

Notes on Mosquitoes of Amami-Oshima Island and the Overwintering of Japanese Encephalitis Virus

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ABSTRACT : Mosquitoes were investigated on Amami-Oshima Island in 1972-1975. Adults were collected by light traps at animal shelters and by dry ice traps in the field, and larvae at their breeding sites in the whole year. In total, 31 species of mosquitoes were found. From the mosquito catches by the above methods together with the rearing records of some larvae collected in the field, the biology of each mosquito particularly in the winter time was reported. Also, the possibility of the overwintering of Japanese encephalitis virus on Amami-Oshima was discussed on the basis of the biology of the vector mosquito, *Culex tritaeniorhynchus*. It was considered that the successful overwintering of the virus is attained only by the succession of the pig-mosquito cycle maintained by the continuous feeding activity of the vector mosquitoes in warm winter.

On Amami-Oshima Island which is located between Okinawa and Kyushu Main Island, mosquitoes were examined in 1972-1975 in relation to the epidemiology of Japanese encephalitis (JE) (see Hayashi et al., 1975). Therefore the primary concern was with the vector mosquito of the disease, *Culex tritaeniorhynchus*, however other mosquitoes were also studied. The mosquito fauna on Amami-Oshima was fairly well investigated, and Sasa and Kamimura (1971) recorded 33 species in total and Tanaka et al. (1975) added 5 species, however little was known about their biology there. In the present paper, we wish to report the biology of the mosquitoes particularly in connection with their overwintering,

and to discuss about the possibility for JE virus to overwinter on the basis of the winter biology of *C. tritaeniorhynchus*.

PLACES AND METHODS

Mosquitoes were collected mostly at four farm villages (Seisui, Atsuse, Kunetsu and Susare) near Koniya in the southern part of Amami-Oshima. Those villages faced to the seaside in the south and to hills in the north, and very small rice fields and marshes were scattered around the villages. Larval collections were also made at Hatsuno and Mt. Yuwan, remote from human dwellings.

The most extensively used method to catch adult mosquitoes was by the light trap (Nozawa-type). A light trap was operated each at four pigsties, a cowshed and a henhouse at indefinite intervals throughout the year in 1972-1975 (Table 1). The total number of nights for light trap operation was 286 at pigsties, 258 at a cowshed and 49 at a henhouse. Besides the light trap catch, adult mosquitoes were collected by hand at animal shelters and in the field and also by a dry ice trap. Larval collections were made by a dipper and a pipette at their breeding sites. Some larvae were reared to adults to help the identification, and to observe the time of emergence in winter.

To know the winter physiology of *C. tritaeniorhynchus* in relation to the ecology of

Table 1. Number of nights when the light trap was operated for mosquito collections at animal shelters, 1972-1975

Village	Year	Jan.	Feb.	Mar.	Apr.	May	Jun.	Jul.	Aug.	Sep.	Oct.	Nov.	Dec.	Total
PIGSTY														
Seisui	1972										1	1		2
	1973		1	1	3								2	7
	1974						10							10
Atsuse	1975	3	8	7	4									22
Kunetsu	1973									3	3			6
	1974	14	24	24										62
Susare	1973						2	9	3	9	12	8	4	47
	1974	14	23	26	16	16	10	5	4	3	4	3	2	126
	1975				3	1								4
Total		31	56	58	26	27	12	14	7	15	20	12	8	286
COWSHED														
Seisui	1972										1	1		2
	1973	1	1	2	5	6	10	13	7	10	11	7	6	79
	1974	14	25	26	16	18	9	5	4	3	4	3	7	134
	1975	5	10	16	10	2								43
Total		20	36	44	31	26	19	18	11	13	16	11	13	258
HENHOUSE														
Seisui	1972											1		1
	1973		1	1	2			3		3	6	2		18
	1974	2	13	4		10	1							30
Total		2	14	5	2	10	1	3		3	6	3		49

JE virus, female mosquitoes collected in winter and early spring were dissected and their physiological ages were determined by Detinova's method and/or Polovodova's method (Detinova, 1962).

RESULTS

The mosquitoes collected in 1972–1975 were listed by species in Table 2. In total, 31 species of mosquitoes were found by the present survey.

