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**Observations on the Diurnal Activity of a Japanese
Common Mosquito, *Culex pipiens* var. *pallens*
Coquillett¹⁾**

By

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(With 5 Text-figures)

Up to the present, it has been repeatedly reported that, out of the environmental meteorological factors the light is the most effective upon the diurnal activity of mosquitoes. But the other environmental factors, i.e., temperature, humidity and water bodies etc. change also their cycles every day. It is, therefore, desirable to investigate the direct influence of the light upon the activity itself. The diurnal cycle of the mosquito has hitherto been scarcely studied: probably because of various difficulties using mosquito in the experiment. Recently Satô (1950) reported the migration of rhabdom due to the change of photic environments in the compound eye of *Culex pipiens* var. *pallens* Coquillett. The present research was designed to secure any information on the relationship between the rhabdom migration and the diurnal activity of the insect.

Before proceeding further, the writer wishes to express his cordial thanks to Professor Tohru Uchida for his instructive guidance and his kindness in reading through the manuscript.

Material and method

The mosquitoes used in this study were collected in the University farm. For the observation of diurnal activity, a large cage (0.6 m in width, 1.8 m in height), with all sides covered with Victorian lawn, was set in a darkroom. Under the natural and artificial light conditions, the number of flying individuals was counted every five minutes and the hourly summation of these counts was employed as an indicator of activity. In the case of the artificial light condition, a 100 watt electric lamp was situated at the distance of 80 cm above the cage and the brightness was 30–40 lux at the center of the cage. In the natural light condition,

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the solar ray was directly taken through the window faced to the north. Light intensity and humidity were measured with a Mazd's photometer (type 1-A) and a wet-and-dry bulb hygrometer respectively. In order to investigate the process of rhabdom migration in the compound eye, the specimens were fixed into Bouin's and Kahle's fluid. After embedding with the ordinary paraffin method, their heads were cut 10μ in thickness and stained with Delafield's iron haematoxylin and eosin.

Results

1. Diurnal activity

The experiment was carried out 4 times during the period from the last decade of August to the first of October 1952. As a representative example, the result of the observation on August 25th will be described in the following (Fig. 1).

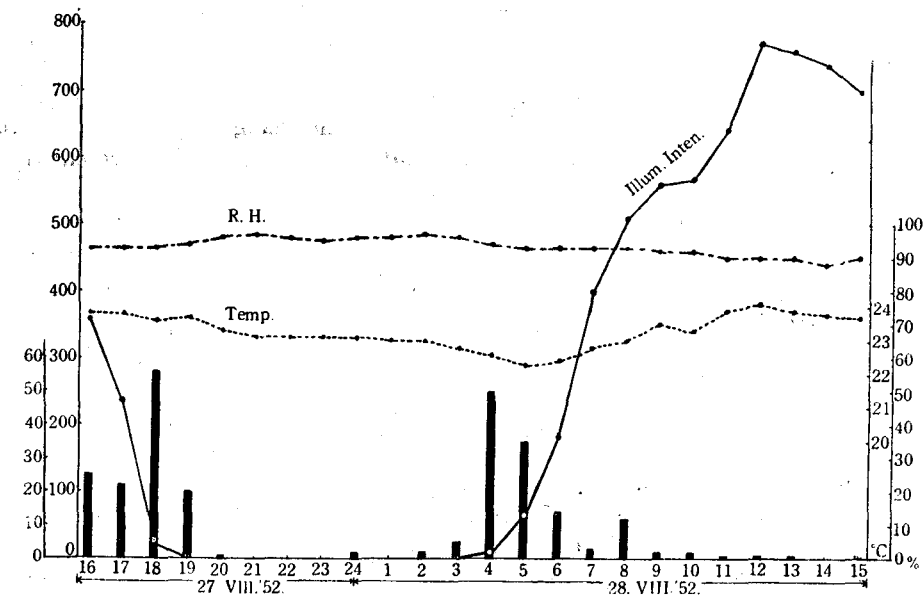


Fig. 1. Diurnal rhythm of flying activity and those of meteorological elements.
 Ordinate: individual number & illumination intensity in lux on left, temperature in °C & relative humidity in % on right.
 Abscissa: time in 24 hours. Black rods show number of flying individual.

