Ecology of Vector Mosquitoes of Japanese Encephalitis, Especially of *Culex tritaeniorhynchus summorosus*. 5. Overwintering of *Culex tritaeniorhynchus summorosus* and *Anopheles sinensis**.

Yoshito WADA, Tsutomu ODA, Motoyoshi MOGI

Department of Medical Zoology, Nagasaki University School of Medicine

Osamu SUENAGA, Ichiro MIYAGI

Department of Medical Zoology, Institute for Tropical Medicine, Nagasaki University

Nanzaburo OMORI

Department of Parasitology, School of Medicine, Teikyo University

Sumiyo ITO

Osaka Prefectural Institute of Public Health

and

Jojiro NISHIGAKI

Laboratory of Applied Entomology, Faculty of Agriculture, Shizuoka University

(Received for Publication February. 10, 1973)

Abstract

The time of awakening from winter diapause in *Culex tritaeniorhynchus summorosus* and *Anopheles sinensis* was examined from the data of mosquito catches in winter and early spring from 1965 to 1971 in the Nagasaki area. The results indicated that at least until the end of February most females of *C. t. summorosus* are still in a diapausing state, i. e., they do not feed on animals even on a warm day, while many of *An. sinensis* females are

*Contribution No. 206 from the Department of Medical Zoology, Nagasaki University School of Medicine and No. 665 from the Institute for Tropical Medicine, Nagasaki University.

in such a physiological state already in January that they can feed on animals if the temperature is high enough for their flight activity. From the observations in the field and the experiments in the laboratory, it was implied that the main overwintering place of *C. t. summorosus*, and probably *An. sinensis* too, is the underground small space of stone walls, banks and the likes which are common in the terraced rice field area.

Introduction

Culex tritaeniorhynchus summorosus, which is the most important vector of Japanese encephalitis at least in Japan and Korea, overwinters undoubtedly as the adult female. Overwintering females were collected in winter, though usually small in number (Bullock et al., 1959 ; Harada et al., 1963 ; Shimogama & Takatsuki, 1967 ; Wada, Yoshitake et al., 1968). In early spring in the Nagasaki area many overwintered females can be collected by using dry ice traps (Omori et al., 1965a), and adult females, kept in a cellar, an animal house and a landing, can overwinter successfully (Omori et al., 1965b). Kawai (1969) observed that all larvae died by the end of the year in the outdoor insectary of the Department of Medical Zoology, Nagasaki University School of Medicine, and in fact all attempts to find larvae of *C. t. summorosus* at their breeding places in mid-winter were with negative results ; the latest record in the field is that 3 pupae were collected on November 14, 1965, from a small pond, of which the water surface was about 1 m below the ground level, at a farm village, Nagasaki.

It is not only due to low temperatures but due to adult diapause that the females of *C. t. summorosus* can scarcely be collectd in mid-winter. Eldridge (1963) and Kawai (1969) demonstrated that the diapause in adult females is induced by short photoperiod in autumn.

As for *Anopheles sinensis* which is an abundant species in rice-field areas, adult females overwinter as in *C. t. summorosus*. Females of *An. sinensis* in winter, though the number was rather small, were observed by Harada et al. (1963), Ishii et al. (1964a, b), Sasa (1949), and Shimogama & Takatsuki (1967) in Japan and by Whang (1961) in Korea. The physiological state of the overwintering females in this anopheline is not so clear as in *C. t. summorosus*. Whang (1961) mentioned in connection with the overwintering of *An. sinensis* "Hackett (1937) and Swellengrebel and deBuck (1938) have stated that some mosquito adults in hibernating condition show different types of behavior, e. g., *A. atroparvus* Van Thiel hibernating in warm stables and houses occasionally biting, *A. messeae* Fall. going into a state of more complete inactivity, etc. The hibernating behavior of the *Anopheles* in Korea seems to belong to the first category, i. e. some hibernating in warm stables and occasionally biting." His statement seems to indicate that the females of *An. sinensis* do not undergo winter diapause. However, the presence of diapause in this mosquito is implied by the facts that the females collected by various methods decrease in number toward late autumn and the feeding activity is usually not observed from

late November to late December even when the temperature is high enough for the flight activity, but, as will be reported in the present paper, in January and thereafter large numbers of the females can be collected at animal sheds or by dry ice traps on warm days. In other words, it seems that the females of *An. sinensis* go into diapause once in autumn but awake very early, perhaps by January.

As mentioned above, it is indicated by much literature that *C. t. summorosus* and *An. sinensis* overwinter as adult females, but little has been reported about the time of awakening from winter diapause. This is an interesting subject in the ecology of the mosquitoes, and therefore was examined in the present paper from the data obtained in the Nagasaki area from 1965 to 1971. Considerations were also given on the overwintering place of mosquitoes, based on the data in the Nagasaki area as well as in the Saga area, Kyushu, Japan.