Table 3 shows the numbers of female and male mosquitoes collected by a light trap each at animal shelters in 1972–1975. The abundant species and the host preference of mosquitoes can be seen in Table 3. The seasonal prevalences of mosquitoes by light

Table 2. Species of mosquitoes collected on Amami-Oshima, 1972–1975

Mosquito species	Adult collections			Larval collections
	By light trap	By dry ice trap	By hand*	
<i>Anopheles sinensis</i>	+	+	+	+
<i>An. aitkenii bengalensis</i>				+
<i>Toxorhynchites yamadai</i>				+
<i>Malaya genurostris</i>			+	+
<i>Ficalbia luzonensis</i>	+			
<i>Mansonia crassipes</i>	+		+	
<i>M. uniformis</i>	+	+	+	+
<i>Uranotaenia bimaculata</i>	+	+	+	+
<i>Orthopodomyia anopheloides</i>				+
<i>Aedes watasei</i>	+			+
<i>Ae. japonicus</i>	+		+	+
<i>Ae. okinawanus</i>	+			+
<i>Ae. togoi</i>	+		+	+
<i>Ae. nipponicus</i>				+
<i>Ae. albopictus</i>	+		+	+
<i>Ae. riversi</i>	+		+	+
<i>Ae. vexans nipponii</i>	+	+		+
<i>Armigeres subalbatus</i>	+	+	+	+
<i>Culex vorax</i>	+	+		+
<i>C. hayashii</i>	+			+
<i>C. okinawae</i>			+	+
<i>C. infantulus</i>				+
<i>C. ryukyensis</i>	+			+
<i>C. bitaeniorhynchus</i>	+	+		+
<i>C. sinensis</i>	+			+
<i>C. whitmorei</i>	+	+		
<i>C. pseudovishnui</i>	+		+	+
<i>C. tritaeniorhynchus</i>	+	+	+	+
<i>C. mimeticus</i>	+	+		+
<i>C. pipiens fatigans</i>	+		+	+
<i>C. vagans</i>				+

* At animal shelters and/or in the field

traps are given in Table 4. Table 5 represents the larval instars of mosquitoes collected in winter and early spring in 1973–1975. Although larvae of common species such as *Aedes togoi* etc. were not surveyed in every month at their breeding sites, Table 5 gives informations on winter biology of mosquitoes. Some larvae collected in January were reared to observe the time of adult emergence, in a room without air conditioning until February 4 and in an insectary of 21°C and 12 hour daylength thereafter, and the results

Table 3. Numbers of female (and male) mosquitoes collected by a light trap at each animal shelter, 1972–1975

Animal shelter Village	Pigsty				Cowshed	Henhouse	Total
	Seisui	Atsuse	Kunetsu	Susare	Seisui	Seisui	
Number of nights	19	22	68	177	258	49	593
<i>Anopheles sinensis</i>	483 (5)	48	20 (1)	5301 (52)	11321 (27)	208 (11)	17381 (96)
<i>Ficalbia luzonensis</i>			14	938 (8)	464 (3)	8	1424 (11)
<i>Mansonia crassipes</i>	7	1	2 (4)	5	27 (2)	89 (3)	131 (9)
<i>M. uniformis</i>	128 (3)	1	5	181 (1)	1447 (39)	59	2821 (43)
<i>Uranotaenia bimaculata</i>			1	2	3		6
<i>Aedes watasei</i>	1			3			4
<i>Ae. japonicus</i>					8		8
<i>Ae. okinawanus</i>				1			1
<i>Ae. togoi</i>	17	5		34	123 (4)	7 (1)	186 (5)
<i>A. albopictus</i>	5		1	28 (1)	47 (1)	3	84 (2)
<i>Ae. riversi</i>				1			1
<i>Ae. vexans nipponii</i>	16		41	506 (2)	153	5	721 (2)
<i>Armigeres subalbatus</i>	102 (23)	3	49 (10)	1294 (186)	4919 (1551)	274 (110)	6641 (1880)
<i>Culex vorax</i>		4	1	5 (1)	5 (1)	2	17 (2)
<i>C. hayashii</i>				2			2
<i>C. ryukyensis</i>				1	1		2
<i>C. bitaeniorhynchus</i>	10			67 (3)	28 (1)	6	111 (4)
<i>C. sinensis</i>				17	1	6	24
<i>C. whitmorei</i>				1			1
<i>C. pseudovishnui</i>	10	1		988 (1)	78	3	1080 (1)
<i>C. tritaeniorhynchus</i>	472 (1)	214	860	21739 (33)	32525 (33)	656	56466 (67)
<i>C. mimeticus</i>				17 (3)			17 (3)
<i>C. pipiens fatigans</i>	155 (28)	10	26 (13)	252 (138)	310 (253)	197 (354)	950 (786)
Total	1406 (60)	287	1020 (27)	31383 (429)	52460 (1914)	1523 (479)	88079 (2909)

are shown in Table 6. The age composition of *C. tritaeniorhynchus* females in winter and early spring was represented in Table 7.

