.0–11.0	II b
7	+	1	8.80	8.0– 9.0	II a– II b
8	+	1	8.16	8.0– 8.8	II a– II b
Mean	+	1–2	8.83	8.0–11.0	II a– II b

In Table 13, the result of examinations was tabulated which were made for the number of parity; the number of follicular tubes in an ovary; the percentage of tubes, with degenerated follicles, being sac-like, shrinking, and with dilatation. The examinations were made with ten unfed parous females captured when they came to bite cattle. The percentage occurrences of follicular tubes being in the four different forms are subjected to

Table 13. Changes in the shape of the posterior part of follicular tubes in unfed parous females which just came to bite cattle (Examinations were made with ten females among those collected during from July to August, 1964).

Mosq. No.	No. of parity	No. tubes in an ovary	Percentage of tubes			
			with degenerated follicle	sac-like	shrinking	with dilatation
1	1	136	9.6	80.9	8.8	0.7
2	2	125	7.2	68.8	16.8	7.2
3	2	107	1.9	86.0	7.5	4.6
4	1	120	4.2	68.3	14.2	13.3
5	1	97	3.1	72.2	14.4	10.3
6	1	108	0.0	97.2	2.8	0.0
7	1	126	3.2	87.3	8.7	0.8
8	1	112	1.8	91.9	4.5	1.8
9	2	104	12.9	54.8	17.3	25.0
10	2	131	0.8	82.4	5.3	11.5
Mean		116.6	3.6	79.2	9.9	7.3

a great variation with individual mosquito and the elapsed time after oviposition. In the female No. 6, nearly all tubes were sac-like showing that she came to feed on cattle just after oviposition. On the contrary, No. 9 seemed to come to feed more than 48 hours after oviposition (cf. Table 6). In an average, however, the females seem to come to feed usually within 24 hours after the oviposition.

III Results of examinations in late autumn.

In late autumn, collections of the mosquito were also made in cow-sheds during from 9 to 12th November. The females collected were found on dissection to be all parous ones as shown in Table 14. Probably by mere chance, we could not collect any nulliparous female. It is of great interest that the current follicles of the unfed (and having just fed) parous females were subjected to a great variation in size and stage, being divided broadly into two forms. The large follicles was 11.58 in an average ranging from 7.5 to 15.0 which was clearly larger than the current follicles of parous unfed females observed at 27°C or in summer in nature (cf. Tables 3, 4, and 12). This was due to the occurrence of a number of larger follicles which, however, remained mostly in stage IIa-IIb as usuall. The small follicles were in stage I and 6.35 in the mean length and a little smaller than the follicles in the first quiescent phase in nulliparous females

Table 14. Size and developmental stage of current follicles of parous females collected in cow-sheds at Kaizu village during November 9−12, 1965 (Females Nos. 1 to 12 were captured immediately after feeding, while Nos. 13 to 16 were done before feeding).

Mosq. No.	Feeding	Parity	Large current follicles				Small current follicles			
			Nos.	Mean length	Range	Stage	Nos.	Mean length	Range	Stage
1	just fed	2	349	11.84	8.0−15.0	II a− II b	0	—	—	—
2	〃	3	235	11.25	7.5−13.0	〃	30	6.32	6.0−6.5	I
3	〃	2	258	11.36	9.5−13.5	II a	4	6.26	5.7−6.8	〃
4	〃	2	312	10.74	8.3−13.0	II a− II b	0	—	—	—
5	〃	1	262	9.64	7.5−11.0	〃	0	—	—	—
6	〃	1	273	12.11	9.3−14.0	〃	0	—	—	—
7	〃	3	341	10.68	9.3−12.0	〃	11	6.72	6.3−7.0	I
8	〃	3	187	11.13	10.0−12.0	II b− II c	0	—	—	—
9	〃	3	275	12.11	11.5−13.0	II a− II b	5	6.17	5.7−6.5	I
10	〃	2	198	13.77	13.0−14.5	II b	0	—	—	—
11	〃	2	358	11.78	10.5−12.5	II a− II b	0	—	—	—
12	〃	2	260	11.23	8.5−13.0	〃	0	—	—	—
13	unfed	2	257	13.85	12.0−15.0	II b	0	—	—	—
14	〃	3	170	10.90	8.3−12.8	II a	4	5.73	5.5−6.0	I
15	〃	4	253	11.51	8.8−14.0	II a− II b	0	—	—	—
16	〃	3	229	11.29	7.8−13.5	〃	6	6.60	6.5−6.8	I
Mean			263.6	11.58	7.5−15.0	II a− II c	3.8	6.35	5.5−7.0	I

