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THE EFFECT OF MATING ON THE FOLLICULAR DEVELOPMENT IN CULEX TRITAENIORHYNCHUS (DIPTERA, CULICIDAE)

MITSUO TAKAHASHI[1] and SADAO YABE[2]

Department of Medical Entomology[1] and Department of Virology and Vickettsiology[2], National Institute of Health, Tokyo 141, Japan

The inhibition of follicular development will represent a conventional clue to the diapause in adult insects. In unautogenous *Culex* mosquitoes in temperate areas which shall have diapause phase in the adult, the blood feeding by females is a prerequisite of follicular development. However, the cessation of blood feeding activity is not always directly connected with the manifestation of diapause, but it sometimes denotes merely quiescence under unfavorable conditions. If the blood meal could be replaced by any suitable diet which accelerates the follicular development, the physiological status of mosquitoes will be grasped with more accuracy.

Basing on the assumption stated above, lactalbumin hydrolysate (LAH) suspended in sugar water was tested for the effect on follicular development in mosquitoes under a non-diapausing condition. The choice of the medium was made from the reason that the component of LAH as analyzed by Takaoka *et al.* (1960) satisfactorily covers the essential amino acid requirements for the egg production in mosquitoes reported by Dimond & Lea (1956) and Singh & Brown (1957). During the course of experiments, we noticed that the insemination of mosquitoes influenced the stimulation of follicular development. The present paper deals with the latter subject in connection with problems of the mechanism of egg production in mosquitoes.

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Materials and Methods

A laboratory colony of *Culex tritaeniorhynchus summorosus* Dyar which has been maintained in the National Institute of Health was used. Larvae were bred to pupae with dried yeast at 28°C under a continuous illumination. Five hundred to 1,000 pupae as a sum of males and females were transferred to a mosquito cage of 30 cm cube and kept in the insectary at 28C and 16 hr light per day. Mosquitoes emerged were bred for first 5 to 7 days with 2% sugar water. Then, each of three concentrations (0.5, 1.5 and 3.0%) of LAH (Wako Pure Chemical Industries) in 2% sugar water was soaked in cotton pad and given to mosquitoes for 6 to 10 days without oviposition site. LAH-sugar water was not sterilized but renewed every day.

After feeding of LAH, females were randomly sampled and dissected in saline. Insemination was examined by the presence of sperms in the spermathecae. The developmental stages of egg follicles were recorded basing upon the categories of Christophers (1911) revised by Clements (1963). When the follicles were observed to be seemingly undeveloped and kept at State II or smaller, ovaries were dissected and observed further for the presence or absence of relics on ovarioles. The statistical analysis was made following Fischer's direct calculation of probabilities (P) in 2×2 tables.

Results

1. Follicular development with different concentrations of LAH

When female mosquitoes were fed LAH suspension, the follicular development was accelerated in various patterns. Mosquitoes with matured eggs were commonly observed, but the number of matured eggs produced by one mosquito was small ranging from only one to about 50. The maturation of follicles was not completed in some mosquitoes and most of follicles stayed at Stage III or IV. In the extreme case, complete degeneration of follicles occurred forming a relic on ovariole and the second follicles were kept at Stage Ia or Ib. Degeneration of follicles was also commonly observed in ovaries with either incompletely or completely matured eggs.

The ratio of follicular development, presented as the percentage of mosquitoes in which the follicular development was stimulated in any sense to the total of mosquitoes examined, gradually increased in accordance with the increase of LAH concentration, if the results of experiments repeated several times were summed up (Fig. 1, left). When those ratios were calculated separately for mated and unmated females, a prominently higher ratio in mated females than in unmated females was observed in every series of LAH concentrations (Fig. 1, right).

There was no difference between ratios in mated females which were fed 1.5% and 3% LAH. The statistical analysis attempted for each experimental cage indicated that the difference in ratio of follicular development between mated and unmated females was most significant in 1.5% LAH series as shown in Table 1.