From Table 3-7 and other observations in the field, the biology of each species of mosquitoes on Amami-Oshima is given in the below.

Anopheles (Anopheles) sinensis Wiedemann: This is a most common species and breeds commonly in rice fields and marshes. Females prefer pigs and cows to hens (Table 3). Because adults were collected by light traps at animal shelters throughout the year (Table 4) and larvae in all instars and pupae were continuously found from December to March (Table 5), it can be said that females feed on animals on warm days and lay eggs and adults

Table 4. Seasonal distributions of female (and male) mosquitoes collected by light traps at animal shelters, 1972-1975. Data in Table 3 are arranged by month

Month Number of nights	Jan. 53	Feb. 106	Mar. 107	Apr. 59	May 63	Jun. 32	Jul. 35	Aug. 18	Sep. 31	Oct. 42	Nov. 26	Dec. 21	Total 593
<i>Anopheles sinensis</i>	85	315 (1)	459 (5)	2436 (16)	5064 (25)	4233 (31)	2271 (6)	636 (1)	686 (4)	923 (5)	224 (2)	49	17381 (96)
<i>Ficalbia luzonensis</i>				3	36 (1)	87	522 (4)	372 (4)	269 (2)	125	10		1424 (11)
<i>Mansonia crassipes</i>				7	13	45 (2)	14	1	11 (5)	39 (2)	1		131 (9)
<i>M. uniformis</i>			5	144 (3)	406	409 (1)	390 (3)	448 (1)	378 (9)	460 (21)	144 (5)	37	2821 (43)
<i>Uranotaenia bimaculata</i>	1		1		1					1	1	1	6
<i>Aedes watasei</i>					4								4
<i>Ae. japonicus</i>		1		7									8
<i>Ae. okinawanus</i>										1			1
<i>Ae. togoi</i>		4	22	46 (2)	48 (2)	29	16	4	1	9	6 (1)	1	186 (5)
<i>Ae. albopictus</i>				1	16	22 (1)	19	4	7	10 (1)	5		84 (2)
<i>Ae. riversi</i>										1			1
<i>Ae. vexans nipponii</i>		3	22	164	33	26	10	14	129	298 (2)	19	3	721 (2)
<i>Armigeres subalbatus</i>	4 (1)	9 (1)	16 (5)	462 (166)	330 (58)	755 (180)	1325 (350)	451 (73)	1103 (326)	1403 (540)	633 (164)	150 (16)	6641 (1880)
<i>Culex vorax</i>	3	4	1	3	1				1 (1)	1 (1)	3		17 (2)
<i>C. hayashii</i>	1				1								2
<i>C. ryukyensis</i>			2										2
<i>C. bitaeniorhynchus</i>		1	2	14	28 (1)	28	15 (1)	4	5 (1)	9 (1)	3	2	111 (4)
<i>C. sinensis</i>		6				4	3		10	1			24
<i>C. whitmorei</i>									1				1
<i>C. pseudovishnui</i>	1	17	10	108 (1)	318	121	27	44	337	94	2	1	1080 (1)
<i>C. tritaeniorhynchus</i>	40	1315	1500	2808 (4)	5053 (7)	5934 (12)	12533 (7)	11488 (22)	9299 (9)	6006 (6)	425	65	56466 (67)
<i>C. mimeticus</i>										17 (3)			17 (3)
<i>C. pipiens fatigans</i>	11 (6)	62 (24)	57 (21)	75 (35)	408 (380)	116 (160)	39 (55)	15 (8)	39 (37)	61 (41)	58 (17)	9 (2)	950 (786)

emerge even in winter.