Places and methods

Mosquitoes were tried to collect in winter and early spring, 1965-1971, at 8 villages in the Nagasaki area. The topography and the area of rice fields at these villages were various, as shown in Table 1. Attempts to collect mosquitoes were done usually on warm days in winter and early spring by three methods, i. e., by using a dry ice trap in the field, by an aspirator at cowsheds, and by an aspirator at pigsties. The dry ice trap was operated from at least one hour before to about 30 minutes after the sunset, so that the peak time of flight activity was included during the operation (see Mogi et al., 1970). Catches at cowsheds and pigsties were done each for 20 minutes by a person usually after the collection by the dry ice trap. Data in 1965 which appeared in Omori et al. (1965a) and Wada et al. (1967) are included in the present paper.

Besides the 8 villages mentioned above, mosquitoes were collected at three places in Saga Prefecture in 1967. Their environmental features are given later when the results are described.

Table 1. Villages where mosquito collections were made in winter and early spring, 1965-1971, Nagasaki area.

Name of village	Distance & direction from main city of Nagasaki	Topography	Area of rice field	Year of collection
Tomachi	4Km, S	between small hills	small	1965-1967
Mogi	6Km, SE	between small hills	rather large	1965-1971
Nishiyama	4Km, NE	between small hills	very small	1968
Fukuda	5Km, W	foot-hill near seaside	small	1965
Koebaru	6Km, NW	between small hills	very small	1968
Kobasaki	15Km, NNW	foot-hill near seaside	small	1966, 1968
Kawabira	8Km, NNE	between small hills	small	1965
Kai u	23Km, NE	foot-hill facing plain area	large	1965-1971

Time of awakening from winter diapause

From the records of mosquito catches in winter and early spring from 1965 to 1971, the earliest examples of the females of *C. t. summorosus* and *An. sinensis* in each year are given in Table 2. The dates for *An. sinensis* were generally earlier than *C. t. summorosus*, and in an extreme case some females of *An. sinensis* were collected in December 11, 1968. The earliest date for *C. t. summorosus* was January 27, 1969.

The difference in the time of awakening from winter diapause between *C. t. summorosus* and *An. sinensis* is seen more clearly by the numbers of females collected in winter, as given in Table 3. *An. sinensis* females were recorded in all attempts of mosquito

Table 2. Earliest catches of *Culex tritaeniorhynchus summorosus* and *Anopheles sinensis* females in winter and early spring of each year from 1965 to 1971, Nagasaki area.

Year	*C. t. Summorosus*	*An. sinensis*
1965	Mar. 18 (Mogi)	Mar. 12 (Kawabira)
1966	Feb. 19 (Tomachi)	Feb. 11 (Mogi)
1967	Mar. 15 (Kai u)	Feb. 20 (Kaizu)
1968	Mar. 21 (Kaizu)	Feb. 28 (Koebaru)
		Dec. 11 (Mogi etc.)
1969	Jan. 27 (Kaizu)	Jan. 27 (Mogi etc.)
1970	Mar. 29 (Kai u)	Feb. 14 (Mogi etc.)
1971	Mar. 29 (Mogi etc.)	Feb. 22 (Mogi etc.)

In parentheses is given the name of village of mosquito collection.

Table 3. Numbers of *Culex tritaeniorhynchus summorosus* and *Anopheles sinensis* females collected in winter, Nagasaki area, 1966-1971.

Year	Date	Max.[1] temp. °C	Village	*C. t. summorosus*			*An. sinensis*		
				DI[2]	Pig[3]	Cow[4]	DI[2]	Piq[3]	Cow[4]
1966	Feb. 11	16.1	Mogi	0			1		
	Feb. 19	14.1	Tomachi	1			8		
	Feb. 28	16.7	Tomachi	0			2		
1967	Feb. 20	12.9	Kaizu		0	0		12	0
	Feb. 21	13.7	Tomachi	0	0	0	8	0	0
1968	Feb. 27	12.5	Nishiyama	0	0		0	0	
	Feb. 28	16.8	Koebaru	0			5		
	Dec. 11	17.8	Mogi	0			9		
			Kai u	0	0	0	2	0	5
			Kobasaki	0			4		
1969	Jan. 27	19.3	Mogi	0	0	0	100	77	7
			Kai u	1	0	0	80	65	56
	Jan. 28	19.3	Mogi	1	0		70	23	
1970	Feb. 14	14.5	Mogi	0	0		5	0	
			Kai u	0	0	0	11	0	3
	Feb. 19	19.8	Kaizu	0		0	945		405
1971	Feb. 22	17.7	Mogi	0	0	0	451	23	
			Kai u	0	0	0	1,338	79	191

1) Daily maximum temperature at Nagasaki Marine Observatory.

