

## Preliminary notes on the collection of hibernated females of *Culex tritaeniorhynchus* in Nagasaki\*

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**ABSTRACT :** The first coming out of hibernated females of *Culex tritaeniorhynchus* may possibly be observed on the side of banks or stone walls between grain fields or rice fields on warm afternoon in mid March. Then, they may be caught in the first place by dry ice method. Some days later they may enter animal sheds to feed on cows, pigs, or hens nearly simultaneously. Soon after that they may be attracted to light traps. In human dwelling houses they may be rarely found in March and April.

The most effective method for collecting

hibernated females of the mosquito is that by dry ice. By this method only unfed females are collected earlier and more numerously than by the other methods.

It is to be noted, however, that the females which hibernated but not yet take their blood meals in the spring can be caught before the 20th of April, though the date may be depend on the temperatures on those days. After that, some females which hibernated but fed and deposited eggs in the spring begin to be collected and after the beginning of May newly emerged females also begin to be captured.

### Introduction

Searches for determining the overwintering habits of *Culex tritaeniorhynchus* have been carried out for many years by many authors

in Japan without any essential results. We have also tried in vein since January, 1965 to collect hibernating adults of this mosquito.

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Ultimately, however, we hit on an idea that the main hibernating place of the mosquito may be in ground holes on the side of banks or stone walls between grain fields or rice fields. We got the hint from the discovery of an adult female of *Culex pipiens pallens* hibernating in a deep and narrow hole being on the side of the bank of earth under the digged out big wood stump leaned against the bank at the corner of a grain field. The female was found in a quite lethargic state, in the depths of a narrow hole, among the earth just crumbled when searching the mosquito. It seemed therefore that it must be quite difficult to find out mosquitoes when they are hibernating in a quite dormant state in such places as above. Then we

planned to collect the females when coming out from the hibernating place as early as possible in spring.

We succeeded to collect a great number of hibernated females attracted to CO<sub>2</sub> gas generated from dry ice in early spring in the field. Catches of mosquitoes by various methods and examinations on the change in age distribution of successive populations are now being continued in the Department of Medical Zoology and the isolation of Japanese encephalitis virus from those caught in the field are being carried out in the Department of Pathology. Here, however, the results of catches of the mosquito in March and April will be only reported preliminary.

### Place and method

For the collection of the mosquito, *Culex tritaeniorhynchus*, such villages were selected where there are preferably plenty of terraced rice fields; villagers are raising cows, pigs, hens, and other domestic animals; there occurred the patient of Japanese encephalitis in the past several years or the mummified fetus of the swine which may have some relation to the infection of the mother animal with the disease. The villages which nearly answer the purpose are as shown in Table 1.

Methods of the collection are: Dry ice; covering of mosquito-nets over the side of banks; cattle sheds; light traps; and human dwelling houses.

(1) Dry ice: CO<sub>2</sub> gas was generated by heating a can containing dry ice and some water. Near or over the generator large or small mosquito-net was set tucking up one side as shown in Fig. 1. Collectors collected with hand nets mosquitoes flying about around the mosquito-net or entering

Table 1 Villages where collections of mosquitoes were made in March and April, 1965

Name and No. of village		Distance from Nagasaki City	Period of Collection	Times of Collections
Aino	1	40km, EEN	Nar. 18-Apr. 20	7
Kawabira	2	8km, NNE	Mar. 17-Apr. 28	7
Mogi	3	6km, SE	Mar. 16-Apr. 27	11
Tomachi	4	4km, S	Mar. 19-Apr. 30	9
Fukuda	5	8km, W	Apr. 15	1
Hotachime	6	13km, NNW	Apr. 16, Apr. 22	2
Kuromaru	7	42km, NNE	Apr. 16-Apr. 24	4
Kibasaki	8	15km, NNW	Apr. 22, Apr. 25	2
Kaizu	9	23km, NE	Apr. 24-Apr. 29	4

Fig. 1 Collection of hibernated *Culex tritaeniorhynchus* by dry ice method in terraced rice fields



it. The collection was usually started from several hours before sun-set.

(2) Banks : Mosquito-nets were covered over the side of grassy banks or stone walls between hills, streams, or road, and rice fields, between rice fields or grain fields. The covering was made a few hours before sun-set and examined for mosquitoes after dark. The covering method under operation is shown in Fig. 2.

(3) Cattle sheds etc : At cow sheds, pig sheds, or hen houses, mosquitoes were collected for about 20 minutes from about 9 p.m.

