

Experimental hibernation of *Culex tritaeniorhynchus* in Nagasaki, Japan*

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Abstract : To examine experimentally the suitable conditions for the hibernation of *Culex tritaeniorhynchus*, adult mosquitoes which received various pretreatment were exposed to different hibernating conditions. The results of the experiment show that : The place where is nearly dark day and night and mild and possibly stable in air conditions seems to be the most favorable for the hibernation of the mosquito ; the place where is nearly dark, though greatly variable in air conditions to be not much unfavorable ; while, the place where is light in daytime to be unfavorable.

Introduction

To examine the favorable conditions for hibernation of adult mosquitoes of *Culex tritaeniorhynchus*, three places of different environmental conditions were selected as experimental hibernating sites. One is nearly dark, and mild and little variable in temperature : the second is nearly dark but colder and much variable in temperature ; the third is light in the daytime and medium in temperature condition. Under

the different environmental conditions fed females on healthy man, unfed females and males were kept and the survival rates of them were examined. The survival rates of females were compared with those obtained with females which were infected with Japanese encephalitis virus (JE virus) and kept at 27°C or 24°C for some days before they were transferred to the above hibernating sites.

Material and Method

Culex tritaeniorhynchus used in this experiment was from our laboratory colony. Pupae from

stock colony at 27°C were transferred to the laboratory on October 23, 1964 where adults

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Table 1. Characteristics of experimental hibernating sites represented by the environmental conditions during from December to February in 1964-65, and the number of *C. tritaeniorhynchus* adults exposed to the conditions

Experimental hibernating site	Temperatures (°C)				Relative humidity		Range of illumination (Lux) inside the cage	No. of adults exposed		
	in general	M.Max.	M.Min.	Mean	Rang	Mean		Fed ♀	Unfed	
									♀	♂
Cellar	Mild	12.3	11.0	11.6	70-93	84.6	0.0-0.1	54	199	186
Animal house	Cold	11.8	4.2	8.0	47-90	69.3	0.0-0.2	61	187	189
Landing	Medium	11.6	8.3	9.9	55-100	78.2	240-360	55	193	193

were emerged on the 27th. They were fed on 5% sugar solution, while some of females were starved one day and fed on healthy man on the 29th. The room temperature from October 23 to 29 averaged 23.2°C ranging from 22.4°C to 24°C. On the 29th, unfed females and males, and fed females were transferred to each hibernating site (Table 1).

As experimental hibernating sites, three places were selected. The first place is the cellar of our Institute where it was mild and less variable in temperature and nearly dark day and night. The second is a room in the house for experimental animals which was attached to the Institute. The room was windswept and cold and much variable in temperature but was nearly dark day and night. The third was a landing of north side stairway leading from the third floor to the roof. The landing was medium in temperature and was light in the daytime and dark at night. The environmental conditions are given in Table 1 together with the number of females, unfed females and males which were transferred to

each hibernating site on October 29, 1964. The mosquitoes were reared on 5 percent sugar solution and examined for the number of the dead throughout the experiment.

The states of survival of these females which received no special treatment before subsection to the hibernating conditions were compared in Table 2 with those of females which were pre-treated, that is, reared at 27°C, infected at 27°C with JE virus, and incubated at 27°C or 24°C for some days before they were transferred to the three hibernating sites by Mifune (1965), one of the authors. In his experiments, females emerged at 27°C on the end of October were fed on 0.5 percent sugar solution for 2 to 3 days and starved for one or two days. On the following day they were fed on JE virus suspension. Some of them were incubated continuously at 27°C or 27°C and 21°C, while others at 24°C or 24°C and 21°C, for some days. After incubation they were reared on 2 percent sugar solution throughout the experiment.

Result of experiments

Weekly changes in percentage survival of fed females, unfed females and males kept at the cellar, animal house, and landing from the end of October 1964 to June 1965 were illustrated in

Figs. 1, 2, and 3 respectively, together with the weekly means of relative humidity and maximum, mean, and minimum temperatures in each site. The characteristics of environmental conditions of

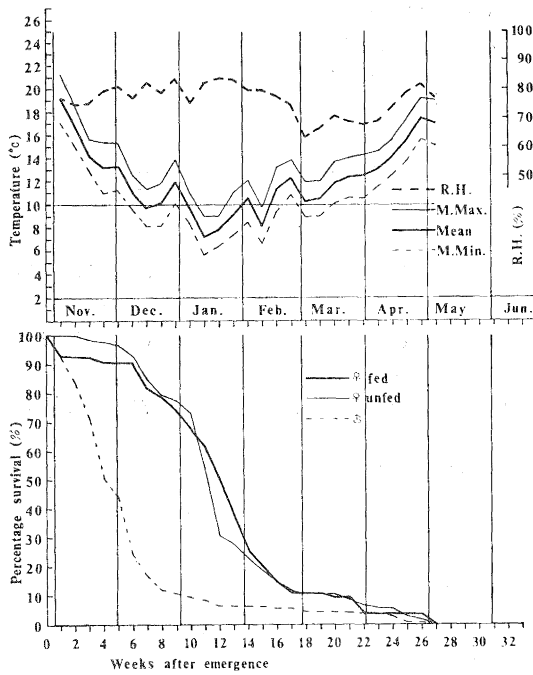


Fig. 1 Percentage survival of adult mosquitoes of *G. tritaeniorhynchus* kept at the cellar in 1964-65