2) By dry ice traps.

3) At pig-sties.

4) At cowsheds.

catches, excepting February 27, 1968 when the daily maximum temperature was as low as 12.5°C. As it was generally warm days that we attempted to collect mosquitoes, it can be said that many of *An. sinensis* females are in such a physiological state that they can feed on animals if the temperature is high enough for their flight activity. In other words, it seems that the females of *An. sinensis* are mostly awaken from diapause already in mid-winter. The fact that only several females were collected on December 11, 1968 in spite of high temperature of 17.8°C may indicate that many were still diapausing in December.

In contrast to this, *C. t. summorosus* females were not collected in winter, excepting one female each on February 19, 1966 and January 27 and 28, 1969. It is, therefore, considered that at least until the end of February most females of *C. t. summorosus* are still in a diapausing state, i. e., they do not come out to feed on animals even on a warm day.

In order to investigate when most of *C. t. summorosus* females were awaken from winter diapause, daily maximum temperatures and the number of *C. t. summorosus* females collected were illustrated in Fig. 1 for each year from 1965 to 1971. From Fig. 1 the time of awakening from diapause in most of *C. t. summorosus* females can be presumed. For example, the time of diapause awakening in 1966 was given as follows. One female was collected firstly on February 19, and later four on March 2 and one on March 5. However, no mosquito was collected on March 3, 4, and 6, though it was very warm, maximum temperatures on those days having been higher than 20°C. This clearly indicates that most females were still in diapause. On March 10-12 one or two only were collected, but on March 13 and later many were collected so long as temperature was high. Thus, March 13 was considered as the time of awakening from diapause in most *C. t. summorosus* females in 1966 (the day was indicated by an arrow in Fig. 1).

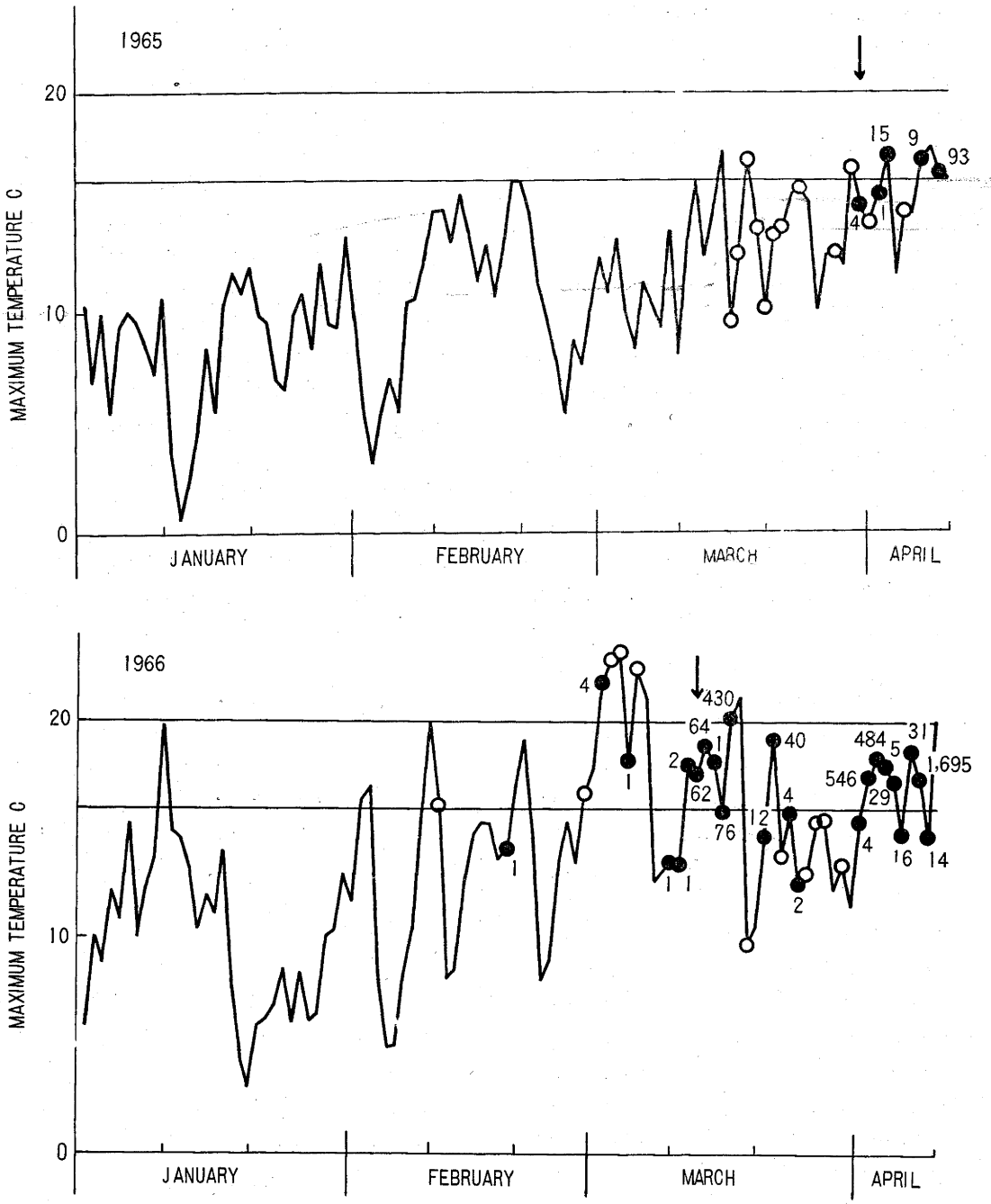
The times of diapause awakening in *C. t. summorosus* obtained in this way during seven years from 1965 to 1971 were considerably different from March 13 in 1966 to April 8 in 1970, and the time seemed generally early in the year in which the temperature in late winter and early spring was high. So, the daily mean temperatures were cumulated from the beginning of January to the time of diapause awakening in each year and given in Table 4. The cumulated values do not differ very much, ranging 621 to 707°C. It may be said from this fact that *C. t. summorosus* females are mostly awaken from winter diapause when cumulated daily mean temperatures from January 1 reach around 650°C.