(4) Light traps : The light traps of the

Fig. 2 Collection of hibernated *Culex tritaeniorhynchus* by covering mosquito-nets over the side of banks and stone walls in terraced rice fields



New Jersey type were set near houses and operated from sun-set to sun-rise.

(5) Houses : Although collections of mosquitoes in human dwelling houses were necessary, it was very difficult because of early sleeping habit of farmers.

It is to be noted that the number of cans with dry ice set in the field, mosquito-nets used, cattle sheds examined, light traps used, the number of collectors engaged in the work, times of collections, hours spent for each catch, were varied inevitably with place and date in this preliminary survey.

### Experimental results

Since the beginning of February, 1965 searches were carried out for hibernating females of *Culex tritaeniorhynchus* in many villages in many places. A fair number of *Culex pipiens pallens* and some of *C. hayashii* were captured in old anti-air-raid trenches. On the 26th, February, Omori and Ito found a hibernating female of *C. p. pallens* in a quite dormant state in the depth of a narrow hole on the side of a bank as mentioned above. Thereafter, banks, stone walls, the side of hills along valley in

terraced rice field areas were mainly searched for coming out of hibernated females.

On the 18th, March, S. Kawai, one of the authors, had a good fortune to find a female *C. tritaeniorhynchus* which started and then rested on a stone wall of a bank. The bank, 3m wide and with a thicket of bamboo grass on the top, was being constructed along a river to lay out rice fields. The day was very warm and the temperature at 4 p.m. when he caught the female was as high as 15.4°C. Searches of banks and stone walls

Table 2 Number of unfed and fed females of *Culex tritaeniorhynchus* collected by various methods and places in March and April, 1965

Date	Place	Air temp. (°C)*			Dry ice		Banks		Cow sheds			Pig sheds			Hen houses			Light traps			Houses			Grand total		Date
		Max	Min	Mean	Unfed	Fed	U	F	U	F	T	U	F	T	U	F	T	U	F	T	U	F	T	Unfed	Fed	Total
Mar. 16	3	9.6	2.0	5.8	0	0			0	0	0	0	0	0	0	0	0	0	0	0			0	0	0	16
17	2	12.5	3.6	8.1	0	0			0	0	0	0	0	0	0	0	0	0	0	0			0	0	0	17
18	3 1	16.9	6.6	11.8	0	0	1	0	1	0	1	0	0	0	0	0	0	0	0	0			1	0	1	18
19	4	13.8	4.1	9.0					0	0	0	0	0	0	0	0	0	0	0	0			0	0	0	19
20	3	10.2	3.2	6.7					0	0	0	0	0	0	0	0	0	0	0	0			0	0	0	20
21	3	13.5	2.2	7.9	0	0			0	0	0	0	0	0	0	0	0	0	0	0			0	0	0	21
22	3	13.9	3.5	8.7	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0			0	0	0	22
23	1	15.4	8.6	12.0	0	0			0	0	0	0	0	0	0	0	0	0	0	0			0	0	0	23
24	2	15.6	6.7	11.2	0	0			0	0	0	0	0	0	0	0	0	0	0	0			0	0	0	24
25	15.0	5.2	10.1																							25
26	10.1	4.5	7.3																							26
27	1	12.6	1.2	6.9	0	0			0	0	0	0	0	0	0	0	0	0	0	0			0	0	0	27
28	4	12.8	2.9	7.9	0	0	6		0	0	0	0	0	0	0	0	0	0	0	0			0	0	0	28
29	12.1	6.0	9.1																							29
30	3	16.6	6.2	11.4	0	0			0	0	0	0	0	0	0	0	0	0	0	0			0	0	0	30
31	3	14.8	10.9	12.9	4	0	4	0	0	0	0												4	0	4	31
Apr. 1	2 1	14.1	6.6	10.4	0	0			0	0	0	0	0	0	0	0	0	0	0	0			0	0	0	1
2	4	15.4	3.9	9.7	1	0	1		0	0	0	0	0	0	0	0	0	0	0	0			1	0	1	2
3	4	17.1	8.8	13.0	15	0	15		0	0	0	0	0	0	0	0	0	0	0	0			15	0	15	3
4	11.7	5.7	8.7																							4
5	3 1	14.6	4.4	9.5	0	0			0	0	0	0	0	0	0	0	0	0	0	0			0	0	0	5
6	14.4	5.0	9.7						0	0	0	0	0	0	0	0	0	0	0	0			0	0	0	6
7	2	16.9	10.2	13.6	9	0	9		0	0	0												9	0	9	7
8	17.5	12.1	14.8																							8
9	4 1	16.3	8.1	12.2	93	0	93		19	44	63	4	4	8	7	0	7	0	0	0			123	48	171	9
10	16.1	5.8	11.0						0	2	2	0	0	0	0	0	0	0	0	0			0	2	2	10
11	15.6	5.5	10.6																							11
12	3	18.8	10.6	14.7	316	0	316	3	0	3	7	13	20	7	7	14		22	10	32			355	30	385	12