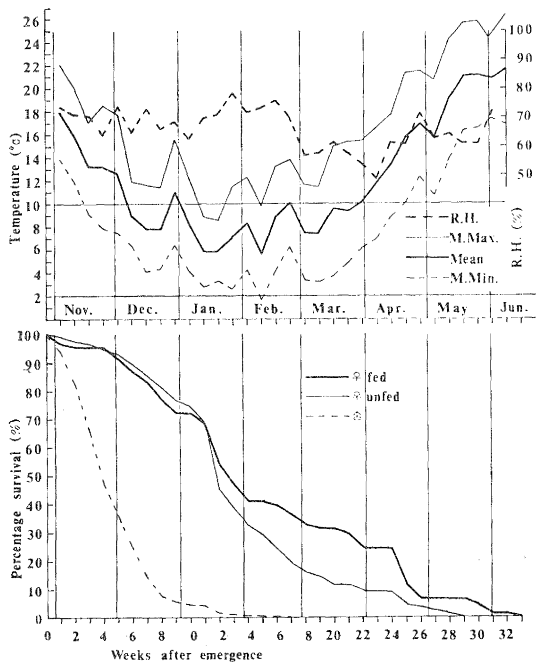


Fig. 2 Percentage survival of adult mosquitoes of *G. tritaeniorhynchus* kept at the animal house in 1964-65

the sites in winter were also shown in Table 1.

The cellar (Fig. 1) was mild and stable in temperature and relatively high in humidity. The survival rate of fed females decreased very slowly during the first 2 months, rapidly during from early January through the end of February, and again very slowly thereafter taking an inverse sigmoid curve. About 20 percent females survived the winter (on the end of February) and the last one lived longest for 231 days or 33 weeks after emergence. In the case of unfed females, the survival rate decreased nearly the same way as fed ones but more slowly during from mid January to mid April and about 32 percent of them could survive the winter, while the last one lived longest for 224 days or 32 weeks. In the case of males the rate decreased rapidly from the beginning taking a concave curve and only 1.6 percent of them could survive the winter but the last two lived for 175 days or 25 weeks.

The animal house (Fig. 2) was windswept, cold and variable in temperature and humidity. The survival rate of both fed and unfed females decreased rather gradually and straightly and about 33 percent of the former and 16 percent of the latter survived the winter. The last one of the former lived for 231 days or 33 weeks and that of the latter did for 206 days or about 29 weeks. The rate in males decreased taking a concave curve nearly the same way as in the cellar but the last one lived only for 119 days or 17 weeks. It is to be noted that in spite of the great changes by day and in day and night temperatures, the females could survive nearly as long as those did in the cellar.

The landing (Fig. 3) was medium in air conditions. The trend of the curves for survival rates of fed and unfed females was nearly similar with each other taking an inverse sigmoid form. Only about 10 percent of both of them, however, could survive the winter and the longest survivor

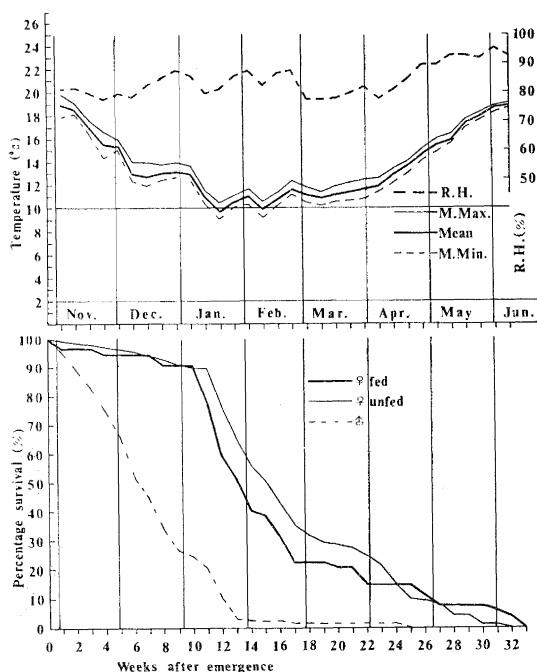


Fig. 3 percentage survival of adult mosquitoes of *C. tritaeniorhynchus* kept at the landing in 1964-65

lived for 189 days or 27 weeks. The decreasing trend of the survival rate of males was similar to those in the other two sites but in this case the last male lived for as long as 189 days or