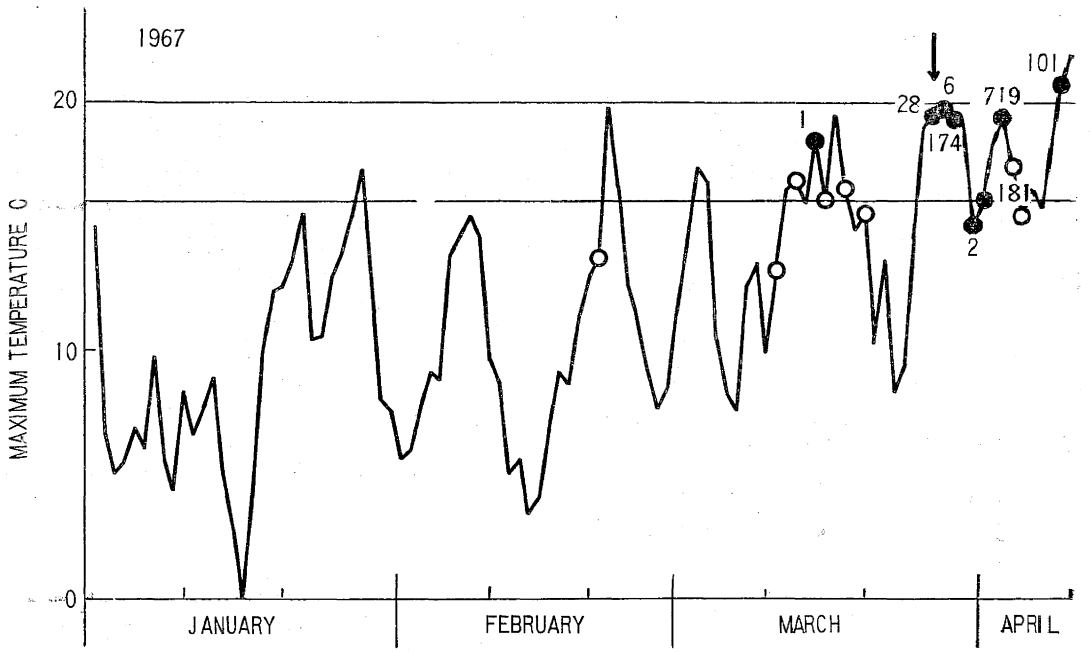
Table 4. Time of awakening from winter diapause in *Culex tritaeniorhynchus summorosus* females, and cumulative temperature to that time, Nagasaki area, 1965-1971.