When the illumination intensity decreased gradually at about 15.00, the individuals that had quietly settled by this time on the net wall scatterly began to fly and at once alighted on the wall of opposite side. At 18.00, according to rapid falling of

light intensity from 230 lux to 5 lux, the flight was aroused frequently and lasted for a fairly long time. Within 5 minutes after decrease of the intensity to 0 lux at about 18.40, a swarming was just found composed of ca. 20 individuals near the ceiling of the cage. In the midnight, only when illuminated with weak light below 1 lux, several flights could be noticed. Again, in the morning twilight the insects began to fly corresponding to the slight change of illumination from 0 to 2 lux and instantly formed a swarming. Thus, in the activity of *Culex pipiens*, as generally is expected, two phases are also recognized in a day, namely in the morning and evening; in which the light intensity rapidly changes and then photic environment completely reverses the order within very short time. Such a transitional light environment seems to give a stimulus to the photoreceptor of mosquitoes and consequently to accelerate the activity of the insects. Therefore, the time of the maximal activity in the evening was between 18.00 and 19.00 on this experiment-day but after about a month i.e., on October 3rd the time shifted to between 17.00 and 18.00 in accordance with the shift of the sunset (Fig. 2).

2. The experimental study with an artificial light source

As stated above, it appears that light is the most effective upon the activity

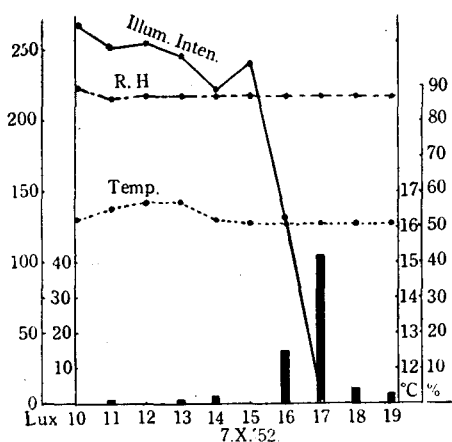


Fig. 2.

Fig. 2. Time of maximal activity at evening became earlier an hour than that of September according to a shift of sunset. Ordinate: left, intensity of illumination in lux & number of flying individual; right, temperature in °C & relative humidity in %. Abscissa: time in a day.

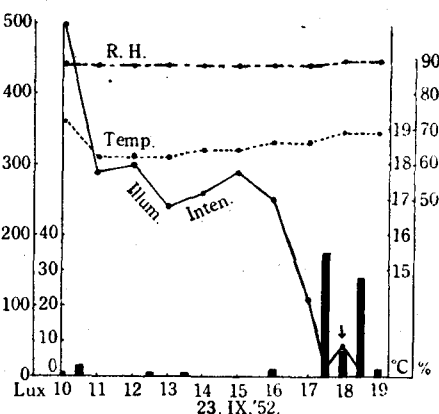


Fig. 3.

Fig. 3. Suppression of activity by an artificial light under natural light conditions. Ordinate: left, individual number & illumination intensity in lux; right, temperature in °C & relative humidity in %. Abscissa: time in a day. Arrow shows the time at which flight of mosquito was remarkably suppressed by lighting after sunset.

of the mosquito. In order to analysis the diurnal activity, the following experiments were executed with an artificial light source. On September 23th (cloudy throughout the day from morning), 40 individuals, including 20 males and 20 females, that had newly hatched out the day before were put all together into a cage. Thereafter the observation was continued from 10.30 under natural light conditions. When the light intensity became 0 lux at 17.30, a swarming was found instantly. After 20 minutes, it became still gloomier in the room and consequently the flying individuals went on increasing. Then, the electric lamp was lighted, and the activity fell off immediately and the majority of the insects alighted on the side net except only one individual that was flying throughout the lighting. The flying activity was, thus temporarily suppressed by lighting but after 30 minutes putting out the lamp, they flied out again and soon became as active as in the previous state. When the darkness was continued, however, flying individuals diminished gradually and disappeared within 30 minutes (Fig. 3).

Then, is the activity suppressed by the light alone? As is anticipated from

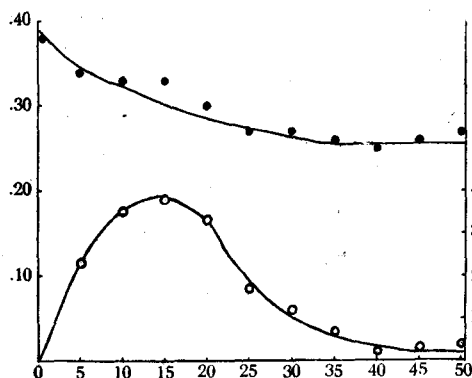


Fig. 4.

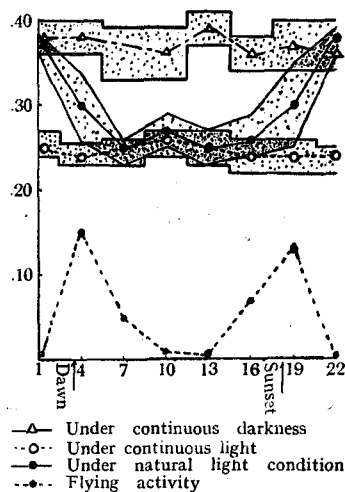


Fig. 5.