Comparison of mortality of *C. tritaeniorhynchus* females which were exposed to different hibernating conditions after being subjected to various pretreatments (Table 2)

Here, pretreated females (Nos. 1 to 12) refer to those which were emerged at 27°C and reared on 0.5 percent sugar solution for 2 to 3 days, starved for next one or 2 days, and fed on the following day on JE virus suspension, and incubated continuously at 27°C or 24°C rearing on 2 percent sugar solution for definite days as illustrated in Table 2. After incubation, females of cages Nos. 1 to 6 were transferred to the cellar, Nos. 11 and 12 to the animal house, and Nos. 7 to 10 to the landing.

Control females (Nos. a to f) refer to those

by the beginning of May.

It was expected at the start of the experiment that, considering the air conditions at the three different sites, the cellar might be the most favorable for hibernation and the landing might be medium but the animal house less suitable. The results of experiments, however, show that the cellar was the most favorable as expected but the animal house was nearly the same as cellar or only a little less, and the landing was the least suitable.

Regarding the experimental hibernation of *C. tritaeniorhynchus*, it is summarized from Figs. 1, 2, and 3 that: Some of the females kept at darkness under natural variable air conditions can survive as long as about 33 weeks after emergence i. e. from the end of October through mid June. The decreasing curve of weekly survival rate of females takes a gentle inverse sigmoid form, while, that of males takes a sharp concave one. Fed females can live a little longer than unfed ones, while males can not live for only much shorter period than the females. Darkness appears to favor mosquitoes to live longer under hibernating conditions.

which were emerged and fed or left unfed at a laboratory mean temperature of 23.2°C before exposure to the three hibernating sites as already mentioned above. The control females were fed on 5 percent sugar solution throughout the experiment. The females of cages a and b, c and d, and e and f are the same as those shown in Figs. 1, 2, and 3 respectively as unfed and fed females.

Pretreated females were observed for longevity every day and some of them (marked with + in Table 2) were used for biting experiments,

Table 2 Comparison of mortality of *Culex tritaeniorhynchus* females kept under different hibernating conditions

(As for the results of infection with JE virus, cf. the paper published by Mifune in 1965)

Hibernating place	Infection	Cage No.	Date of emergence	Date of feeding on virus suspension	Days kept at 27°C, 24°C, or 21°C, from just after being infected		No. of females	Dates in weeks after emergence on which 50%, 80%, 90% and 100% mortalities were observed during subjection to different natural temperature conditions																																
					27°C	24°C or 21°C		11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33										
Cellar	with JE virus	1	Oct. 24	Oct. 27	1-7	8-13	42						50	80	90	100																								
		2	Oct. 25	Oct. 29	1-7	8-13	93				50	90	100				90	+	(8)	100																				
		3	Oct. 25	Oct. 29	1-7	8-13	51				50	80	90	100																										
		5	Nov. 1	Nov. 4	1-7	8-13	93				50	80	90	+	(19)																									
		4	Nov. 1	Nov. 4	1-7	8-13	95				50	80	90	+	(3)																									
		6	Nov. 1	Nov. 5	1-7	8-13	73				50	80	90	+	(3)																									
Animal house	with JE virus	a	Oct. 27	**	1-7	8-13	199						50	80	90	100																								
		b	Oct. 27	Oct. 29*	1-7	8-13	54				50	80	90	100																										
		11	Oct. 30	Nov. 2	1-7	8-13	90				80	90	100																											
		12	Nov. 1	Nov. 4	1-7	8-13	105				50	80	90	+	(5)																									
Landing	with JE virus	c	Oct. 27	**	1-7	8-13	187				50	80	90	100																										
		d	Oct. 27	Oct. 29*	1-7	8-13	61				50	80	90	100																										
		8	Oct. 30	Nov. 2	1-7	8-13	74				80	90	100																											
		7	Oct. 25	Oct. 29	1-7	8-13	94				90	100																												
Landing	with JE virus	10	Nov. 1	Nov. 6	1-7	8-13	67				80	90	100																											
		9	Nov. 1	Nov. 4	1-7	8-13	111				80	90	100																											
		e	Oct. 26	**	1-7	8-13	193				50	80	90	100																										
Landing	no infection	f	Oct. 26	Oct. 29*	1-7	8-13	55				80	90	100																											
												80	90	100																										

* : Females were fed on healthy man. ** : Females were reared only on sugar solution.
+ : On the date in weeks the survived females (given in parentheses) were used by Mifune (1965) to have them bite on susceptible pigs.

while control ones were observed once a week throughout the experiment. To make easy to compare with each other the states of longevity of females of different batches, dates in weeks on which 50%, 80%, 90%, and 100% mortalities were observed were marked with figures 50, 80, 90, and 100 with each batch respectively.