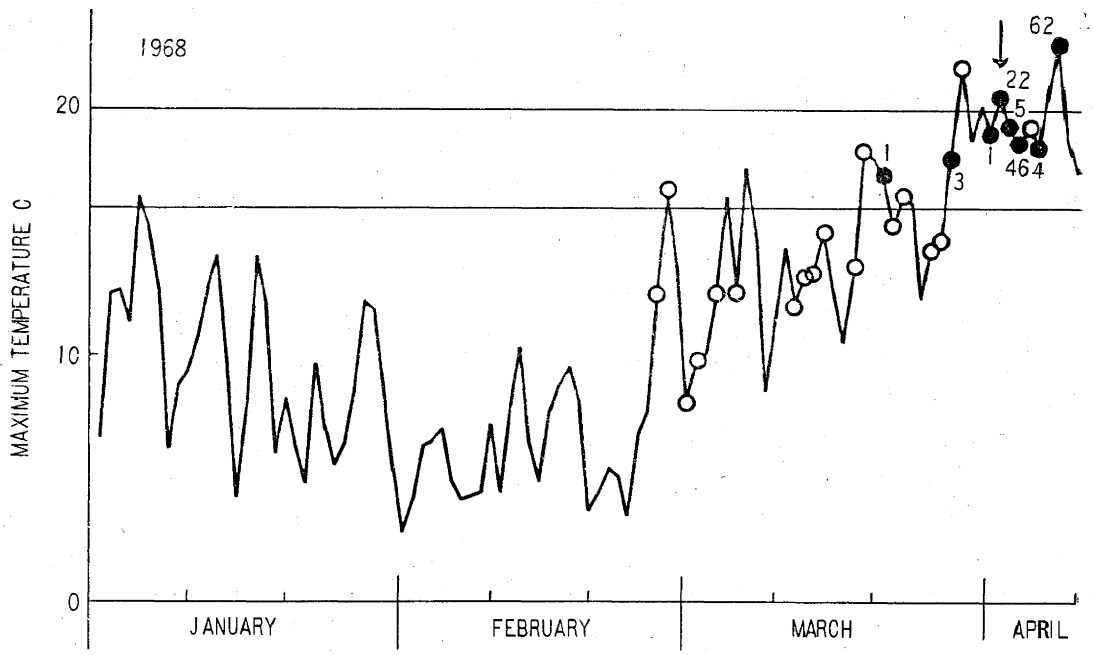
Year	Time	Cumulative temp. °C[1)]
1965	Mar. 31	649
1966	Mar. 13	621
1967	Mar. 27	622
1968	Apr. 2	651
1969	Mar. 26	671
1970	Apr. 8	707
1971	Mar. 29	636

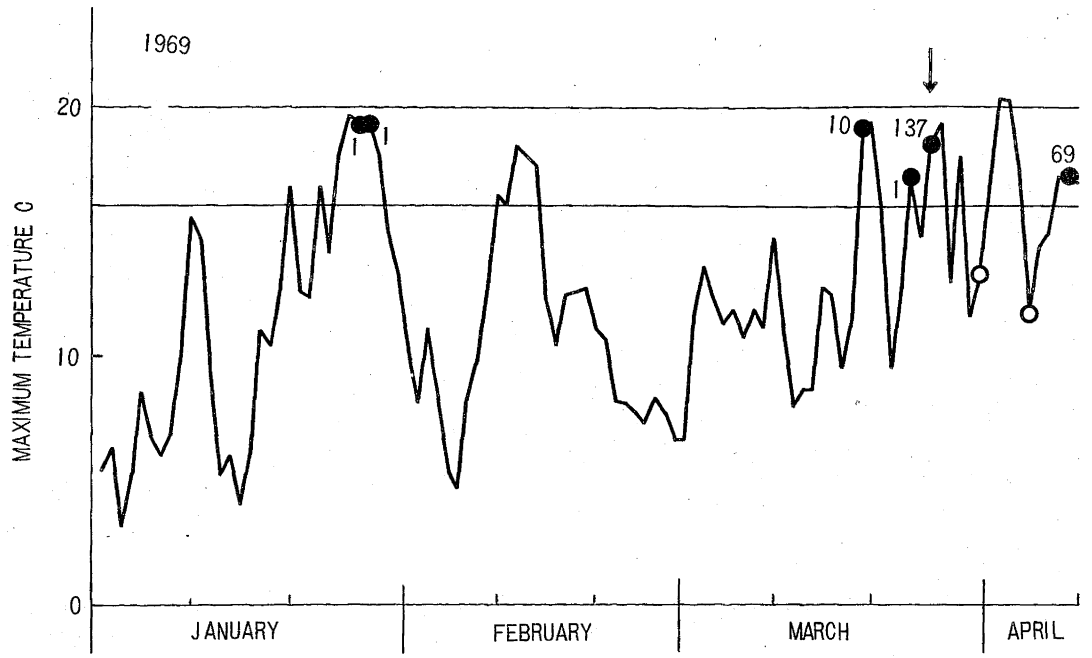
1) Cumulative daily mean temperature from January 1 to the time of diapause awakening.