Fig. 4. Light adaptation of flying activity and that of rhabdom migration after lighting. *Ordinate*: index of rhabdom migration on left, number of flying individual on right. *Abscissa*: time in minutes after lighting. At 25 minutes after lighting, flying activity fell suddenly in accordance with the accomplishment of rhabdom migration.

Fig. 5. Daily change of I.R.M. and of the confidence limits under various conditions, that of flying activity. *Ordinate*: time in 24 hours. Black frames show the confidence limits of I.R.M. under various conditions. Time of maximal activity well corresponds to the time, in which rhabdom migration is in progress.

the natural light conditions, the activity of the individuals that were in dark environment seems rather to be accelerated by lighting. To verify this assumption, the flying number of the dark-adapted individuals was counted every 5 minutes after lighting of 100 watt electric lamp and the result was shown in Fig. 4. Simultaneously with lighting the activity was promptly raised and attained to the maximum after 15 minutes. At the time of 25 minutes the fall of the activity was striking and since then a remarkable change was scarcely seen. In this way the activity was suppressed or accelerated by light in accordance with the physiological state of the insect.

According to Satô (1950), the rhabdom in the dark-adapted eye of *Culex pipiens* migrates to the distal part of the retina and enters into the iris part, while it migrates proximally in the light-adapted eye. In order to investigate the process of the light adaptation in the eye, the writer after having placed the mosquitoes in a darkroom for 24 hours, decapitated every 5 minutes under lighting and fixed the heads. 8-10 specimens adequately prepared were selected from each of the series mentioned above, and 5 ommatidia were selected from every specimen and measured with the following manner. The method used by Satô was slightly modified as follows: the distance from the basement membrane to the proximal end of the rhabdom was divided with that from the basement membrane to the distal end of the iris pigment layer, which is hardly movable by the change of photic environment. The ratio thus obtained was named as the index of rhabdom migration (I. R. M.). The results were statistically arranged and the confidence limits of the means were determined (Tab. 1). From the table the rhabdom migration is evidently recognized, namely the rhabdom moves instantly with lighting and seems almost to accomplish the light adaptation in 25 minutes. Comparing the results with the curve of flying activity previously secured under light environment, it is interesting that the flying activity is the highest a little before the light adaptation of rhabdom and then suddenly falls with the complement of the migration (Fig. 4).

Table 1. Change of mean of I.R.M. and the confidence limits under 100-watt electric lamp, (in 95% reliability)

Min.	Mean	Confidence limits
0	.38	.40—.36
5	.34	.36—.32
10	.33	.34—.32
15	.33	.34—.31
20	.30	.31—.29
25	.27	.30—.23
30	.27	.29—.25
35	.26	.28—.24
40	.25	.27—.23
45	.26	.27—.23
50	.27	.29—.25

3. Diurnal rhythm of the rhabdom migration

It has been distinctly recognized that the flight is closely related to the rhabdom migration. Then we should pay an attention to the daily migration of the rhabdom under natural light conditions, namely to the problem how the daily migration goes and whether the migration is caused by the inner physiological

rhythm or only by the daily variation of environmental factors. Hence, the daily progress of the rhabdom under the natural light condition, continuous lighting or continuous darkness has been respectively measured with the previously stated method as in Tab. 2 & Fig. 5. The diurnal rhythm of the migration was, as ex-

Table 2. Daily change of means of I.R.M. and the confidence limits under various conditions, (in 95% reliability)

Hour	Natural light	Continuous darkness	Continuous lighting
16.00	.27 (.29—.24)	.36 (.38—.34)	.24 (.26—.22)
19.00	.30 (.35—.25)	.37 (.40—.34)	.24 (.26—.22)
22.00	.38 (.39—.37)	.36 (.38—.34)	.24 (.25—.22)
1.00	.37 (.38—.36)	.38 (.40—.36)	.25 (.27—.24)
4.00	.30 (.34—.26)	.38 (.40—.36)	.24 (.24—.24)
7.00	.25 (.26—.23)		.25 (.26—.23)
10.00	.27 (.29—.25)	.36 (.39—.33)	.26 (.27—.24)
13.00	.25 (.27—.24)	.39 (.41—.37)	.25 (.27—.23)

pected, clearly confirmed. I. R. M. in both the daytime and night are almost constant, i.e., about 0.25 and 0.37 respectively. At 4.00 in the morning and at 19.00 in the evening, however, the values are in the middle of the both mentioned above, furthermore the ranges of the confidence limits are also remarkably extensive as compared with that of other times. These facts seem to show that in some individuals the rhabdom migration was finished at these times and in other the migration was not yet started or now progressive. In comparing this result with the diurnal flying activity, it is clear that the rapid migration of the rhabdom well corresponds to the phase of the activity. On the other hand, the values of I. R. M. under continuous lighting or darkness only fluctuate within the narrow range of the confidence limits, and such a periodicity observed in the natural light condition did not take place. Accordingly, it can be concluded that the migration is caused by the change of light intensity but not by the inner physiological rhythm.