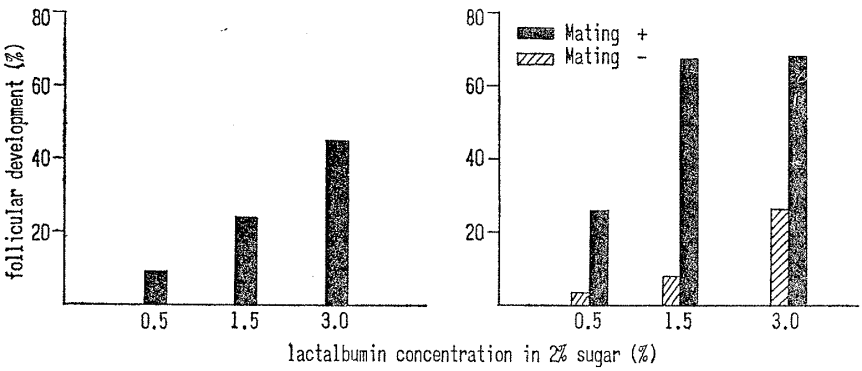

Fig. 1. Stimulation of follicular development in *Culex tritaeniorhynchus* which was fed three different concentrations of lactalbumin hydrolysate (LAH). Left: percentage of follicular development in total females; right: differentiation of follicular development between mated and unmated females.

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Table 1. Statistical analysis of the mating effect on the follicular development of *Culex tritaeniorhynchus* which was fed three different concentrations of lactalbumin hydrolysate (LAH).

Lactalbumin concentration 0.5 %

		follicle development		
		+	−	
mating	+	2	2	4
	−	1	25	26
		3	27	30

P=0.038
Difference +

	+	−	
+	1	5	6
−	1	23	24
	2	28	30

P=0.366
Difference −

	+	−	
+	1	3	4
−	1	25	26
	2	28	30

P=0.253
Difference −

	+	−	
+	6	4	10
−	0	10	10
	6	14	20

P=0.005
Difference +

	+	−	
+	3	12	15
−	0	5	5
	3	17	20

P=0.399
Difference −

Lactalbumin concentration 1.5 %

		follicle development		
		+	−	
mating	+	6	1	7
	−	4	19	23
		10	20	30

P=0.002
Difference +

	+	−	
+	2	3	5
−	0	25	25
	2	28	30

P=0.022
Difference +

	+	−	
+	6	4	10
−	3	17	20
	9	21	30

P=0.018
Difference +

	+	−	
+	7	3	10
−	1	19	20
	8	22	30

P<0.001
Difference +

	+	−	
+	6	2	8
−	1	21	22
	7	23	30

P<0.001
Difference +

Lactalbumin concentration 3.0 %

		follicle development		
		+	−	
mating	+	12	4	16
	−	1	3	4
		13	7	20

P=0.101
Difference −

	+	−	
+	1	2	3
−	8	9	17
	9	11	20

P=0.05
Difference ?

	+	−	
+	11	5	16
−	0	4	4
	11	9	20

P=0.026
Difference +

2. Further observations of the effect of mating

The study was thus extended to confirm the acceleration of follicular development by the insemination of mosquitoes. This was made in two series of experiments with different conditions to control the mating rate.

In the first series of experiment, mosquito cages were kept in the insectary with 16 hr light plus 3 hr dim light per day which could be expected to produce a high rate of mating. Two cages were prepared for each of three LAH concentrations and 60 to 80 females were examined from each cage. As shown in Table 2, the difference in ratio between mated and unmated females were highly significant in every concentration of LAH.