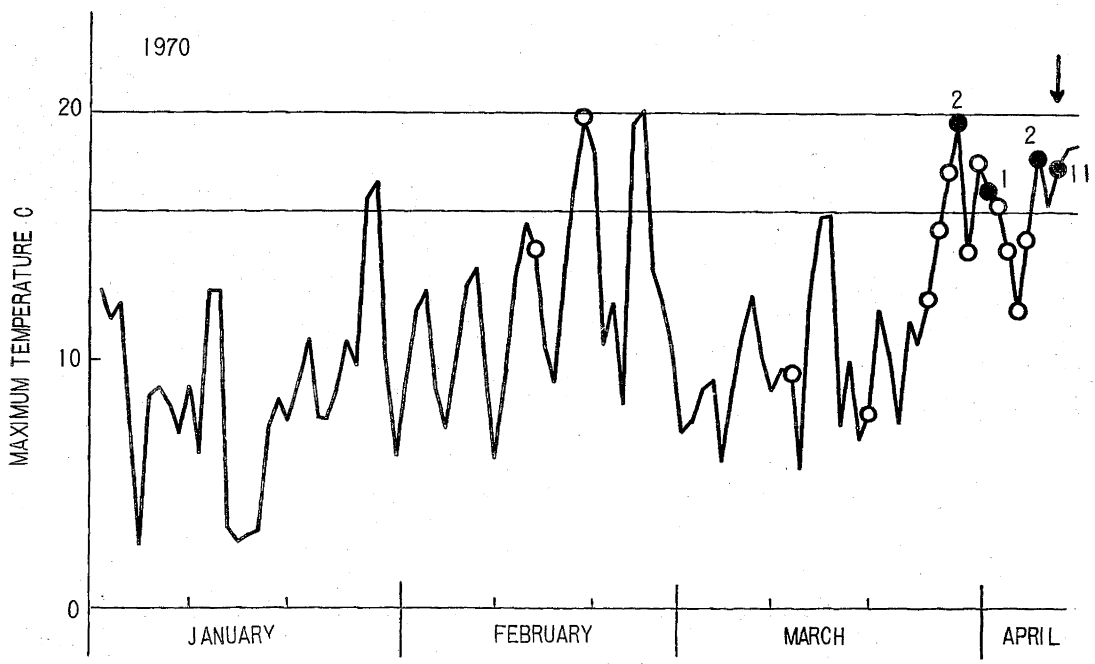
Fig. 1. Daiy maximum temperatures from winter to early spring, 1965-1971g, Nagasaki area, with the results of mosquito catches by dry ice traps. White circles show the days on which *C. t. summorosus* females were not collected, black circles and attached figures the days and numbers of catches. An arrow indcates the presumed time of awakening from winter diapause.

