Tropical Medicine, 14(1) : 55～63, March, 1972

The follicular development of *Culex tritaeniorhynchus summorosus* females after taking various amounts of blood in reference to feeding and oviposition activity*

Motoyoshi MOGI, Yoshito WADA

Department of Medical Zoology,
Nagasaki University School of Medicine

and

Nanzaburo OMORI

Department of Parasitology, School of Medicine, Teikyo University

(Received for Publication February 12, 1972)

Abstract

The follicular development of *Culex tritaeniorhynchus summorosus* females after taking various amounts of blood was observed, and the following facts were demonstrated. Even in the females which have taken merely a trace amount of blood insufficient for filling up the midgut, many follicles do initiate the development, but none of them mature. When the uptake amount of blood increases, some follicles develope to mature eggs, and generally the more amount of blood is taken, the more mature eggs are produced; those follicles which have initiated the development but have failed to mature are degenerated. The females which have smaller number of mature eggs than the normal will lay a small egg raft or will take a second blood meal, depending on which is given the oviposition place or the blood source.

Introduction

Some female mosquitoes can take blood up to about the twofold of the body weight at one feed (Bekku, 1953; Woke *et al.*, 1956), so that the possible range of the uptake amount of blood is rather wide. Dow *et al.* (1957) collected *Culex tarsalis* females attracted to baited chickens and said that with the increasing number of the collected females, the rate of the fed females in all the females decreases but tne rate contain-

*Contribution No. 199 from the Department of Medical Zoology, Nagasaki University School of Medicine.

ing merely a trace of blood in the fed females increases. More recently, Fujito *et al.* (1971) reported similar tendency in the females of *Culex tritaeniorhynchus summorosus* collected by light traps at pigsties. In connection with these interesting phenomena, it was thought to be necessary to examine whether those females that take only a small amount of blood will develop mature eggs and whether they feed again on animals. This paper deals with the results of experiments along this line with *Culex tritaeniorhynchus summorosus*, the principal vector of Japanese encephalitis in Japan.

Materials and Methods

Culex tritaeniorhynchus summorosus used in the experiments were obtained from the colony which had been derived from engorged females collected at animal sheds in Nagasaki area in April, 1965. The colony had been maintained under a daily photoperiod from 14 to 15.5 hours and a constant temperature of 27°C. The present experiments were carried out in 1970 under 14 hour photoperiod and 27°C. The larvae were reared with the equally mixed powder of Ebios (brewer's yeast) and mouse pellet, and the

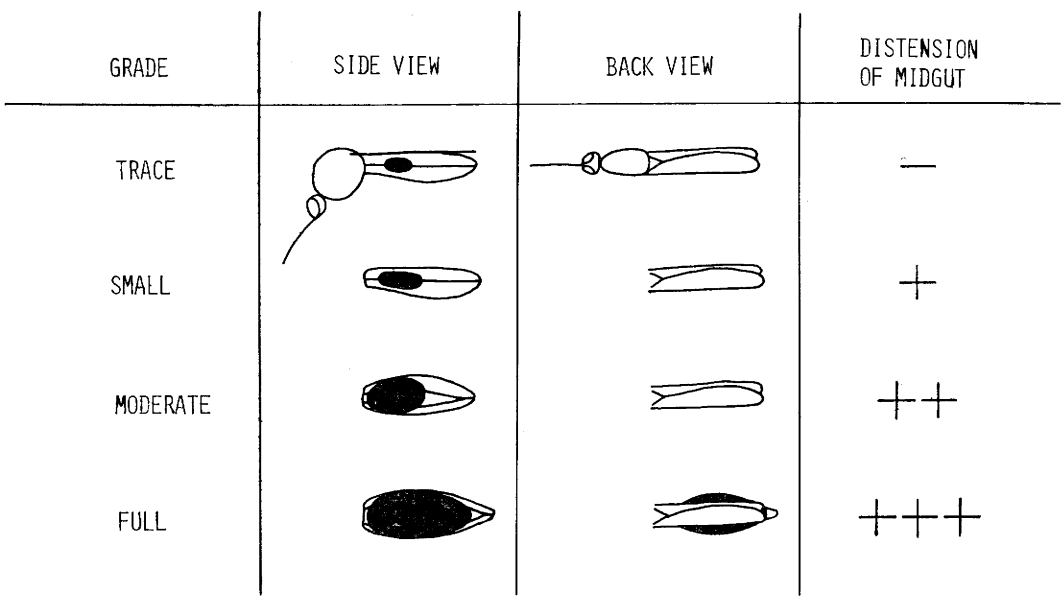

Fig. 1. Schematic representation of the grades of the amount of blood in the females of *Culex tritaeniorhynchus summorosus* immediatery after feeding.

females were given 1% sugar solution.

