

Epidemio-entomological Surveys in Vector Mosquitoes of Japanese Encephalitis in Kyongbuk, Korea*

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Abstract : The seasonal prevalence and population dynamic of *Culex tritaeniorhynchus* (*Cx. tritaeniorhynchus*) in relation to the epidemic of Japanese encephalitis, and the epidemio-entomological surveys of this vector mosquito in Kyongbuk Province, Korea were made during the period of 15 years spanruing from 1984 to 1998. As in the general patterns of seasonal prevalence, *Cx. tritaeniorhynchus* was first collected between early and late June, and peaks in the population densities of *Cx. tritaeniorhynchus*, occurred during the period from late July in 1987 and 1994, early- to late-August in 1984-1986, 1990-1993 and 1995, and early- and mid-September in 1988, 1989 and 1996-1998. they showed a simple sharply pointed one-peak curve. There was a gradual decrease from mid-September, accompanied by with a very small number of them collected until late October or early November in every year, except 1990.

Cx. tritaeniorhynchus with nocturnal biting habits had two peaks in their daily activities, one soon after the sunset and the other during a few hours' period from 2-3 o'clock, although the relative numbers collected varied and the highest peak collection occurred at different time in a night. The total number of larval *Cx. tritaeniorhynchus* in rice fields was the highest in mid-August, and its number progressively decreased in early September due to insecticides sprays. After late September, the larvae was rarely found until rice plants were harvested. The larval *Cx. tritaeniorhynchus* showed high resistance against five organophosphorus compounds. In the adult horizontal life table characteristics of Kyongsan colonies of *Cx. tritaeniorhynchus* under insectary conditions, life expectancy was 28.3 days for males and 59.8 days for females. The net reproductive rate was 7.8 days, and generation time was 25.6 days.

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Key Words : *Culex tritaeniorhynchus*, Horizontal life table, Japanese encephalitis, Kyongbuk Province, Light trap, Population dynamics, Seasonal prevalence, Vector mosquito

Introduction

Culex tritaeniorhynchus Giles (Diptera: Culicidae) has a number of problems encountered by medical and entomological scientists. The present study was undertaken as part of our continued investigation of the epidemiology and control of mosquito-borne diseases, since the species was found to be the main vector of Japanese encephalitis (JE) in Korea, Japan, China and South-Eastern Asia.

Sabin *et al.*[1] first reported the isolation of JE virus from the American military personnels in Kunsan area, and also showed the presence of JE antibody among Korean residents in several areas, including Kunsan. After the Korean War, the *Culex* mosquitoes have been the primary target for many investigations in order to get more information or to control JE in Korea: Kono and Kim[2] summarized the epidemiological features of JE in Korea from 1949 to 1966; Kim[3] reported that the annual incidence of JE in 1982 was unusually high, a total of 1,197 cases with a mortality rate of 3.1; Joo and Wada[4] studied the seasonal prevalence of vector mosquitoes of JE in Kyongbuk Province. The seasonal prevalence and population dynamics of *Cx. tritaeniorhynchus* in relation to the epidemics of JE, and the ecology of these

vector mosquitoes in Kyongbuk Province [5], suggested that the population density of *Cx. tritaeniorhynchus* in the Province was decreasing over the seven-year period from 1984 to 1990.

This paper presents data obtained on seasonal variation and population density of adult and larval mosquitoes collected in Kyongbuk Province during 1984 and 1998, and on the number of reported JE cases during that period.

Materials and Methods

1. Surveyed areas:

Kyongbuk Province is situated in the southeast part of the Korean peninsula, having an area of 19,700 km². Two areas were selected as vector mosquito survey stations (Fig. 1). The main survey area was Keimyung University training farm in Kyongsan county located about 15 km E.S.E. of Taegu City. The other area was the Agricultural research farm in Kyongbuk provincial office of Rural development, located in Dongho village, a northern district of Taegu city. The more detailed geographical conditions of surveyed areas were presented by Baik and Joo[5].

2. Meteorological data:

Meteorological data for the period of

the present survey was provided by the Taegu branch of the Korea Meteorological Agency.