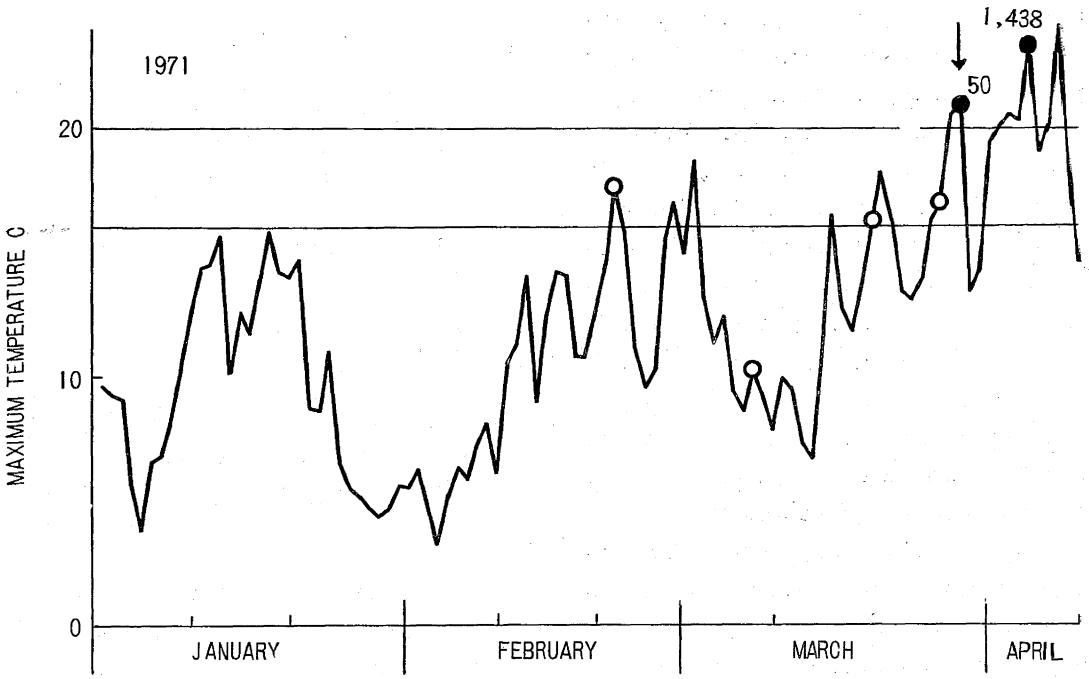



1967
MAXIMUM TEMPERATURE C
20
10
0
JANUARY
FEBRUARY
MARCH
APRIL
1
28
6
174
719
181
2
101


1968
MAXIMUM TEMPERATURE C
20
10
0
JANUARY
FEBRUARY
MARCH
APRIL
1
3
1
22
5
464
62


1969
MAXIMUM TEMPERATURE C
20
10
0
1
1
10
137
1
69
JANUARY
FEBRUARY
MARCH
APRIL


1970
MAXIMUM TEMPERATURE C
20
10
0
2
2
1
11
JANUARY
FEBRUARY
MARCH
APRIL



Overwintering place

The place where *C . t. summorosus* females overwinter has long been a great concern of many medical entomologists, and much work has been done in Japan and Korea. Overwintering *Culex pipiens pallens* females are frequently encountered in artificial or natural caves in a large number, but ***C. t. summorosus*** females are not found (Harada et al., 1963; Ishii et al., 1964a, b; Whang, 1961) or, if any, the number is usually very small (Bullock et al., 1959; Shimogama et Takatsuki, 1967). Only one exception is seen in a paper by Wada, Yoshitake et al. (1968) which reported 60 overwintering *C. t. summorosus* females from several caves in Izu Peninsula, Shizuoka Prefecture, in January, 1967. However, the number of females found in the caves seems still too small to explain the fact that a great number of overwintered females can be collected in early spring (Omori et al. ,1965a). Thus, it is considered that there must be some other places as a typical overwintering site.

Since the success of collecting many overwintered females of C. *t. summorosus* in the Nagasaki area in early spring, 1965 (Omori et al., 1965a), our attention has been paid to find the environmental features for the suitable overwinterng place of this mosquito. The results obtained by repeated observations at various places indicate that it is at the terraced rice field area with stone walls, banks and the likes that many overwintered females can be collected. Here, an example of this is givn in Table 5.

In April and May, 1967, mosquitoes were collected by a dry ice trap each at three places, Saga City, Kinryu and Yamato, in

Saga Prefecture. The collection site at Saga City was situated on a plain area, near to a moat of the old ruined castle which provided *C. t. summorosus* with a suitable breeding place in summer, and was surrounded by residential quarters of the city. The site at Kinryu was also on a plain area, but surrounded by a large areaof the rice field which is the main breeding place of this mosquito in Japan. On the other hand, the collection site at Yamato was between hills along a river, and a small area of terraced rice fields with stone walls and the likes was developed there. The distance between Saga City and Yamato is ca. 12 km, kinryu being situated halfway between the two. The beginning date of the appearance of newly-emerged females was presumed to be between April 26 and May 10, judged from the appearance date of males in Saga Prefecture and from the data obtained in Nagasaki Prefecture where more detailed work on the overwintering ecology was performed. Accordingly, it can be said that more overwintered females and less newly-emerged ones were collected at Yamato in a foothill area with terraced rice fields than at Saga City in a plain area, the situation at Kinryu being intermediate between the two. The same tendency was observed repeatedly in Nagasaki and Saga Prefectures.

Bullock et al. (1959) collected 11 overwintering females of *C. t. summorosus* in November, 1957 through February, 1958 mostly among brush piles on Kanto Plain around Tokyo, and Harada et al. (1963) obtained 4 overwintering ones in December, 1962 and January, 1963 by sweeping the thick grass land with many dead leaves. Accordingly, dead vegetation above ground may be used for the overwintering of *C. t. summorosus*. However, this does not seem to provide with a main overwintering site, because the number of overwintering females collected by them was small in number in spite of much labor taken. Moreover, if the small space between dead vegetation above ground had been a typical overwintering site, the numbers of overwitnered females collected at the three places in Saga Prefecture would have been nearly the same, as dead vegetation was equally abundantly found on the ground of all the three collection sites. However, the results obtained showed very different catches of overwintered females at the respective sites, as seen in Table 5.

Table 5. Difference in the numbers of hibernated and newly-emerged females of *Culex tritaeniorhynchus summorosus* collected by a dry ice trap at three places with different environmental situations, Saga Prefecture, 1967. Beginning of the appearance of newly-emerged females is presumed to be between April 26 and May 10.

Date	Saga City	Kinryu	Yamato
Apr. 18	0	6	140
Apr. 26	0	9	20
May 10	64	1	0
May 16	300	139	25
May 22	320	37	18
May 31	1,700	215	70

The result of the experiment on the overwintering of *C. t. summorosus* by Omori et al. (1965b) indicated that darkness throughout day and night is probably a very important condition for the female to survive winter successfully. This seems, in turn, to indicate that the suitable overwintering place for this mosquito in nature is somewhere underground, where it is dark.