4. The influence of temperature and humidity

The factors above given seem to be not so considerable effect upon the flying activity of the mosquitoes. The result coincides with the experiment in the field of Yoshida et al. (1947) in the point that temperature and humidity only play the secondary rôle on the diurnal activity of *Culex pipiens*. Therefore, only the temperature and humidity in the laboratory were estimated as in Tab. 3. In the table, the data of 4.00 and 18.00 have been excluded, because the activity might be evoked by the sudden change of the light intensity. Judging from the table, the optimal temperature seems to be 22–23°C in both the mean and the total number of flying individuals. Judging from the means of the flying individual, the optimal range of humidity appears to be 93–94 % and does not attain even

Table 3. Correlation table between temperature and humidity
Relative Humidity in %

Relative Humidity in %													
	77-78	79-80	81-82	83-84	85-86	87-88	89-90	91-92	93-94	95-96	97-98	Tot.	Mean
Temperature °C													
16-17						18						18	6
18-19						9	37	33				79	4.6
20-21													
22-23	1	5	3	3			13	21	126	8	2	182	7.9
24							1					1	1
Tot.	1	5	3	3		27	51	54	126	8	2	280	6.3
Mean	1	5	3	3		3.3	5.1	5.0	16.5	2.6	2		

a half of the total. The optimum is possibly not confined to only the range of 93-94 % and seems to extend over the range of 89-94 %.

Discussion

Collins (1934) reported that in the compound eye of Codling moth, *Carpocapsa pomonella* Linn., the pigment movements well correspond their vital activity i.e., mating, oviposition, feeding etc. According to him, the moth becomes active proportional to the degree of the pigment movements caused by a rapid change of light intensity in the morning and evening twilight. In the compound eye of *Culex pipiens* in which the pigment moves hardly except the rhabdom-pigment, the rhabdom migrates swiftly according to the change of light intensity and seems to keep the brightness which is accepted by the eye approximately constant. The rhabdom migration is finished in a comparative short time, namely within 10-20 minutes (Satô, 1950), but in the present research it seems to take at least 25 minutes for the completion under the change from 0 to 30-40 lux. In progress of the migration the flying activity is accelerated and reaches the maximum just before the completion, but abruptly falls simultaneously when the migration was finished. Such a phenomenon is obviously seen also in the natural light condition. That is to say, during each period of 4.00-5.00 and 18.00-19.00, in which the photic environment is changing rapidly and the rhabdom migration is in progress, the flight takes place most frequently and even the swarming is often formed. Once the eye, however, finished the adaptation, the flight was observed rarely to occur (Fig. 5).

Considering from the response of *Culex pipiens* to the light, it seems that the excitability considerably differs in the daytime and in the night. In the daytime, so far as the considerable difference of light intensity (200-300 lux) does not occur, the insects fly unfrequently but in the night they are declined to fly out easily according to the slight change of the intensity (from 0 to 1-2 lux). Yoshida et al. (1947) observed that in the midnight the active flight of *Culex pipiens* was

arosed only by the moonlight, in spite of the invariability of other environmental factors. These facts seem to show that the photo-sensitivity of the dark-adapted individuals is highly increased than that of the light-adapted ones.

At present, our information is very little as regards the mechanism of rhabdom migration. It has been merely known that the rhabdom migration is caused by changes of environmental factors, especially by changes of light intensity and not by inner physiological rhythm. Wigglesworth (1950) stated that the light entered into the eye brings about some photochemical changes in the substance of the rhabdom, whose products stimulate the surrounding sense cell. In the eye of *Culex pipiens* a fine fibrillar structure is seen at the intermediate zone between the rhabdom and the surrounding pigment layer. These fibrils have been regarded as the nerve ending of optic ganglion (Hesse 1091, Aino 1933, Satô 1950). Accordingly, from the facts stated above, it is deduced that the light directly causes photochemical reactions in the rhabdom, whose products stimulate the optic nerve ending, and through this innervation the rhabdom migration takes place immediately and evokes the flying activity indirectly.

Summary

1. The diurnal flying activity of the Japanese common mosquito, *Culex pipiens* var. *pallens* Coquillett well coincides with the daily progress of the rhabdom in the eye. Namely, corresponding to the rapid change of the light intensity in the morning and evening twilight, the rhabdom quickly migrates from either adapted position to other adapted position. During the migration, the insects fly about most vigorously and become suddenly inactive simultaneously with the completion of the migration.

2. The rhabdom migration is caused by the change of light intensity and has no physiological rhythm in itself. It seems, therefore, to be permissible to conclude that the flying activity of the insects is governed only by the outer environmental factors, mainly the light.

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