3. Light trap operation:

In order to observe the seasonal prevalence of the vector mosquito populations, light trap collections were performed as follows: A light trap was fixed 1.5 m above the ground at the trapping spots, including the piggery, the cow-shed and the house-dwelling, and operated from dusk to dawn on one-night per week schedules. Mosquitoes collected at each station were counted by species.

4. Indices of mosquito abundance:

In order to compare the annual abundance of *Cx. tritaeniorhynchus*, mean percent index (MPI) proposed by Maeda *et al.* [6] was used.

5. Collection of larvae:

In order to estimate the species and density of mosquito larvae and pupae, 30 fixed rice paddies were dipped from April to October at one week intervals in 1991-1993. The dipper was 15 cm in diameter and 5 cm in depth with a wooden handle of 60 cm in length.

At the outset a collector stood at a point on the side of rice paddies, and took a dip on the water surface, which is thought to be the most favorable for the breeding of the larvae and pupae within the reach of the dipper. In each rice paddies, the dipping was made ten times which is suggested to be necessary to determine the distribution pattern of the

numbers of mosquito larvae in rice paddy [7]. The total number of mosquito larvae in the study areas was estimated according to the methods described by Wada and Mogi [8]. Total number (No.) in the study area at the median age of each stage in the area was the average No. per dip $\times 186 \times$ total area with water in m^2 /mean period of the stage at the then temperature. Ten day average temperatures were used here in.

6. Resistance level for organophosphorus compounds:

In order to determine the insecticidal resistance, blood fed females of *Cx. tritaeniorhynchus* were collected from human and animal shelters with an insect net or sucking tube and were transferred into the cages. The mosquitoes were allowed to oviposit in an insectary at $30 \pm 1^\circ C$ and of 70-80% relative humidity with 16 hours of illumination per day. Approximately 300 first instars were reared in enamel pans measuring 50×40 cm filled to 2 cm depth of water and fed on crushed powders of laboratory mouse pellets and the adults were fed on sugar solution. Insecticidal resistance assay was the modified one from those described by Yasutomi and Takahashi [9]: Please check for accuracy. Toxicities of five organophosphorus compounds were determined with fourth instar, and LC_{50} were calculated from the average of two replicates. From these data the regression of the probit mortality on log dosage was computed and the LC_{50} were obtained.

7. Life table characteristics of *Cx. tritaeniorhynchus*:

In order to estimate the effects of mating and/or male-female association on the lifespan of adult *Cx. tritaeniorhynchus*, the longevity of adults was tested by comparing six groups of males and females. All experiments were repeated two times and conducted in an insectary in which the temperature and relative humidity were maintained at $27 \pm 1^\circ\text{C}$ and $75 \pm 5\%$, respectively. A 16L:8D photoperiod was established with 40 watt fluorescent light and 1 hour simulated dusk and dawn [10]. Adults were continuously offered 5.0% sucrose solution in flask with cotton wads, and the solution was changed every 5 days. Each morning, all dead individuals and egg rafts were removed and recorded. The calculation procedures, formulae, and rationale used in this study followed the

methods described by Reisen *et al.* [11]

Results

The earliest date when *Cx. tritaeniorhynchus* were first collected by light traps, and the meteorological data at that time in Kyongsan county are shown in Table 1. During the period from 1984 to 1998, *Cx. tritaeniorhynchus* were collected first between the 3rd and 8th of June in 1987, 1988, 1991 and 1994, and in the mid- and late-June in another years. At that time, the air temperature ranged from 17.2 to 35.1°C , and relative humidity from 65 to 90%. The average number of *Cx. tritaeniorhynchus* per trap-night ranged from 0.3 to 2.5.