Preliminary notes on the collection of hibernated females of *Culex tritaeniorhynchus* in Nagasaki

151

Date	Place	Air temp. (°C)*			Dry ice		Banks		Cow sheds			Pig sheds			Hen houses			Light traps			Houses			Grand total			Date	
		Max.	Min.	Mean	Unfed	Fed	Total	U	F	T	U	F	T	U	F	T	U	F	T	U	F	T	Unfed	Fed	Total			
13		16.2	9.4	12.8																					13			
14	2	17.5	6.6	12.1	15	0	15	0	0	0	1	2	3	0	0	0	1	0	1	0	0	0	17	2	19			
15	5	18.9	7.9	13.4	100	0	100															100	0	100				
16	{ 6	21.8	9.1	15.5	1056	0	1056		17	23	40											1073	23	1096				
	{ 7				4	0	4	0	2	2	0	0	0				4	2	6									
17	4	19.5	11.9	15.7	**74	0	74		322	146	468	40	1	41	25	0	25	53	544	597	514	691	1205	17				
18		15.6	13.3	14.5																				18				
19	{ 4	19.8	10.9	15.4	300	0	300	0	0	0	67	166	233	15	72	87	30	2	32	0	8	8	1	0	1			
	{ 7				4	0	4	0	2	2	0	0	0				4	2	6									
20	{ 3	19.1	9.0	14.1	209	0	209	0	0	0	3	2	5	0	1	1				0	0	0	212	3	215			
	{ 1				2	0	2	0	0	0	0	0	0				0	0	0									
21	2	17.8	6.8	12.3	2	0	2		0	0	0	0	0	0	0	0	0	0	0	0	0	2	0	2				
22	{ 6	20.8	13.3	17.1	795	0	795	2	0	2	23	92	115	0	0	0				0	0	0	820	92	912			
	{ 8				409	0	409	0	0	0				1	28	29	410	28	438									
23	{ 7	19.3	14.9	17.1	189	0	189															189	0	189				
	{ 4				6769	0	6769	0	0	148	368	516	102	74	176	114	41	155	341	1545	1886	10	0	10				
24	{ 7	21.5	12.8	17.2	218	0	218															7484	2028	9512				
	{ 9																						218	0	218			
25	{ 8	25.3	14.5	19.9	**9	0	9															152	769	921				
	{ 9																						118	143	261			
26		21.2	12.4	16.8																		939	953	1892				
27	{ 3	21.4	11.5	16.5	7	0	7	1	1	2	2	28	30	0	0	0							50	132	182			
	{ 9				298	0	298																	40	103	143		
28	2	20.1	10.2	15.2	98	0	98	1	0	1	12	31	43	1	2	3	1	0	1	25	54	79	138	87	225			
	9	15.9	7.3	11.6	2	0	2																79	452	531			
30	4	18.0	7.7	12.9	131	0	131	0	0	0	96	370	466	4	9	13	38	63	101	7	8	15	276	450	726			
Total					11127	0	11127	8	1	9	717	1291	2008	1465	2600	4065	216	106	322	488	2272	2760	11	0	11	14032	6270	203022

Remarks U.F.T. refer to Unfed, Fed, and Total respectively. Figures enclosed by rectangle show the first catch by each collecting method.

\* Max. temp. is that at p.m. of the day of collection, while, Min. temp. is that at a.m. of the next day.

\*\* Collection was discontinued by rain fall.

in terraced rice field areas were continued by various methods especially by the dry ice method. Ultimately we succeeded to capture hibernated females of the mosquito in question by dry ice method and gradually thereafter increasing number of females were captured. The result of collection by this method together with those by the other methods are given in Table 2.

#### (1) Result of collection by dry ice

During late March and early April the mosquito could collect only on the evening of higher minimum temperature, and in general, greater numbers were collected in warmer evening. All the females attracted to  $\text{CO}_2$  gas were unfed ones and about 60–70% of them collected on mid April seemed to have taken blood meals before entering hibernation (details will be reported on the other occasion).