Anopheles (Anopheles) aitkenii bengalensis Puri: Larvae were found not rarely in streams with clean water, not only in mountaneous areas but also near villages. It is certain that this species can overwinter in the larval stage (Table 5; see also Kanda & Kamimura, 1967).

Toxorhynchites (Toxorhynchites) yamadai (Ouchi): Larvae were collected from tree holes and an earthen jar in the forest of Mt. Yuwan. The fourth instar larvae in December (Table 5) may indicate that the overwintering is possible in the larval stage.

Malaya genurostris Leicester: Larvae were commonly found in leaf-axils of the taro and the banana. This species overwinters in the larval stage (Table 5), probably in the state of diapause, because the fourth instar larvae collected on January 24 started to pupate as late as on February 21 and 1 male and 6 females emerged till March 26, but still 5 larvae remained, even though they were kept under 21°C and 12 hour daylength after February 4 (Table 6).

Ficalbia (Etorletiomyia) luzonensis (Ludlow): Adults including engorged females were

Table 5. Larval instars of mosquitoes collected in winter and early spring, 1973-1975

Mosquito species	December	January	February	March
<i>Anopheles sinensis</i>	I, II, III, IV, P	I, II, III, IV, P	I, II, III, IV, P	I, II, III, IV, P
<i>An. aitkenii bengalensis</i>	I, II, III, IV	II, III, IV	II, III	I, II, III, IV, P
<i>Toxorhynchites yamadai</i>	IV			
<i>Malaya genurostris</i>	I, II, III, IV	II, III, IV	III, IV	
<i>Mansonia uniformis</i>				P
<i>Uranotaenia bimaculata</i>	I, II, III, IV, P	I, II, III, IV, P		IV
<i>Orthopodomyia anopheloides</i>	IV	IV	IV	IV
<i>Aedes watasei</i>				IV
<i>Ae. japonicus</i>		I, II, III, IV	I, II, III, IV	I, II, III, IV
<i>Ae. okinawanus</i>	IV	I, II, III		IV
<i>Ae. togoi</i>	I, II, III, IV	II, III, IV		
<i>Ae. nipponicus</i>			IV	
<i>Ae. albopictus</i>	III, IV	I, II, III, IV, P	I, II, III, IV	I, II, III, IV
<i>Ae. rivarsi</i>	III, IV	I, II, III, IV, P	I, II, III, IV	I, II, III, IV
<i>Ae. vexans nipponii</i>			IV	II, III, P
<i>Armigeres subalbus</i>	I, II, III, IV, P	I, II, III, IV	I, II, III, IV	I, II, III, IV
<i>Culex vorax</i>				II, III, IV
<i>C. okinawae</i>		IV	III, IV	
<i>C. infantulus</i>				III
<i>C. ryukyensis</i>		I, II, III, IV	IV	IV
<i>C. bitaeniorhynchus</i>	II, III, IV	IV	IV	III, IV, P
<i>C. pseudovishnui</i>				III, IV
<i>C. tritaeniorhynchus</i>	II, III, IV, P			I, II, III, IV, P
<i>C. mimeticus</i>	I, II, III, IV, P	I, II, III, IV, P	I, II, III, IV, P	I, II, III, IV, P
<i>C. pipiens fatigans</i>	I, II, III, IV, P	I, II, III, IV, P	I, II, III, IV, P	I, II, III, IV, P
<i>C. vagans</i>				IV, P

commonly collected by light traps set at animal shelters. The female seems to prefer pigs and cows to hens for blood feeding (Table 3). This species overwinters probably in the larval stage, because adults were collected during the period only from April to November (Table 4).

Mansonia (Coquillettidia) crassipes (van der Wulp): This species was encountered not rarely at animal shelters. Females including engorged ones were collected by light

Table 6. Rearing records of mosquito larvae collected in winter, 1974.
Larvae were reared in a room without air conditioning until February 4, and in an insectary of 21 C and 12 hour daylength thereafter.