The dates of peak population of *Cx.*

Table 1. Fifteen-year observation of the earliest date, when *Culex tritaeniorhynchus* appeared in Kyongsan county, Kyongbuk Province, together with meteorological data

Year	Earliest date	Temperature ($^\circ\text{C}$...)	Humidity (%)	Average No. per trap-night
1984	June 19	$21.9^\circ \pm 29.4$	77	2.5 (5/2)*
1985	June 27	$19.4^\circ \pm 21.5$	90	0.5 (1/2)
1986	June 12	$12.0^\circ \pm 28.8$	65	0.3 (1/3)
1987	June 4	$19.0^\circ \pm 35.1$	65	1.5 (3/2)
1988	June 8	$23.2^\circ \pm 29.6$	83	0.3 (1/3)
1989	June 28	$17.5^\circ \pm 29.7$	75	2.3 (7/3)
1990	June 21	$18.7^\circ \pm 28.8$	82	1.0 (3/3)
1991	June 3	$17.2^\circ \pm 20.8$	86	0.3 (1/3)
1992	June 17	$17.4^\circ \pm 26.0$	71	0.3 (1/3)
1993	June 23	$18.3^\circ \pm 27.3$	80	1.0 (3/3)
1994	June 8	$18.8^\circ \pm 26.4$	74	0.3 (1/3)
1995	June 21	$18.5^\circ \pm 23.1$	67	0.3 (1/3)
1996	June 13	$19.2^\circ \pm 29.4$	71	0.7 (2/3)
1997	June 27	$22.7^\circ \pm 30.3$	79	0.3 (1/3)
1998	June 23	$20.0^\circ \pm 28.8$	69	0.3 (1/3)

* Number in parentheses means the total number of female /traps.

tritaeniorhynchus and the meteorological data at that time are listed in Table 2. The highest population density of *Cx. tritaeniorhynchus* during 1984 and 1998 was observed in early-August to mid-September, and in the late July in 1987 and 1994. The air temperature was between 21.9 to 25.2°C and humidity from 82 to 85%. The maximum numbers of *Cx. tritaeniorhynchus* in 1984 and 1994 were 706.6 and 887.7 per trap-night, respectively. Subsequently, the number decreased markedly in 1989, 1993 and also in 1997, reaching a minimum of 120.7, 115.0 and 130.3 per trap-night, respectively.

Table 3 shows the dates when *Cx. tritaeniorhynchus* were not collected in the surveyed area. *Cx. tritaeniorhynchus* were not found in the area between September 26 in and November 7 in 1990-

1996. The temperature of the air at that time ranged from 2.6 to 28.5 °C and humidity ranged from 54 to 83 %.

Table 4 summarizes the seasonal prevalence of *Cx. tritaeniorhynchus* collected by light traps. In general, *Cx. tritaeniorhynchus* were collected in months every year, five months spanning from June to October, in every year in 1995, the average number of female *Cx. tritaeniorhynchus* per trap-night in June was 0.2, increased to 21.8 in July, and reached its maximum number, 322.3 in August. In September the average number decreased to 92.8, to 2.5 in October, and none was collected in November. The general pattern of monthly changes of *Cx. tritaeniorhynchus* per trap-night in other years was similar to those in 1995, but the patterns of monthly changes in 1990, 1993 and 1994 were dissimilar to those in

Table 2. Date of peak population of *Culex tritaeniorhynchus* and meteorological data

Year	Date of peak population	Temperature (°...)	Humidity (%)	Average No. per trap-night
1984	Aug.31	22.6°±27.1	86	706.6 (3,548/5)*
1985	Aug.15	23.5°±29.4	89	229.5 (659/3)
1986	Aug.14	22.2°±30.9	81	171.7 (515/3)
1987	July. 23	20.6°±28.6	88	205.3 (616/3)
1988	Sep. 1	18.7°±30.7	72	253.7 (761/3)
1989	Sep. 6	18.5°±27.6	75	120.7 (362/3)
1990	Aug. 22	24.5°±34.2	71	205.7 (617/3)
1991	Aug. 9	20.0°±21.5	85	178.0 (534/3)
1992	Aug. 5	21.9°±25.2	82	255.7 (767/3)
1993	Aug. 25	24.5°±31.9	79	115.0 (345/3)
1994	July. 27	24.8°±31.4	80	887.7 (2,663/3)
1995	Aug. 24	24.3°±31.7	83	535.3 (1,606/3)
1996	Sep. 5	20.0°±26.5	73	318.0 (954/3)
1997	Sep. 18	16.6°±27.5	57	130.3 (391/3)
1998	Sep. 15	20.3°±31.5	61	51.7 (1,655/3)

* Number in parentheses means the total number of female per trap-night.