Remarks: Six to ten large follicles and 3−5 small ones were measured randomly with each female.

reared at 27°C (cf. Table 2). The occurrence of the varied conditions in size and stage in the current follicles of unfed (and having just fed) parous females might perhaps be caused by progressive decrease in temperature and day length in autumn.

With each of the same 16 females shown in Table 14, the percentage of the follicular tubes of various forms in the process of producing dilatation was computed as shown in Table 15. The percentage of tubes with degenerated follicles varied with individual mosquito. The percentage of tubes being sac-like was relatively lower but that of those being shrinking was higher, and consequently the percentage of total tubes of being sac-like and shrinking was 86.0%. The percentage of tubes with dilatation was only 10.0%. The proportion of the above two figures is nearly identical to that of those observed 24 or 36 hours after oviposition in females reared at 27°C (see Table 6). This may suggest that the females might have come to feed on cattle mostly one or two days after the oviposition.

The survey made on November was probably too late to collect unfed nulliparous females and if we had any chance to collect mosquitoes on about October, we might be able to learn the transitional condition in development of the first follicles of unfed nulliparous females, between the normal condition of being in stage I as in females in summer and the condition which was observed in February to March in nature (In hibernated nulliparous females the first follicles were found mostly to be in stage

Table 15. Changes in the shape of the posterior part of follicular tubes in parous females shown in Table 14.

Mosq. No.	Feeding	No. tubes in two ovaries	Percentage of tubes			
			with degenerated follicle	sac-like	shrinking	with dilatation
1	just fed	349	1.1	41.5	51.9	5.5
2	〃	265	16.2	35.9	32.1	15.8
3	〃	262	5.3	33.2	43.9	17.6
4	〃	312	2.2	42.3	48.4	7.1
5	〃	262	1.1	70.2	23.3	5.3
6	〃	273	0.7	68.9	23.8	6.6
7	〃	352	2.3	9.1	67.3	21.3
8	〃	187	0.0	0.0	88.8	11.2
9	〃	280	16.1	71.4	8.6	3.9
10	〃	198	0.0	51.5	38.9	9.6
11	〃	358	0.0	0.0	83.2	16.8
12	〃	260	0.0	54.2	45.4	0.4
13	unfed	257	2.7	40.5	53.3	3.5
14	〃	174	4.0	5.2	83.9	6.9
15	〃	253	0.0	44.7	43.5	11.8
16	〃	235	14.0	33.6	41.7	10.7
Mean		267.3	4.0	37.7	48.3	10.0

IIa-IIb as will be mentioned later).

IV Results of examinations in from Februaiy to March

The females emerged from hibernation were collected when they were attracted to CO_2 gas generated from dry ice (dry ice method). The collections were made on from February 11 to March 9, 1966. The results of examinations for the length and developmental stage of the first follicles of nulliparous females and those of the second follicles of 1-parous females (no 2- or more parous females were collected) are presented in Table 16.

The first follicles of the nulliparous 31 females were 11.32 in the mean length and, interesting to say, they were mostly in stage IIa having some exceptions in which some were in stage IIb and a few in IIc or even in I. These follicles were nearly identical in size to those of large current ones of parous females in late autumn. Another interesting thing is that some of the first follicles were found to be degenerated ones as shown in the footnote of Table 16. The degenerated follicles were smaller in length than the first follicles of newly emerged females in summer, very thin in width, and irregularly rugged in both sides. The undifferentiated 8 cells and epitherial cells were entirely being disintegrated into cell debris. These degenerated small first follicles will have possibly been indistinguishable from dilatation when observed after the coming gonotrophic cycle.