Mosquito species	Date & stage of collection	Rearing records
<i>Malaya genurostris</i>	Jan. 24 4th instar	Started to pupate on Feb. 21, 1 male and 6 females emerged on Feb. 25–Mar. 26, still 5 larvae on Mar. 27.
<i>Uranotaenia bimaculata</i>	Jan. 30 4th instar	1 male emerged on Feb. 26.
<i>Orthopodomyia anopheloides</i>	Jan. 26 4th instar	Started to pupate on Feb. 17, 4 males emerged on Feb. 24–27 and 2 females on Mar. 7 and 19, still 3 larvae on Mar. 27.
<i>Aedes japonicus</i>	Jan. 30 & 31 4th instar	1 female emerged on Feb. 7 and 1 male on Feb. 8.
<i>Aedes okinawanus</i>	Jan. 22 & 23 1st–3rd instars	3 males emerged on Feb. 9–15.
	Feb. 1 1st instar	1 male emerged on Feb. 22.
<i>Aedes albopictus</i>	Jan. 22 Pupa	1 male and 3 females emerged on Jan. 27–29.
	Jan. 28–31 2nd instar	Males and females emerged on Feb. 5–16.
<i>Aedes riversi</i>	Jan. 22 4th instar & pupa	Males and females emerged on Jan. 27–29.
	Jan. 30 & 31 4th instar	Males and females emerged on Feb. 8–16.
<i>Culex ryukyensis</i>	Jan. 22 3rd–4th instars	2 males emerged on Feb. 6–9, and 1 female on Feb. 17.

Table 7. Age composition of *Culex tritaeniorhynchus* in winter and early spring, 1972–1975

Date	No. females examined	No. parous females	% parous females
Dec. 2–10	23	3	13.0
Jan. 18–31	28	0	0.0
Feb. 1–7	63	15*	23.9
Feb. 11–14	27	0	0.0
Feb. 24–27	16	3	18.8
Mar. 3–9	167	44	26.3
Mar. 11	113	13	11.5
Mar. 22–28	157	73	46.5

* One female retained two mature eggs.

traps more abundantly at the henhouse than at the pigsties or the cowshed (Table 3). The overwintering stage seems to be the larva, because of the absence of adults in winter (Table 4).

Mansonia (Mansonioides) uniformis (Theobald): This was a common species, and adults were collected by light traps and by hand at animal shelters, and also by diy ice traps in the field. One pupa was obtained at a marsh in the end of March, which seemed to indicate the beginning time of adult emergence (see Table 4). No adults were encountered in winter, therefore, larvae were considered to overwinter.

Uranotaenia (Pseudoficalbia) bimaculata Leicester: The number of collected individuals in either imaginal or larval stage was not large. Breeding sites were earthen jars and tree holes. Apparently larvae survive in winter (Table 5), and adults emerge throughout the year (Table 4 and 6).

Orthopodomyia anopheloides (Giles): Larvae were collected mainly from tree holes. The larvae found in December to March were all in the fourth instar (Table 5). When the fourth instar larvae collected on January 26 were reared at room temperatures till February 4, and at 21°C after that, 4 males emerged on February 24–27 and 2 females each on March 7 and 19, but still 3 larvae remained on March 27 (Table 6). These facts seem to indicate that the fourth instar larvae overwinter in the state of diapause.

Aedes (Finlaya) watasei Yamada: Larvae were not rare in tree holes and depressions of gravestone markers. Four females were collected by light traps at pigsties in May (Table 3 and 4). The fourth instar larvae in March (Table 5) may indicate that this species overwinters in the larval stage.

Aedes (Finlaya) japonicus (Theobald): Larvae were commonly found in depressions of gravestone markers, and in tree holes. It is clear that the larva can overwinter successfully, because larvae in all instars were found from January to March (Table 5), and adults emerged in February (Table 6). However, the first instar larvae in winter and early spring (Table 5) may show the overwintering also in the egg stage.

Aedes (Finlaya) okinawanus Bohart: From tree holes and earthen jars, larvae were collected in December, January and March (Table 5). The adult emergence from overwintering larvae was observed in February (Table 6).

Aedes (Finlaya) togoi (Theobald): Larvae were found in rock holes and a boat in the seaside. It is certain that the larva can overwinter successfully. Adult females were obtained by light traps throughout the year except for January (Table 4), but it is not known whether the adult emergence occurs even in winter.

Aedes (Finlaya) nipponicus LaCasse et Yamaguti: Old larvae were collected from tree holes near the seaside and an earthen jar in the forest of Mt. Yuwan. The fourth instar larvae in February (Table 5) seem to indicate that the overwintering stage is the larva.