In the second series, mosquitoes from the same batch of larval breeding were separated into two cages and kept in the insectary with 16 hr light plus 8 hr dim light to give nearly 100% mating rate, although every newly emerged male was removed from one of

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these two cages to inhibit mating. Thirty females fed on LAH were sampled from each cage and the ratio of follicular development in mated females from one cage was compared with that in unmated females from the other without males. This series of experiment was also made for 3 concentrations of LAH but not repeated. The difference was also quite clear as shown in Table 3, and the significance was most prominent in mosquitoes fed 1.5% LAH.

Table 2. Further analysis of the mating effect on the follicular development of *Culex triteaniorhynchus* which was fed three different concentrations of lactalbumin hydrolysate (LAH), first series. Refer to text.

		Lactalbumin concentration 0.5 %			Lactalbumin concentration 1.5 %			Lactalbumin concentration 3.0 %		
		follicle development								
		+	−							
mating	+	24	27	51	38	8	46	54	2	56
	−	0	9	9	4	10	14	2	2	4
		24	36	60	42	18	60	56	4	60
		P=0.0064 Difference +			P=0.0003 Difference +			P=0.019 Difference +		
		+	−		+	−		+	−	
	+	17	15	32	54	1	55	68	0	68
	−	4	24	28	2	3	5	6	6	12
		21	39	60	56	4	60	74	6	80
		P=0.0027 Difference +			P=0.0011 Difference +			P=0.000003 Difference +		

Table 3. Further analysis of the mating effect on the follicular development of *Culex tritaeniorhynchus* which was fed three different concentrations of lactalbumin hydrolysate (LAH), second series. Refer to text.

		Lactalbumin concentration 0.5 %			Lactalbumin concentration 1.5 %			Lactalbumin concentration 3.0 %		
		follicle development								
		+	−		+	−		+	−	
mating	+	16	9	25	24	0	24	24	2	26
	−	10	20	30	11	19	30	14	16	30
		26	29	55	35	19	54	38	18	56
		P=0.022 Difference +			P=0.0000003 Difference +			P=0.0002 Difference +		

Discussion

The suppression of follicular development in virgin mosquitoes after their blood feeding was firstly recorded by Roy (1940) in *Anopheles subpictus*. This phenomenon was also found by Bates (1954) in *Anopheles superpictus* and *A. maculipennis* group. But it was not accepted by Clements (1963), who states: "It has been found in many species that virgin females develop their ovaries in the normal manner after feeding but that in many cases they will not lay these eggs until they have mated, and it is probably either this fact or the difficulty of stimulating ovary development in some species in the laboratory which has led to claim that insemination is a prerequisite of ovary development." In fact, as reported in many species of culicine mosquitoes, there is no difference in follicular development between mated and unmated females after blood feeding but the oviposition may be inhibited in virgins. The situation is quite same even in *Culex tritaeniorhynchus*. Although we have not mentioned it in Results, both mated and unmated females of our material equally stimulated follicular development into maturation when they engorged laboratory mice.

In some hemimetabolous insects, as reviewed by Engelmann (1970), the insemination will stimulate the neuroendocrine system and the consequent oocyte growth. The most typical case is in Cimicidae as reported by Mellanby (1939) and Davis (1964, 1965a, 1965b), where the mating and blood feeding are acting synergistically for egg maturation. The

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synergism may also be maintained potentially in mosquitoes, but it may be masked in most cases. It has been convinced that the mechanical "mid-gut stimuli" are the trigger for the activation of endocrine system in mosquitoes. When mosquitoes took blood meals, the mid-gut stimuli might be probably so much high as to conceal the mating stimuli. Thus, the mating stimuli could be only manifested positively with a quantitative relation to the mid-gut stimuli. This could be ascertained from the present findings which show the most significant effect of mating at feedings of a proper concentration of LAH in contrast to the cases at higher and lower concentrations. However, the mechanism of mating stimuli in mosquitoes is left unclear.