The reason why most of the first follicles of 0-parous females emerged from hibernation advanced in size and stage and some of them degenerated is uncertain now but is assumed to have been probably caused by the progressively decreasing temperatures in the days of their entering into hibernation in autumn. The assumption is based on the fact that the two females emerged as adults from wild caught larvae on the end of October, 1966 and reared under natural temperatures on 20% or 40% sugar solution for 4 days were found to have considerably varied follicles in size and stage: In one female, the follicles were all in stage I but 5% of them were in degeneration; in another, 63.3% were in stage I-II and 36.7% in stage I, while 9.1% of the stage I follicles were very small.

Another group of 36 females shown in Table 16 were collected during from February 19 to March 9, 1966 and found all 1-parous. The current follicles of these unfed 1-parous females were found having 2 types: Follicles of one type were large, 11.77 in an average length and in stage IIa-IIb, rarely in IIc, while, those of another type were small, 8.6 in an average length and in stage I. The varied states of current follicles had been similarly observed already in late autumn (cf. Table 14).

With 25 females out of the 36 ones, changes in the posterior part of follicular tubes were examined with the results presented in Table 17. The percentage of the tubes having degenerated follicles was greatly varied with individual mosquito, averaging 9.3%. Tubes of being sac-like, shrinking, and with dilatation were 11.9, 0.0, and 78.8% respectively in an average. It is of interest that sac-like tubes had remained in most females till the days of emergence from a long-term hibernation. However, since it was thought very strange that some females had the sac-like tubes in as high as 20 to 25% in the extreme cases, examinations were carried out for the shrinking process of the tubes in relation to the elapsed time after oviposition with the females which fed on cow on emerging from hibernation and laid eggs, with the results shown in Table 18. The process was found very much slow and even 20

Table 16. Size and developmental stage of follicles of hibernated females collected by dry ice method in nature during from February 11 to March 9, 1966, at villages near Nagasaki City and Kaizu Village. About ten follicles were randomly measured for each female.

Date of catch	Nulliparous females			Parous females (all 1-parous)						
	Mosq. No.	First follicles		Mosq. No.	Large 2nd follicles			Small 2nd follicles		
		Mean length	Stage		Nos.	Mean length	Stage	Nos.	Mean length	Stage
Feb. 11	1*	10.68	Ⅱa							
Feb. 19	2		〃	1	230	12.75	Ⅱa−Ⅱb	4	8.15	Ⅰ
〃	3		〃							
〃	4		〃							
〃	5		〃							
Feb. 21	6*		〃							
Feb. 28	7*	12.00	〃							
Mar. 2	8	9.27	〃	2	210	11.76	Ⅱa	60	6.01	Ⅰ
〃	9	11.95	〃	3			〃			
〃	10	12.65	〃							
〃	11	13.35	〃							
〃	12	11.75	〃							
〃	13	10.55	〃							
〃	14	11.75	〃							
〃	15	11.50	〃							
〃	16	12.53	〃							
〃	17	11.67	〃							
〃	18	10.83	〃							
〃	19	12.27	〃							
〃	20	12.67	〃							
〃	21	12.15	Ⅱb							
〃	22	15.43	Ⅱa							
〃	23	15.33	Ⅱa−Ⅱc							
〃	24	12.67	Ⅱa							
〃	25		〃							
〃	26		〃							
Mar. 4	27	10.00	〃	4	191	9.62	Ⅱa	3	7.00	Ⅰ
〃				5			Ⅱb			
〃				6	204	11.70	Ⅱa−Ⅱb	3	7.15	Ⅰ
〃				7	191	9.00	Ⅱa	9	6.00	〃
〃				8	215	9.36	Ⅱa−Ⅱb	12	5.50	〃
〃				9	127	10.60	〃	31	6.00	〃