It was found that the method is the most effective for collecting hibernated females of *C. tritaeniorhynchus* as seen in total number collected. Besides this mosquito, fair numbers of *C. vishnui*, *An. sinensis* and a few of *C. whitmorei*, *C. p. pallens*, *C. bitaeniorhynchus*, *An. sineroides*, *An. lindesayi japonicus*, *Ae. togoi*, and *Ae. japonicus* were also collected by this method.

#### (2) Result of collection at banks

Many banks and stone walls occur at skirts of hills, between grain fields or rice fields, along river or road around hilly farm villages. Since our discovery of the first hibernated female resting on a stone wall, searches were often made covering mosquito-nets over the side of these banks. Although many hibernated females seemed coming out from the side of these banks, the numbers collected by this method were rather small. It is now not understandable but may possibly be due to the hindrance by the covering from coming out of mosquitoes.

On the 27th April, a fed female was cap-

tured by this method. This may suggest that the hibernated females are using their old hibernating places as shelters even after their coming out from there.

#### (3) Result of collection in cow sheds, pig sheds, and hen houses.

The mosquito began to be collected simultaneously in these animal sheds from the 9th of April about 9 days after the catch by dry ice. It appeared that the mosquito prefers cows and pigs in nearly the same degree. Although the numbers of the mosquito collected are usually smaller in pig sheds in farm villages because of being less closed than in cow sheds, the numbers were much greater in the well built pig house in Nagasaki Agricultural and Forestry Experiment Station as seen in figures obtained at place No. 9 on the 24th, 25th and 29th of April. The pig house has a passage at lower part of the side but upper glass windows were being closed on those days. In hen houses the numbers were much smaller apparently because of the mosquito being less fond of hen's blood and also of difficulty of collecting it in hen houses.

The engorged females collected on the 9th, April were reared in the laboratory. Some of them deposited viable eggs on the 18th or 9 days after engorgement. Some of the eggs developed to adults on the 3rd of May or 24 days after the engorgement of mother mosquitoes.

The above facts suggest that the unfed females captured before the 18th or a few days later in April can be said to be those which overwintered but not yet take blood meals in spring, while unfed ones captured after the 20th or so in April must be said to be a mixed population of those as above and those fed and deposited eggs in spring. After the beginning of May, a population of unfed females must be composed of those of the above mixed population and

also those newly emerged in the spring.

(4) Result of collection by light traps

Hibernated mosquitoes of this species began to be collected lastly by this method. Although the numbers collected varied greatly with date and place, the total numbers appeared to be roughly near to those in cow sheds and pig sheds.

(5) Result of collection in Houses

In human dwelling houses, collections of mosquitoes were rarely tried because farmers said no mosquito enters houses usually in March and also because they usually go to bed in early evening. It appears that the mosquito may be not necessarily fond of human blood especially in these months.

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コガタアカイエカの越冬した♀成虫の採集成績についての予報、大森南三郎・和田義人・河合潜二・伊藤寿美代・小田 力・末永 敏・西垣定治郎・林 薫・三舟求真人。

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総 括

段々畑や段々水田の間、丘の麓、道路や川沿いにある雑草の茂った土手とか土手や石垣の側面の石の間や草木の根の間にある無数の小穴がコガタアカイエカ♀の主な越冬場所だと考えられる。このような小穴の奥に、飛び立ち得ない状態で越冬中の成虫を、冬期採集することは殆んど不可能に近いと考えられる。従って越冬から覚めて出て来るものを出来るだけ早期に採集することが望まれる。3月中旬の温暖な日の午後この様な越冬場所から出て来る♀成虫を観察できることがある。

Dry ice を使用することによって、♀成虫を早期に越冬場所からおびき出して採集することができる。その時期は3月下旬であるが、年により気温によって多少異なるであろう。その後1週間位すると牛舎、豚舎、鶏舎などへ吸血に来るようになり、更にその後間もなく Light trap でも採れるようになる。人家へは3月、4月中は殆んど侵入しない様である。

この様に Dry ice 法は越冬した♀成虫を早期に採集できるばかりでなく、他の何れの採集方法によるよりも驚くべき多数に採集することができる。然し注意しなければならないことは、越冬から覚めて春末だ吸血しない♀成虫のみを採集できるのは3月20頃迄であって、それ以後は、越冬したものではあるが春先吸血し産卵した♀が混り始め、5月上旬からは更に新生♀成虫も出始めることが考えられることである。