Aedes (Stegomyia) albopictus (Skuse): Common breeding sites of this species were depressions of gravestone markers and vases in the cemetery, but larvae were found also in tree holes and earthen jars. Adult females were collected by light traps from April to November (Table 4), but larvae were found in winter (Table 5). The adult emergence

occurs even in winter (Table 6). The first instar larvae collected from January to March may indicate that this species can overwinter also in the egg stage.

Aedes (Stegomyia) riversi Bohart et Ingram: Breeding sites were similar to those of *A. albopictus*, but larvae were more common in tree holes in the forest of Mt. Yuwan. As in *A. albopictus*, this species overwinters in the larval stage, and possibly in the egg, and adults emerge even in winter.

Aedes (Aedimorphus) vexans nipponii Theobald: Larvae were found in rice fields and marshes. Many adults were collected by light traps. Females seem to prefer pigs and cows to hens (Table 3). The main overwintering stage is considered to be the egg from the fact that a large number of the second and third instar larvae were encountered in a fallow rice field flooded a few weeks before, and no larvae were found in December and January in spite of much effort to catch mosquito larvae in rice fields and marshes (Table 5).

Armigeres (Armigeres) subalbatus (Coquillett): Larvae were common in foul water in villages. Adults were also abundant at animal shelters. It is certain that larvae can overwinter successfully (Table 5). The presence of males throughout the year (Table 4) indicates that adults probably emerge even in winter.

Culex (Lutzia) vorax (Edwards): Larvae were found in association with *C. pipiens fatigans* in March (Table 5). Seven females collected by light traps in January and February may show that the female adult is the overwintering stage.

Culex (Eumelanomyia) hayashii Yamada: A few larvae were collected from marshes in summer. Since one female was collected in January (Table 4), the overwintering stage is probably the female adult.

Culex (Eumelanomyia) okinawae Bohart: Many larvae were found at a ground pool within an artificial cave on the slope of Mt. Yuwan, and a number of adults were resting on the inside wall of the cave, in September. In the same cave, old larvae were collected also in January and February (Table 5) and one female was flying in January. This seems to indicate the successful overwintering in the larval stage, and perhaps in the adult stage.

Culex (Lophoceraomyia) infantulus Edwards: Only one third instar larva was obtained from a fallow rice field.

Culex (Culiciomyia) ryukyensis Bohart: Larvae were common in earthen jars and tree holes. This species is considered to overwinter in the larval stage, because larvae were found in January-March (Table 5) and adults emerged in February (Table 6).

Culex (Culex) bitaeniorhynchus Giles: Larvae were abundant in rice fields, marshes and road-side ditches, usually in association with green algae. Adults were collected by light traps at animal shelters and also by dry ice traps in the field. Throughout the year except for January, females were obtained by light traps (Table 4), and larval collections were made in January-March (Table 5). Therefore, it seems that this species can overwinter in the larval stage, and perhaps also in the female adult.

Culex (Culex) sinensis Theobald: Two old larvae were collected in May at a ground

pool with clean water near rice fields, and reared to adults in the laboratory. Some females obtained by light traps.

Culex (Culex) whitmorei (Giles): Only one female was collected by the light trap at a pigsty in September.

Culex (Culex) pseudovishnui Colless: Larvae were collected from rice fields. Females were encountered at animal shelters throughout the year (Table 4). Old larvae appeared in rice fields in the end of March, but no larvae in December-February. Females seem to overwinter.

Culex (Culex) tritaeniorhynchus Giles: Larvae were common in rice fields and marshes. This species was the dominant mosquito at animal shelters. Females were found throughout the year (Table 4), and the blood feeding was observed, on warm days, even in winter. Fairly large numbers of larvae in the second to the fourth instars and pupae were found in rice fields and marshes in December, but the extensive larval survey in January and February at potential breeding sites including the above rice fields and marshes did not yield any immature stages of this mosquito (Table 5). In March, larvae and pupae were collected, and the emergence of adults was confirmed in the end of March. From these facts, it is certain that this species overwinters in the female, but not in the larva. The parous rate of females from December to February was variable (Table 7), but it was demonstrated that parous females are included in the overwintering population at least in some circumstances. One female collected in the beginning of February retained two mature eggs (Table 7), indicating the blood feeding some days before.

Culex (Culex) mimeticus Noe: Larvae were very common in rice fields and marshes very often in association with green algae, but the number of adults at animal shelters was small. Larvae in all instars and pupae from December to March (Table 5) seem to show the successful overwintering in the larval stage, and the continuous adult emergence and egg laying even in winter.