The blood in midgut can be observed from the outside as a red part in the abdomen, so the relation was examined between the appearance of abdomen by observation and the state of midgut with blood by dissection. From the results, the grades of the amount of blood taken were determined as trace, small, moderate and full as shown in Fig. 1, based on the size of red part seen from the side and back of a female and the part of midgut blood occupied. In none of the females with a trace amount of blood, the midgut is distended by blood even immediately after taking blood. On the contrary, all the females with a moderate or full amount of blood have the midgut filled up and distended by blood. The state of the females with a small amount of blood lies between trace and moderate, i. e., the midgut is filled up by blood, but the distension of midgut is very small. The grade of each female was decided actually only based on its appearance.

Female mosquitoes, which had been kept in a small cage with 1% sugar solution 5 to 10 days after emergence, were fed on a human hand. When the amount of blood taken reached one of the grades stated above, the mosquito was taken up by an aspirator, and thereafter reared again with sugar solution. The developmental stages of follicles after blood feeding were recorded following Kawai (1969).

Results obtained

Prior to describing the development of follicles in female mosquitoes after taking various amounts of blood, the follicular development before blood meal is given in Table 1. Under the conditions of 27°C and 14 hour photoperiod, the first follicles become N~Ib in stage, i. e., the first quiescent phase, 3 days after emergence, and rarely develop to more advanced stages unless taking blood.

Follicular developments after taking various amounts of blood are shown in Table 2. In the females 1 day after taking a trace amount of blood, the first follicles developed slightly from the first quiescent phase and were at N~Ib to IIb, mostly I-II and IIa in stages, and the blood had already disappeared from the midgut. On the following

Table 1. Follicular development of the females of *Culex tritaeniorhynchus summorosus* before blood feeding, under 27°C and 14 hour photoperiod (from Kawai, 1969 and his unpublished data).

Days after emergence	No. of females dissected	Percentage of first follicles falling in each developmental stage		
		No1~No2	N~Ib	I-II
1	10	94.5	5.5	0.0
2	15	37.6	62.4	0.0
3	15	0.0	100.0	0.0
4	15	0.0	99.4	0.6
5	15	0.0	96.3	3.7
10	5	0.0	99.8	0.2

Table 2. Follicular development of the females of *Culex tritaeniorhynchus summorosus* after taking various amounts of blood under 27°C and 14 hour photoperiod.

Grades of amount of blood	Days after taking blood	No. of females dissected	Mean number of follicles per ovary	Percentage of first follicles falling in each developmental stage										Percentage of the space in midgut occupied by blood when dissected	Distension of midgut when dissected
				N~Ib	I-II	IIa	IIb	IIIa	IIIb	IV	Va	Vb	degenerated		
trace	1	13	127	7.2	52.3	39.5	1.0							0	—
	2	16	127	2.8	33.2	57.0	6.9	0.1						0	—
	3	13	126	2.9	48.6	40.9	7.5						0.1	0	—
	4	10	130	4.0	23.7	51.7	20.5						0.1	0	—
	8	9	113	6.8	61.2	31.9	0.1							0	—
	12	6	130	6.1	29.3	59.0	4.6						1.0	0	—
small	1	9 [1)]	128	8.2	45.4	46.3								0	—
	4	9	125	4.3	20.9	44.2	28.7	1.9						0	—
	8	16 [2)]	117	0.8	33.1	56.6	9.1							0	—
	12	12	116	0.9	24.1	58.7	14.8							0	—
	1	4 [3)]	130	0.6	26.9	26.3	22.5	23.6						52	—
	4	5	105	*	*						0.8	12.0	87.1*	0	—
	8	2 [4)]	93	*	*							18.4	81.6*	0	—
	12	2	105	*	*	5.2						3.8	91.0*	0	—
moderate	1	6	110	4.7	39.8	30.2	10.6	14.7						70	—
	4	6	100	*	*							27.0	73.0*	0	—
full	1	3	117	1.1	4.0	57.0	11.4	26.5						100	+
	4	3	110	*	*							80.3	19.7*	0	—

1) Females with no blood in midgut 1 day after taking blood.
2) Females with no mature eggs 4, 8 and 12 days after taking blood.
3) Females with blood in midgut 1 day after taking blood.
4) Females with mature eggs 4, 8 and 12 days after taking blood.
* Some first follicles in N to I-II stages are or might be included in the degenerated follicles. For further explanation see text.

days a very few follicles developed to IIIa, but the others remained continuously in N~Ib to IIb, mostly in I-II and IIa, until about 2 weeks elapsed. In some females which had survived more than 2 weeks after taking a trace amount of blood, some number of follicles were degenerated. The degenerated follicle, when contraction had been completed, was very similar to the dilatation formed after oviposition. None of the follicles in the females having taken a trace amount of blood developed to mature eggs after all.