Table 16. (Continued)

Date of catch	Nulliparous females			Parous females (all 1-parous)						
	Mosq. No.	First follicles		Mosq. No.	Large 2nd follicles			Small 2nd follicles		
		Mean length	Stage		Nos.	Mean length	Stage	Nos.	Mean length	Stage
Mar. 4				10	219	9.73	Ⅱa	38	5.50	Ⅰ
〃				11	198	10.76	〃	0	—	—
〃				12	194	9.77	〃	0	—	—
〃				13	197	19.60	Ⅱc	0	—	—
〃				14			Ⅱa			
〃				15			Ⅱb			
〃				16			〃			
〃				17			Ⅱa			
Mar. 5	28*	12.27	Ⅰ	18	162	11.72	Ⅱa−Ⅱb	2	8.65	Ⅰ
〃	29*	9.77	Ⅱa	19	235	10.91	〃	2	7.75	〃
〃	30		〃	20	187	12.20	〃	2	7.50	〃
〃				21	200	11.15	〃	4	8.15	〃
〃				22	212	12.47	Ⅱa−Ⅱc	8	7.50	〃
〃				23	197	12.85	〃	9	9.10	〃
〃				24	168	11.33	Ⅱa−Ⅱb	10	6.50	〃
〃				25	239	12.55	〃	14	7.50	〃
〃				26	270	12.56	Ⅱc	0	—	—
〃				27	246	12.93	Ⅱa−Ⅱb	0	—	—
〃				28	246	11.97	〃	0	—	—
〃				29	241	12.90	Ⅱb	0	—	—
〃				30	199	13.83	Ⅱa	0	—	—
〃				31			〃			
〃				32			〃			
〃				33			Ⅱb			
〃				34			Ⅱa			
〃				35			〃			
Mar. 9	31*	12.67	Ⅱa	36	212	11.90	Ⅱa−Ⅱb	3	8.27	Ⅰ
No. ♀♀ examined		23	31	36	25	25	36	25	25	25
Mean		11.32			207.6	11.77		8.6	7.19	
Range		9.0−16.0	Ⅰ−Ⅱc		127−270	6.5−19.8	Ⅱa−Ⅱc	0−60	5.0−9.8	Ⅰ

Remarks: With randomly selected six females marked with * out of 31 0-parous ones, the number and shape of the first follicles were examined, with an interesting finding that: Female Nos. 1, 6, 7, 28, 29, and 31 had 89, 36, 66, 83, 57, and 36 degenerated first follicles respectively out of 228, 219, 208, 251, 229, and 170 ones, i. e., in an average, 61.2 or 28.12% degenerated ones out of 217.5 follicles.

says after oviposition about a half of tubes were found to be sac-like and shrinking.

Here, it is to be noted that the females No. 6 and 12 which were nulliparous when they were captured, were found after oviposition having tubes with degenerated follicles each in a very low percentage, and those with dilatation each in a relatively high percentage.

This seems possibly due to the inevitable mistake of degenerated very small follicles having been produced among current follicles of unfed females in autumn, for the dilatation produced after the oviposition following the blood meal in the spring.

V Considerations on the shrinking pattern of follicular tubes in relation to temperature and season

Table 17. Changes in the shape of the posterior part of follicular tubes of the 25 unfed parous females (among 36 ones shown in Table 16) collected by dry ice method near Nagasaki City and Kaizu Village during from February 19 to March 9, 1966