Culex (Culex) pipiens fatigans Wiedemann: This was a common mosquito around villages. Males and females were collected by light traps throughout the year (Table 4) and larvae in all instars and pupae were found even in winter (Table 5). Apparently, blood feeding, oviposition, and adult emergence continue in winter.

Culex (Culex) vagans Wiedemann: Larvae were collected in fallow rice fields in March (Table 5). Adult emergence was observed in late March.

DISCUSSION

Hayashi et al. (1975) reported the successful isolation of JE virus on Amami-Oshima from *C. tritaeniorhynchus* females in the winter of 1973, but the failure in isolation in the winter of 1974 in spite of much effort exerted. This finding in 1973 was surprising in view of the complete disappearance of the virus in winter and spring in the Nagasaki area (Fukumi et al., 1975).

Experimentally, JE virus can survive through the winter in *C. tritaeniorhynchus*

females infected in autumn (Mifune, 1965; Shichijo et al., 1972). However, for the successful overwintering of the virus within the vector mosquito in the field, the presence of viremic animals in autumn and the overwintering of the female mosquitoes with the experience of blood feeding are required. In the Nagasaki area, viremic animals are scarcely found in autumn, and the great majority of, if not all, overwintering females of *C. tritaeniorhynchus* do not have the experience of blood feeding (Wada et al., 1975). Therefore, it is understandable that no JE virus was isolated from many *C. tritaeniorhynchus* females having emerged from hibernation in spring from 1965 to 1973 (Fukumi et al., 1975).

On Amami-Oshima, not a small number of susceptible pigs still remain in autumn, owing to the low density of *C. tritaeniorhynchus* in summer. Unpublished data show that the females of *C. tritaeniorhynchus* go into weak diapause after emergence in autumn but acquire their feeding activity soon after. The acquisition of the feeding activity in diapausing females will occur successively, because the time of emergence and the weakness of diapause differ in individuals. In this way, it is expected that the females active in blood feeding are always present in winter. In fact, as shown in the present paper, the females of *C. tritaeniorhynchus* feed on animals frequently on warm days in winter. The parous females found in winter (Table 7) indicate the repetition of blood feeding and oviposition. These situations on Amami-Oshima are much more favorable than in the Nagasaki area for the persistence of JE virus during winter.

However, even when pigs are infected by the bites of overwintering mosquitoes, a sufficient number of mosquitoes must be present at the time of viremia in those infected pigs, for JE epizootic to start in the pig population. In other words, the successful overwintering of JE virus on Amami-Oshima would be achieved by the succession of the pig-mosquito cycle. It is considered that the temperatures in winter play an important role in the virus overwintering, because the feeding activity of *C. tritaeniorhynchus* is

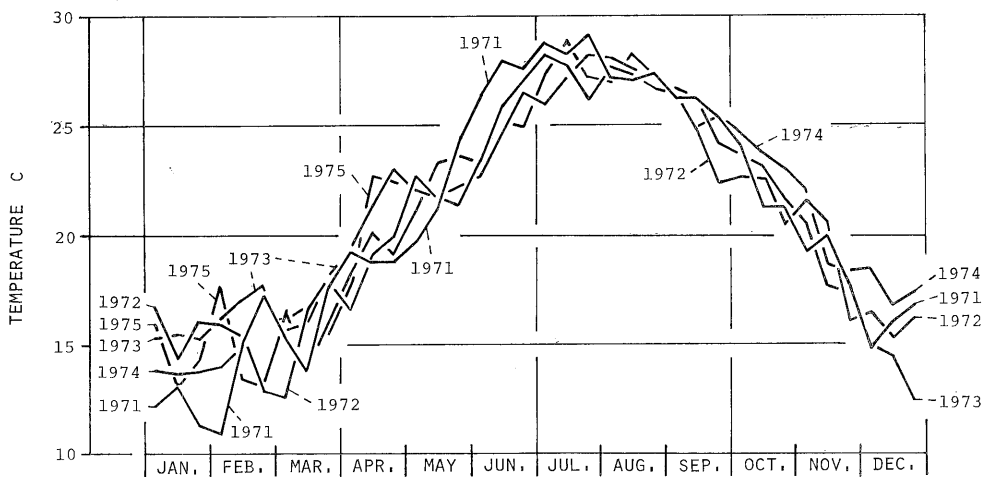


Fig. 1. Ten-day average temperatures at Naze, Amami-Oshima, 1971-1975.

influenced much by prevailing temperatures. Therefore, ten-day average temperatures are shown for the years from 1971 to 1975 in Fig. 1. It is apparent that temperatures in winter differed remarkably in years, and the winter of 1973 was much warmer than usual years. This warm winter is probably the reason why JE virus was isolated from *C. tritaeniorhynchus* in 1973.