In the females having taken a small amount of blood, the majority had already no blood in the midgut 1 day after blood meal, and had the first follicles in stages of N~Ib to IIb, mostly in I-II and IIa. This states were similar to those observed in the females having taken a trace amount of blood. However, the some individuals were found to contain some blood still in the midgut and a considerable number of their follicles were in stage IIIa. The greater part of the females which had survived more than 4 days after feeding had no mature eggs and the states of follicular development of these females were similar to those having taken a trace amount of blood, i. e., the first follicles remained mostly in N~Ib to IIb and very rarely developed to IIIa. But some females had mature eggs, though the number of eggs was small, and the states of follicular development of these females were rather complicated, being classified in 3 groups : (1) the first follicles developed to the mature eggs and the second follicles also developed to the second guiescent phase ranging from N to I-II in stages, (2) the first follicles degenerated and the second follicles developed to the second guiescent phase, and (3) the first follicles remained in N~Ib to I-II and the second follicles did in No1~No2. The second follicles in the group (1) were liable to be separated from the mature eggs on dissection. Such separated follicles had no pedicels and could not be discriminated from the current follicles falling in the latter two groups, when their pedicels were cut off. Therefore, the total number of ovarioles falling in the groups (2) and (3) was obtained by subtracting the number of mature eggs from that of all follicles in stages from N to I-II including those connected to mature eggs. Moreover the follicles of the groups (2) and (3) in the same developmental stages were not always clearly discriminated from each other, because the second follicles of group (2) were undistinguishable from the first follicles of group (3), when the current follicles were cut off, on dissection, from the pedicels to which degenerated ones might have been attached. Therefore the both groups were included for convenience in the colomn of degenerated follicles in Table 2. The real percentage of the degenerated follicles were unknown, but the number falling in the group (2) was greater than the group (3) under the conditions of the present experiment.

The process of the follicular development and degeneration in the females having taken a moderate or full amount of blood was essentially the same as the above description for the females which produced some mature eggs, naturally, however when the more amount of blood had been taken the more mature eggs were produced. Even in the females which had fed to repletion, there were found a considerable number of follicles which fell into degeneration after

once initiating the development. The rate of such degenerated follicles was about 20% as shown in the last line in Table 2, but this figure might be higher than the actual one, for some follicles remaining in N~Ib to I-II might be included in the degenerated ones by the reason as stated above. Of course, this can be applied to the females having taken a moderate amount of blood.

Mature eggs were, if once formed, not deteriorated even in the females which survived 40 days after taking blood.

Next, the oviposition and the feeding activity of the females having taken various amount of blood will be mentioned. Table 3 gives the rate of the females which laid an egg raft after having taken various amounts of blood and the number of eggs in the raft. The rates of the females harboring any number of mature eggs 4 to 12 days after feeding shown in Table 2 were 19.6, 100.0, and 100.0% for those taking a small, moderate and full amount of blood respectively. Therefore the rates of the females which laid an egg raft in the females with mature eggs were not so different between respective groups by blood amount. Very small rafts were laid by the females having taken small amount of blood.

Regarding the feeding activity of once fed females, the females having taken a trace and moderate amount of blood took blood again at a considerably high rate when exposed to human hand 1, 4 and 8 days after the first blood meal as shown in Table 4. On the contrary, none of the fully fed females took the second blood meal 1 and 4 days after the first meal, although on the 8th day some took. Certainly the fully fed females do not take blood repeatedly as easily as those taking a trace to moderate amount of blood.

Table 3. Number of eggs in a raft laid by the females of *Culex tritaeniorhynchus summorosus* having taken various amounts of blood, under 27°C and 14 hour photoperiod.