Mosq. No.	No. tubes in two ovaries	Percentage of tubes			
		with degenerated follicles	sac-like	shrinking	with dilatation
1	234	15.8	20.5	0.0	63.7
2	270	5.2	0.0	0.0	94.8
4	194	0.5	11.3	0.0	88.2
6	207	1.9	3.4	0.0	94.7
7	200	7.5	10.5	0.0	82.0
8	227	4.4	7.5	0.0	88.1
9	158	3.9	0.0	0.0	96.1
10	257	8.6	7.8	0.0	83.7
11	198	17.7	5.6	0.0	76.7
12	194	13.9	9.3	0.0	76.8
13	197	10.7	11.7	0.0	77.8
18	164	24.4	10.4	0.0	65.2
19	237	2.1	18.1	0.0	79.8
20	189	14.8	19.0	0.0	66.2
21	204	25.5	16.7	0.0	57.8
22	220	9.5	10.5	0.0	80.0
23	206	15.5	20.4	0.0	64.1
24	178	14.6	16.3	0.0	69.1
25	253	14.2	12.3	0.0	73.5
26	270	5.9	25.2	0.0	68.9
27	246	4.5	18.7	0.0	76.8
28	246	2.8	19.1	0.0	78.1
29	241	10.8	0.0	0.0	89.2
30	199	5.0	4.0	0.0	91.0
36	215	0.5	14.4	0.0	85.1
Mean	216.2	9.3	11.9	0.0	78.8

In Table 19, the shrinking patterns of the posterior part of follicular tubes in *Anopheles sinensis* are compared with the females examined under different laboratory and natural conditions. The most striking feature in the shrinking of the tubes in this mosquito species is that the

Table 18. Condition of the posterior part of follicular tubes examined on the indicated days after oviposition. (The females which emerged from hibernation and fed on cows on March 2 and 4, 1966, were reared at laboratory under natural air conditions).

Mosq. No.	No. tubes in two ovaries	Percentage of tubes		
		with degenerated follicles	sac-like+shrinking	with dilatation
Examined about 3−5 days after oviposition				
1	181	4.4	64.7	30.9
2	250	0.4	64.0	35.6
3	254	23.6	68.5	7.9
Mean	228.3	10.1	65.8	24.1
Examined about 7 days after oviposition				
4	243	7.4	48.1 17.7	26.7
5	316	13.9	27.8 12.7	45.6
6	232	6.9	31.9	61.2
7	242	2.5	57.4	40.1
Mean	258.3	8.1	48.5	43.4
Examined about 10 days after oviposition				
8	264	6.1	50.7	43.2
9	178	13.5	46.1	40.4
10	212	19.3	55.2	25.5
11	242	27.3	47.9	24.8
12	198	3.5	41.4	55.1
Mean	218.8	14.1	48.5	37.4
Examined about 20 days after oviposition				
13	224	8.0	51.4	40.6
Mean	233.5	10.7	52.6	36.7

Remarks: Females Nos. 6 and 12 were nulliparous, while the others were uniparous when they were captured.

shrinking process is very much slow in comparison with the other mosquitoes. The process appears to become a little slower with decrease in temperature.

When compared the mean percentages of the tubes with dilatation observed in nature in summer and autumn with those observed at 27°C, it is presumable that the females usually take their blood meals within 24 hours after oviposition.

The females which emerged from the long-term hibernation were found to have the tubes of being still sac-like in about 12 percent. It appears strange but will be admitted as true, considering the fact that the females after oviposition following the blood meal on emergence from hibernation were found having sac-like tubes in about 40 to 50 percent till 10 to 20 days after the oviposition.

The percentage of tubes with degenerated follicles varies with individual mosquito as already mentioned above, though, in general, it takes a mean value of about 4% similarly at 27°C, in summer, or even in late autumn, while in early spring it increases to 9.3%. The females which passed a gonotrophic cycle after the emergence from hibernation were found having the tubes with degenerated follicles in 8 to 14% which was roughly near to the percentage or 9.3%. The reason why the percentage of tubes with degenerated follicles becomes high in hibernated females is quite unknown now.

VI Considerations on the changes in size and developmental stage of follicles of females in relation to the temperature and season

In Table 20, the size and developmen-

Table 19. Shrinking pattern of the posterior part of follicular tubes in parous females in relation to the elapsed time after oviposition or the season, together with the tubes with degenerated follicles.