On Amami-Oshima, the infection of mosquitoes with JE virus and the appearance of 2-ME sensitive antibody in pigs were observed in close association, even in winter (Hayashi et al., 1975). Therefore, it is strongly implied again that the persistence of the pig-mosquito cycle is required for the successful overwintering of JE virus, and the possibility of the virus overwintering by other means is very small. It seems reasonable to assume that in the year when JE virus did not overwinter, the virus is introduced from some other areas to initiate the epizootic in pigs in summer.

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REFERENCES

- 1) Fukumi, H., Hayashi, K., Mifune, K., Shichijo, A., Matsuo, S., Omori, N., Wada, Y., Oda, T., Mogi, M., & Mori, A. (1975): Ecology of Japanese encephalitis virus in Japan I. Mosquito and pig infection with the virus in relation to human incidence. *Trop. Med.*, 16, 97-110.
- 2) Kanda, T. & Kamimura, K. (1967): New record of *Anopheles bengalensis* from Amami Islands, southern Japan. *Jap. J. Sanit. Zool.*, 18, 108-113 (in Japanese with English summary).
- 3) Hayashi, K., Mifune, K., Shichijo, A., Suzuki, H., Matsuo, S., Makino, Y., Akashi, M., Wada, Y., Oda, T., Mogi, M. & Mori, A. (1975): Ecology of Japanese encephalitis virus in Japan III. The results of investigation in Amami island, southern part of Japan in the East China Sea, from 1973 to 1975. *Trop. Med.*, 16, 129-142.
- 4) Mifune, K. (1965): Transmission of Japanese encephalitis virus to susceptible pigs by mosquitoes of *Culex tritaeniorhynchus* after experimental hibernation. *Endem. Dis. Bull. Nagasaki*, 7, 178-191.
- 5) Shichijo, A., Mifune, K., Hayashi, K., Wada, Y., Oda, T. & Omori, N. (1972): Experimental infection of *Culex tritaeniorhynchus summosus* mosquitoes reared in biotron with Japanese encephalitis virus. *Trop. Med.* 14, 218-229 (in Japanese with English abstract).
- 6) Sasa, M. & Kamimura, K. (1971): Index and consideration on the taxonomy of Japanese mosquitoes. *In Progress in Sanitary Zoology*, edited by Sasa, M., I, 1-47, Keigaku Shuppan Co. Ltd., Tokyo (in Japanese).
- 7) Tanaka, K., Saugstad, E. S. & Mizusawa, K. (1975): Mosquitoes of Ryukyu Archipelago (Diptera : Culicidae). *Mosquito Systematics*, 7, 207-233.
- 8) Wada, Y., Oda, T., Mogi, M., Mori, A., Omori, N., Fukumi, H., Hayashi, K., Mifune,

K., Shichijo, A. & Matsuo, S. (1975): Ecology of Japanese encephalitis virus in Japan II. The population of vector mosquitoes and the epidemic of Japanese encephalitis. *Trop. Med.*, 16, 111-127.

奄美大島の蚊と日本脳炎ウイルスの越冬について

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奄美大島において1972-1975年に蚊の調査を行なった。成虫は畜舎にかけたライトトラップ及び野外でのドライアイストラップにより, 幼虫はその発生場所において, 1年を通じて採集を行なった。その結果31種の蚊が得られた。上記の方法による採集の記録と, 野外で採集した幼虫の飼育の記録とから, 各々の種の, 特に冬季における, 生態について記載した。また, 奄美大島での日本脳炎ウイルスの越冬について, 伝搬蚊コガタアカイエカの生態の面から考察を加え, ウイルスの越冬が可能なのは, 冬の気温が高く, 蚊-豚の感染サイクルが持続する場合においてのみであると結論した。

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