In LAH-fed mosquitoes, the stimulation of follicular development did not result in the maturation of every egg, but degeneration of follicles was commonly observed. The number of matured eggs produced by LAH feeders was far smaller than in the case of blood feeding. Former authors believed that egg maturation or degeneration of follicles may be determined directly by nutritional factors (Nicholson 1921, Hosoi 1954). From this viewpoint, the LAH-diet might be nutritionally defficient for mosquitoes. But it will be also possible to consider that such defficient nutrient may act indirectly through the hormonal regulations.

The finding presented herein shall offer some other problems for the reproductive physiology of mosquitoes. Firstly, it is recommended that only mated females should be used for studies of the hormonal control of follicular development when blood meals are not given to unautogenous species. Secondly, even if follicles are seemingly undeveloped, the absence of follicular degeneration must be checked by dissection of the ovaries.

Summary

The follicular development was promoted when females of *Culex tritaeniorhynchus* were fed lactalbumin hydrolysate (LAH) suspended in sugar water. The ratio of the follicular development increased in accordance with the increasing concentration of LAH, and it was always higher in mated than in unmated females in every concentration of LAH-diet. The effect of mating appeared most significantly when mosquitoes were fed 1.5% LAH, whereas it was less prominent at higher (3.0%) and lower (0.5%) concentrations. It is discussed that in mosquitoes the mating may be potentially acting synergistically with blood feeding for egg production as in the case of Cimicidae.

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^{14}C INTAKE OF NILAPARVATA LUGENS (STÅL) (HOM., DELPHACIDAE) OF DIFFERENT SEXES AND WING-FORMS FROM ^{14}C-LABELLED RICE SEEDLINGS

Osamu Mochida

Kyûshû National Agricultural Experiment Station, Chikugo-shi, Fukuoka-ken, 833 Japan

The difference between the male and female, and the macropterous and brachypterous forms of *N. lugens*, a serious insect pest on rice, has been mainly discussed in the morphological (Hasegawa 1955; Kisimoto 1957; Mochida 1970), bionomical (Kisimoto 1957; Suenaga 1963; Mochida 1964ab, 1970), anatomical, and histological aspects (Mochida 1970). However, the difference in the amount of plant-sap ingested by the males and females of the two wing-forms has not yet been studied despite the economic importance of the delphacid. The present paper deals with the amount of ^{14}C ingested by *N. lugens* of different sexes, wing-forms, and developmental stages.

Methods

Seven days' seedlings of the paddy rice variety 'Toyotama' were photosynthesized in $^{14}CO_2$ (100 ml chamber, 1% CO_2, ^{14}C 10 μCi or 3000 ml chamber, 1% CO_2, ^{14}C 1000 μCi). Adults or 5th-instar nymphs were kept on the ^{14}C-labelled seedlings for 24 hours. After feeding, the insects were immersed in a scintillation solution, Toluene — PPO (2, 5-diphenyloxazole) — POPOP (1, 4-bis-2-(5-phenyloxazolyl)-benzene), and the ^{14}C in the insects was measured by a Tri-Carb liquid scintillation counter (Packard, model 2002). The ^{14}C in the seedlings used was assayed in a scintillation solution, PPO (4 g)-POPOP (0.1 g)-Naphthalen (75 g) in Toluene:Dioxane:Ethylcellosolve (1:1:1 v/v), after they were homogenized. All the insects were reared at about 27°C.

Results and discussion

A group of 12 adults, 3 for each sex and wing-form, was reared on one ^{14}C-labelled seedling (about 8.3×10^5 cpm) for 24 hours, and the amount of ^{14}C of the insects was measured. The amount of ^{14}C of 5th-instar nymphs was also measured from a group of nine 5th-instar nymphs and nine macropterous males kept together on another ^{14}C-labelled seedling for 24 hours.

The means and relative values for the amount of ^{14}C ingested are given in Table 1. In the ability for the ^{14}C intake males were smaller than females, and macropterous adults of each sex were smaller than brachypterous ones. The ^{14}C intake of 5th-instar nymphs was about 2 to 6 times as large as that of adults.

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