Table 2 shows that: In general, the control females could survive for much longer period than the pretreated ones and the females pretreated at 24°C appear to be able to live a little longer than those pretreated continuously at

27°C, showing that rearing at higher temperature before exposure seems to consume to some extent the vitality in mosquitoes. The animal house seems to be nearly the same or a little less favorable than the cellar, while, the landing to be the least suitable for pretreated females as well as control ones, suggesting that the place where is dark and stable in air condition is the best, dark though variable in air condition is next, and light in the daytime though medium in air condition is the least suitable for the hibernation of the mosquito.

Summary

Hibernation experiments were carried out to find out the most favorable hibernating conditions for adults of *Culex tritaeniorhynchus*. For that purpose, three places of different environmental conditions were selected: The cellar was nearly dark day and night, mild and rather stable in air conditions; the animal house was also nearly dark but rather cold and greatly variable in air conditions; and the landing was light in the daytime and medium in air conditions. Fed females and unfed females and males emerged and fed in the laboratory at about 23.2°C on the end of October, 1964 were transferred to the experimental hibernating sites, counting the dead once a week. The results of the above control experiments were compared with those obtained with the pretreated females i.e. those emerged and infected with JE virus at 27°C and incubated continuously at 27°C or at 24°C for some days before they were exposed to the hibernating conditions.

The results of the control experiments show that: Some of the females which were kept at

darkness under stable or even greatly variable air conditions could survive as long as about 33 weeks after emergence i.e. from late October to mid June; fed females could live a little longer than unfed ones, while males could not survive for only much shorter period than the females.

The results of experiments on pretreated females show that: They could not live for only shorter period by one or two months than control ones and mostly died by mid or late April; females incubated at 27°C were a little shorter in longevity than those incubated at 24°C; for pretreated females, the cellar seems also to be the most favorable for hibernation, while the animal house and landing to be less suitable.

In conclusion, the place where is nearly dark day and night and mild and stable in air conditions seems to be the most favorable for the hibernation of the mosquito, and the place where is nearly dark and greatly variable in air conditions seems only a little less favorable, while the place where is light in the daytime though medium in air conditions seems not favorable.

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コガタアカイエカの越冬実験. 大森南三郎, 伊藤寿美代, 武富正彦, 長崎大学医学部医動物学教室, 長崎大学風土病研究所衛生動物部. 三舟求真, 七条明久, 林薫, 長崎大学風土病研究所病理学部

総 括

コガタアカイエカの越冬に好適な条件を究明するために1964年10月末に羽化した成虫を夫々条件の異なる3ヶ所に入れて、その生存状況を比較した。3ヶ所の内、第一の地下室は昼夜共殆んど暗黒で、冬期の温度が比較的高く、温湿度の変化及び日差の非常に少ない所で、第二の動物舎内の一室は昼夜共殆んど暗黒ではあったが、外気が直接影響して冬期の温度が可成り低く、変化の激しい所、第三の三階から屋上への北側階段の踊り場は、昼間は明るく夜間のみ暗く、温湿度条件は前二者の中間にあった。

実験に供した成虫の一半は、羽化から越冬場所へ移す迄の間室内(約23°C)で飼育したもので、他の一半は著者の一人三舟(1965)が日本脳炎の感染実験に使用したもので越冬場所へ移す前27°Cで羽化させウイルス懸濁液を摂取させた後27°C又は24°Cで一定期間飼育したもので、即ち10数日高温度で処理したものである。これらを10月末又は11月初めに各越冬環境に移し、その後前者では毎週1回、後者では毎日死亡数を調べた。その結果、室温で羽化、吸血させた蚊は最長33週即ち10月末から6月中旬迄生存し、未吸血蚊より多少長く生存し得た。生存率曲線は共にゆるやかな逆シグモイド型をとる。冬の生存期間は一般に極めて短く、生存率曲線はconcave型をとる。高温で前処理した早群では室温で処理した早より約1.2ヶ月短命で4月中・下旬迄には殆んど死滅する。ウイルス摂取後27°Cで飼育したものは24°Cの場合より多少短命である。

地下室で越冬させたものは、予想通り最も長期間生存したが、動物舎でのものは、予想に反して必ず

しも短命ではなく、室温で前処理したものでは地下室での場合と殆んど変らない位長寿であった。これに反して、踊り場でのものは最も短命であった。これらの事からコガタアカイエカの越冬にとって最も好適な条件は、その場所が昼夜共暗黒に近いことであって、温度の多少の高低或いは温湿度の変化の度合などは、その次に問題になる条件であるように思われる。

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