Elapsed time after oviposition		Percentage of tubes: sac-like	shrinking	with dilatation	with degenerated follicles	Reference Table
Hours after oviposition at 27°C	0 hr	95.4	2.6	0.0	1.9	Table 6
	12 hrs	83.0	13.6	1.7	1.7	
	24 hrs	65.8	22.2	9.5	2.5	
	48 hrs	55.0	16.1	26.3	2.5	
	72 hrs	56.5	16.5	23.7	3.3	
Females coming to bite on cow after oviposition in summer		79.2	9.9	7.3	3.6	Table 13
Females coming to bite on cow after oviposition in late autumn		37.7	48.3	10.0	4.0	Table 15
Hibernated females collected before feeding in early spring		11.9	0.0	78.8	9.3	Table 17
Days after oviposition at natural temperatures in early spring	3−5 days	65.8		24.1	10.1	Table 18
	7 days	48.5		43.4	8.1	
	10 days	48.5		37.4	14.1	
	20 days	51.4		40.6	8.0	

Table 20. Changes in size and developmental stage of follicles of unfed females in relation to the temperature and season

Temp. (°C) or season	Reference table	1st follicles of 0-parous females			2nd follicles of 1-parous females			3rd follicles of 2-parous females: Large follicles			3rd follicles of 2-parous females: Small follicles		
		Mean length	Range	Stage	Mean length	Range	Stage	Mean length	Range	Stage	Mean length	Range	Stage
27°C	2, 3, 4	8.19	6.2–11.5	I	9.28	7.8–10.3	IIa–IIb	9.69	8.0–11.3	IIa–IIb			
		(Table 2, 48 hrs after*)			(On the day of 1st oviposition)			(On the day of 2nd oviposition)					
Summer	11, 12	9.12	7.0–13.2	I	8.77	8.0–11.0	IIa–IIb	9.00	8.0–9.5	IIa–IIb			
		(Table 11)			(With 6 ♀♀ in Table 12)			(With 2 ♀♀ in Table 12)					
Late autumn	14				10.88	7.5–14.0	IIa–IIb	11.69	7.5–15.0	IIa–IIc	6.35	5.5–7.0	I
		(Could not collect)			(With 2 ♀♀, Nos. 5 & 6)			(Current follicles of 2- to 4-parous females)					
Feb.-March (hibernated)	16	11.32	9.0–16.0	I–IIc	← **			11.77	6.5–19.8	IIa–IIc	7.19	5.0–9.8	I
					←			(2nd follicles of 1-parous females)					

* : Mean value for females reared by 2, 20, and 50% sugar solutions.
** : The figures are those for 2nd follicles of 1-parous females. Two- or more parous females could not collect.

tal stage of the first, 2nd, and 3rd follicles of unfed 0-, 1-, and 2-parous females examined under different rearing temperature and seasons are summarized. The size and stage of follicles being in the first or second quiescent phase are nearly similar in both unfed females reared at 27°C and those collected in summer in nature. The fact that the current follicles of unfed parous females advance into stage IIa-IIb, or the second quiescent phase, is one marked peculiality of this mosquito species.

In autumn the current follicles of unfed parous females are subjected to a great variation in size and stage: Many of them are large and in stage IIa-IIb, and some are small and in stage I, while some may degenerate. The occurrence of great variation of the current follicles in unfed parous females in autumn is another peculiality of this species.

The first follicles of unfed nulliparous females which emerged from the long-term hibernation are greatly varied in size and stage: Many of them are in stage IIa; some are degenerated; and a few are in stages IIb, IIc or I. The occurrence of great variation and especially the advance into stage II in the first follicles of hibernated unfed nulliparous females are the most interesting and the 3rd peculiality of this mosquito.

Summary

1) Ovariole and age changes were examined with the females of *Anopheles sinensis* Wiedemann, 1828 reared or captured under different conditions of temperature and season during mainly from May, 1964 to March, 1966.