Grades of amount of blood	No. of females observed	No. (%) of females laid an egg raft	Mean number (range) of eggs per raft
small	20	2 (10.0)	26.5 (23—30)
moderate	20	7 (35.0)	57.8 (38—72)
full	20	14 (70.0)	169.1 (128—199)

Table 4. Percentage of the repeated blood feeding in the females of *Culex tritaeniorhynchus summorosus* having taken various amounts of blood and kept without giving water for egg laying under 27°C and 14 hour photoperiod.

Grades of amount of blood at the first feeding	% females fed (No. females exposed*)		
	1 day after the first feeding	4 days after the first feeding	8 days after the first feeding
trace	70.0 (30)	80.0 (25)	92.3 (26)
moderate	96.2 (27)	57.1 (28)	69.6 (23)
full	0.0 (30)	0.0 (30)	12.0 (25)

* Females were exposed to a human hand for 3 hours.

Discussion

Many species of mosquitoes require a blood meal for the production of eggs and it has been found by various investigators that there is a close relationship between the amount of blood taken and the number of eggs produced (Roy, 1936; Hosoi, 1954; Barlow, 1955; Woke *et al.*, 1956; Detinova, 1962; Kupriyanova, 1966 and Volozina, 1967). Most of these investigations showed that the mature eggs are produced only when the relative amount of blood taken to the body weight exceeds a certain value, characteristic to the species.

Such observations seem to support the hypothesis proposed by Larsen & Bodenstein (1959) that in anautogenous mosquitoes the midgut distension for considerable duration is necessary as the first step stimulus inducing the secretion of gonadotropic hormone and consequently for initiating the development of follicles to eggs. Recently, however,Bellamy & Bracken (1971) proposed another hypothesis that the digested blood itself is the factor responsible for initiating the development to eggs. The results of the present study with *Culex tritaeniorhynchus summorosus* seem to throw some doubts on the former hypothesis, because even in the females having taken a trace amount of blood insufficient for the distention of the midgut, the initiation of follicular development was observed (see Fig. 1 and Table 2)

Volozina (1967) found in *Aedes* species a threshold amount of blood for the initiation of follicular development. In the present experiment, however, the threshold amount for the initiation of the development was not detected, but follicles may have not initiated development if the amount of blood taken is smaller than the minimum amount taken in the present experiment.

From the ecological viewpoints, the results obtained raise some problems. It is said that a considerable number of the females of *Culex tritaeniorhynchus summorosus* take only a trace amount of blood in some conditions as referred in the introduction of this paper. If this is not the rare case in nature, its possible role in the population dynamics of mosquitoes and the prevalence of the disease they transmit must be studied in detail. Here, it is interesting that the females of *Culex tritaeniorhynchus summorosus* which took only a small or moderate amount of blood by interruption and then produced mature eggs can either lay an egg raft or take the second blood meal, depending on which is given the oviposition place or the blood source, as shown in Tables 3 and 4. However, the behavior of the females having taken a small to moderate amount of blood is rather inapparent in natural conditions. The females with a few mature eggs are not rarely found in the females collected by dry ice traps or at animal sheds,but these eggs are thought to be remained in the ovaries when the other eggs were laid. The females with 10 to 30 eggs per ovary are hardly found in the females attracted to CO_2 or animals. These facts may possibly suggest that in natural conditions the most of the females interrupted to take a full blood meal feed again on blood. The feeding and refeeding will occur probably in the same night, as the females with considerable number of follicles falling in stages from III to IV are also scarcely found in such females. If the variation of the number of mature eggs in the females attracted

to the oviposition place can be examined by some adequate methods, the results will give some suggestions to the problems.

As for *Aedes aegypti*, one of the most extensively investigated species, even the females which took blood to repletion take blood repeatedly before oviposition at considerably high rate, when exposed to the blood source in laboratory conditions (Judson, 1968). Moreover in this species repeated feedings occur usually in natural conditions on the course of blood digestion and follicular development (MacDonald, 1956; Tuchinda *et al.*, 1969; and Yasuno & Tonn, 1970).

For the exact determination of physiological age in female mosquitoes by counting the number of dilatations of the ovarioles, it is desirable to know fully on the follicular development and degeneration and also on the behavior of mosquitoes from blood feeding to oviposition in various conditions in nature.

References

1) **Barlow, C. A.** : The fecundity of *Aedes hexodontus* in the laboratory. Canad. J. Zool., **33** : 420-427, **1955**.

2) **Bekku, H.** : On the amount of blood taken up by a female mosquito of *Culex pipiens pallens*. J. Nagasaki Med. Soc., **28** : 1036-1037, **1953**.

3) **Bellamy, R. E. & Bracken, G. K.** : Quantitative aspects of ovarian development in mosquitoes. Canad. Ent., **103** : 763-773, **1971**.