From those mentioned above, it may be

concluded that the main overwintering place of *C. t. summorosus* females is the underground small space in stone walls and the likes which are common in the terraced rice field area. Although *An. sinensis* females appear to feed on animals earlier in season than *C. t. summorosus* females, many overwintered *An. sinensis* can be collected at the place where overwintered *C. t. summorosus* females are abundantly encountered. Therefore, it may be said that the overwintering place of *An. sinensis* is very similar to that of *C. t. summorosus*.

References

1) **Bullock, H. R. et al.** : Notes on the overwintering of *Culex tritaeniorhynchus* Giles in Japan. Mosq. News, 19 : 184-188, **1959**.

2) **Eldridge, B. F.** : The influence of daily photoperiod on blood-feeding activity of *Culex tritaeniorhynchus* Giles. Amer. J. Hyg., 77 : 49-53, **1963**.

3) **Harada, F. et al.** : Results of the collections of overwintering mosquitoes in Kanagawa Prefecture. 1962 Kanagawaken-Eiseikenkyusho-Nenpo, 12 : 66-79 (In Japanese), **1963**.

4) **Ishii, T. et al.** : Ecological note on the hibernation of mosquitoes in caves. Sci. Rep. Tohoku Univ. Ser. IV (Biol.), 30 : 151-157, **1964a**.

5) **Ishii, T. et al.** : Hibernation of mosquitoes in rock caves on Miyato Island. Sci. Rep. Tohoku Univ. Ser. IV (Biol.), 30 : 159-165, **1964b**.

6) **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**.

7) **Mogi, M. et al.** : Ecology of vector mosquitoes of Japanese encephalitis, especially of *Culex tritaeniorhynchus*. 3. Seasonal changes in the time of being attracted to dry ice in the females of *Culex tritaeniorhynchus*. Trop. Med., 12 ; 122-127, **1970**.

8) **Omori, N. et al.** : Preliminary notes on the collection of hibernated females of *Culex tritaeniorhynchus* in Nagasaki. Endem. Dis. Bull. Nagasaki, 7 : 147-153, **1965a**.

9) **Omori, N. et al.** : Experimental hibernation of *Culex tritaeniorhynchus* in Nagasaki, Japan. Endem. Dis. Bull. Nagasaki, 7 : 288-295, **1965b**.

10) **Sasa, M.** : Zoophilism, hibernation and appearance of mosquitoes of Japan. Jap. Med. J., 2 : 99-107, **1949**.

11) **Shimogama, M. et Takatsuki, Y.** : Seasonal changes in the distribution and abundance of mosquitoes especially of *Culex pipiens pallens* in a cave in Nagasaki City. Endem. Dis. Bull. Nagasaki, 8 : 159-165, **1967**.

12) **Wada, Yoshitake et al.** : Mosquitoes overwintering in Izu Peninsula and Mt. Nokogiri. Japan. J. Sanit. Zool., 19 : 82-83 (In Japanese with English summary), **1968**.

13) **Wada, Y. et al.** : Ecology of vector mosquitoes of Japanese encephalitis, especially of *Culex tritaeniorhynchus*. 1. Results obtained in 1965. Trop. Med., 9 : 45-57, **1967**.

14) **Whang, C. H.** : Hibernation of mosquitoes in Korea. Mosq. News, 21 : 17-20, 1965.

Yoshito WADA, Tsutomu ODA, Motoyoshi MOGI, Osamu SUENAGA,
Ichiro M3YAGI, Nanzaburo OMORI, Sumiyo ITO, Jojiro NISHIGAKI

日本脳炎伝搬蚊，特にコガタアカイエカの生態
5. コガタアカイエカとシナハマダラカの越冬

和田義人・小田　力・茂木幹義
長崎大学医学部医動物学教室

末永　敏・宮城一郎
長崎大学熱帯医学研究所衛生動物学研究室

大森南三郎
帝京大学医学部寄生虫学教室

伊藤寿美代
大阪府立公衆衛生研究所

西垣定治郎
静岡大学農学部昆虫学教室

摘　要

コガタアカイエカとシナハマダラカの雌成虫が冬期の休眠から覚醒する時期を，長崎地方で1965-1971年の冬及び早春に採集した蚊の資料を用いて吟味して，次の結果を得た．コガタアカイエカの大多数の雌成虫は少なくとも2月の末までは休眠状態にあり，暖い日であっても吸血に来ることは殆んどない．これに反してシナハマダラカの雌成虫の多くは1月には既に休眠から覚醒していて，気温が飛翔活動に充分なほど高くさえあれば，多数の吸血蚊が観察される．[野外及び実験室での観察から，コガタアカイエカが（そしておそらくシナハマダラカも）越冬するのは，階段状の水田が多くある地方の石垣や土手などの中の小さなすき間であるように思われる．