2) The first follicles of newly emerged females were about 40μ in length and in stage No having 8 undifferentiated cells. They reached the initial quiescent phase (stage I) 36-48 hours after emergence and were about 75μ. The second follicles in fed females appeared on the next day of the blood meal and reached the 2nd quiescent phase (stage II) by the day of the first oviposition.

3) The females of this mosquito can not lay eggs unless they are fertilized. The fertilized females can oviposit when they take full blood meal only once.

4) It took from emergence to blood taking usually 2 days in experiment and 2-3 or more days in nature. The first gonotrophic cycle from feeding to oviposition took 4 days, while the second and subsequent cycle did each 3 days at 27°C or in summer in nature. Oviposited females seemed to take next blood meal commonly within 24 hours in experiment and also in nature.

5) The shortest calendar age or the longevity after emergence of the females at 27°C or in summer in nature is obtained by the equation: The [$3+4n+\alpha$]th day. Where, n: parity, α: days from the last oviposition to the day of being captured.

6) In late autumn, the current follicles of unfed parous females were subjected to a great variation in size and stage: Large ones were in stage II as usual and small ones were in stage I. The variation appeared to be caused by the progressive decrease in temperature and day length in that season. The first follicles of unfed nulliparous females could not be examined bacause none of these females were collected in the late autumn.

7) The first follicles of unfed nulliparous females just emerged from hibernation on February to March were unexpectedly mostly in stage II, while some were degenerated and very small, and a few were in stage I. The current follicles of hibernated unfed parous females were mostly large as usual and in stage II, while some were small and in stage I.

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シナハマダラカの卵巣小管及び年令の変化，特に温度及び季節との関係について．武富正彦，長崎大学医学部医動物学教室及び長崎大学風土病研究所衛生動物部（主任：大森南三郎教授）

総　　　　括

シナハマダラカ♀成虫の卵巣小管及び年令の変化を，恒温 27°C で幼虫時代から飼育したものについて調べた成績を基にして，自然界で，夏，晩秋及び早春越年から覚めた個体群について調べた結果を比較吟味した。この研究は主として1964年5月から1966年3月の間に行なった．それらの結果は次のように要約できる．

1) 27°C では，羽化直後の第1口胞はNo期，長径約 40μ で，未分化の8細胞がみとめられ，口胞上皮細胞は生殖巣との連結部を除いて殆んど完成している．羽化後18時間頃からⅠ期に進むものがみられ，36—48時間後には悉くⅠ期となり約 75μ に達して所謂第1静止期となる．

2) 本種の♀では，授精の有無に拘わらず吸血後口胞の発育は同様に進むが，授精していなければ絶対に産卵されない．授精した♀が産卵するためには只1回の満腹吸血で充分である．

3) 羽化から，多数個体の吸血迄には 27°C で約2日，夏自然界では2，3日又はそれ以上を要する．第1生殖環即ち初回の吸血から産卵迄は4日，産卵後吸血迄の時間は約1日である．第2回以後の生殖環即ち吸血から産卵迄は3日を要する．それ故，羽化日を第1日とすると，多数個体の吸血は第3日目にみられ，第1生殖環は第4—7日に完了，第2回の吸血は第8日目に，第2回の生殖環は第9—11日に完了することになるから，最短の Calendar age は次の式から得られる．

calendar age＝第〔$3+4n+\alpha$〕日

但し，n＝経産回数，α＝最後の産卵から，採集された日迄の日数．

4) 晩秋11月には，未吸血の経産♀の口胞は大きさ，発育期共に大きく変異する．大型口胞は 27°C 又は夏自然界でのものより更に大きく，発育期はⅡ期であるが，小型口胞は 27°C での第1静止期のものより小さくⅠ期に止まる．このような変異は秋の低減する温度と日長の影響によるものと思われる．

5) 早春，越年から覚めた未吸血，未経産♀の第1口胞の多くはⅡ期に進んでおり，又，可成りのものが既にⅠ期以前の状態から退化しているが，これらのことは特に興味あり注意すべきことである．未吸血の経産♀では既に越年前に見られたような口胞の大きさと発育期の大きな変異がみられる．