4) **Detinova, T. S.** : Age-grouping methods in Diptera of medical importance. Wld Hlth Org. Monogr. Ser., 47, Geneva, **1962**

5) **Dow, R. P., Reeves, W. C. & Bellamy, R. E.** : Field tests of avian host preference of *Culex tarsalis*. Amer. J. Trop. Med. Hyg., **6** : 294-303, **1957**.

6) **Fujito, S., Buei, K., Nakajima, S., Ito, S., Yoshida, M., Sonoda, H. & Nakamura, H.** : Effect of the population density of *Culex tritaeniorhynchus* on blood sucking rates in cowsheds and pigpens in relation to its role in the epidemic of Japanese encephalitis. Jap. J. Sanit. Zool., **22** : 38-44, **1971** (In Japanese with English summary).

7) **Hosoi, T.** : Egg production in *Culex pipiens pallens* III. Growth and degeneration of ovarian follicles. Jap. J. Med. Sci. Biol., **7** : 111-127, **1954**.

8) **Judson, C. L.** : Physiology of feeding and oviposition behavior in *Aedes aegypti*. Experimental dissociation of feeding and oogenesis. J. Med. Ent., **5** : 21-23, **1968**.

9) **Kawai, S.** : Studies on the follicular development and feeding activity of the females of *Culex tritaeniorhynchus* with special reference to those in autumn. Trop. Med., **11** : 145-169, **1969**.

10) **Kupriyanova, E. S.** : On the gonotrophic cycle in mosquitoes of the genus *Culex* I. The influence of the amount of human blood ingested on the development of the eggs and fecundity of *Culex pipiens molestus* and *Culex pipiens pipiens*. Med. Parazit., **35** : 310-316, **1966** (In Russian with English summary, Rev. Appl. Ent. Ser. B, **57** : 257-258, **1969**).

11) **Larsen, J. R. & Bodenstein, D.** : The humoral control of egg maturation in the mosquito. J. Exptl. Zool., **140** : 343-378, **1959**.

12) **MacDonald, W. W.** : *Aedes aegypti* in Malaya II. Larval and adult biology. Ann. Trop. Med. Parasit., **50** : 399-414, **1956**.

13) **Roy, D. N.** : On the role of blood in ovulation in *Aedes aegypti*. Bull. Ent Res., **27** : 423-429, **1936**.

14) **Tuchinda, P., Kitaoka, M., Ogata, T. & Kurihara, T.** : On the diurnal rhythmus of

biting behavior of *Aedes aegypti* in relation to the age and hemorrhagic fever in Bangkok, 1964 (Preliminary report). Jap J. Trop. Med., **10**: 1-6, **1969**.

15) **Volozina, N. V.**: Jhe effect of the amount of blood engorged and of supplementary carbohydrate feeding on the process of oogenesis in the females of blood-sucking mosquitoes in the genus *Aedes* of different weights and ages. Ent. Obozr., **46**: 49-59, **1967**. (In Russian with English summary, Rev. Appl. Ent. Ser. B, **56**: 182-183, **1968**).

16) **Woke, P. A., Ally, M. S. & Rosenberger, C. R.**: The numbers of eggs developed related to the quantities of human blood ingested in *Aedes aegypti*. Ann. Ent. Soc. Amer., **49**: 435-441, **1956**.

17) **Yasuno, M. & Tonn, R. J.**: A study of biting habits of *Aedes aegypti* in Bangkok, Thailand, Bull. Wld Hlth Org., **43**: 319-325, **1970**.

種々の量の血液を摂取したコガタアカイエカの濾胞の発育及びその後の吸血，産卵活動

茂木幹義・和田義人

長崎大学医学部医動物学教室

大森南三郎

帝京大学医学部寄生虫学教室

摘　　要

種々の量の血液を摂取したコガタアカイエカ雌成虫の濾胞の発育について調べた結果次の点が明らかとなった．中腸を満たすに足りないほど少量しか吸血しなかった雌でも，多くの濾胞が発育を始めるが，成熟卵にまで発育する濾胞はない。吸血量が増えると成熟卵まで発育する濾胞が出現し，その数は．吸血量が増えるほど多くなる．その場合に，成熟卵にならない濾胞は発育途中から退化する．少数の卵しか持たない雌は，産卵場所を与えると，きわめて小さな卵塊を産卵するが，産卵場所を与えずに吸血源のみを与えると抱卵したまま再吸血する．