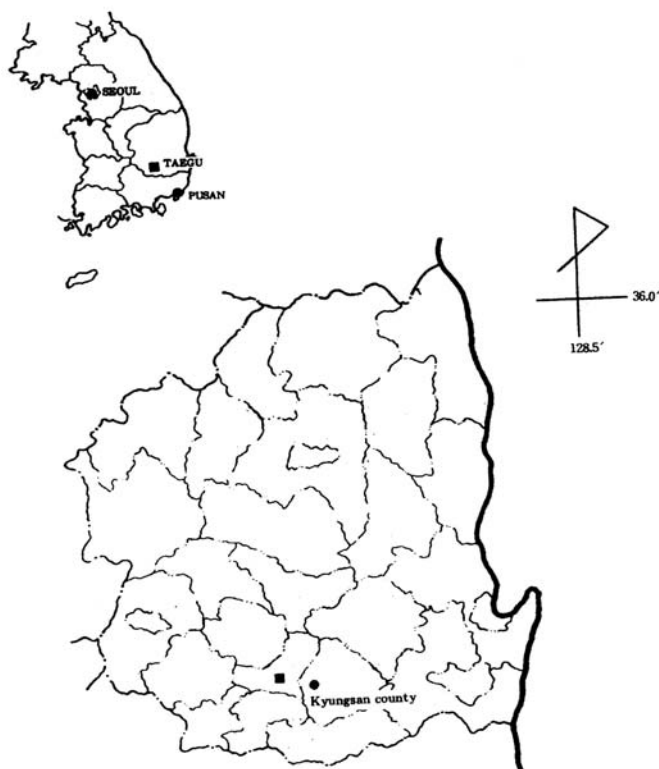


Fig. 1. Surveyed areas (●) in Kyungpook Province, Korea.

Table 3. Date of disappearance of *Culex tritaeniorhynchus* and meteorological data

Year	Date of peak population	Temperature (°...)	Humidity (%)
1984	Oct. 6	8.0°±22.9	67
1985	Oct.24	9.3°±23.0	68
1986	Oct. 2	11.5°±17.4	83
1987	Oct. 8	13.2°±28.5	70
1988	Oct. 26	8.4°±23.1	70
1989	Oct. 11	10.6°±25.1	81
1990	Sep. 26	15.8°±24.8	65
1991	Oct. 17	11.7°±21.0	54
1992	Oct. 21	8.6°±20.1	69
1993	Oct. 6	16.3°±17.9	63
1994	Oct. 19	11.4°±18.9	71
1995	Oct. 25	8.8°±20.2	59
1996	Nov. 7	2.6°±11.2	71
1997	Oct. 16	8.5°±24.8	56
1998	Nov. 5	6.4°±18.8	63

Table 4. Seasonal prevalence of *Culex triaeniorhynchus* by the average numbers collected in each trap during fifteen years in Kyongsan county, Kyongbuk Province

Year	Average number of female mosquitoes per trap-night								
	March	Apri	May	June	July	Aug.	Sep.	Oct.	Nov.
1984	0	0	0	1.1	19.2	133.5	55.8	0.1	0
1985	0	0	0	0.1	23.6	156.0	92.2	3.1	0
1986	0	0	0	0.2	17.9	113.8	63.4	0.1	0
1987	0	0	0	1.3	111.9	107.3	16.6	0.2	0
1988	0	0	0	0.4	50.3	36.5	70.3	1.5	0
1989	0	0	0	0.6	13.7	24.3	45.6	1.5	0
1990	0	0	0	0.6	22.0	141.5	67.5	0	0
1991	0	0	0	0.8	28.5	65.5	44.3	1.5	0
1992	0	0	0	0.1	38.5	164.1	80.2	0.8	0
1993	0	0	0	0.7	13.1	46.1	33.8	0	0
1994	0	0	0	2.2	284.2	689.4	79.8	0	0
1995	0	0	0	0.2	21.8	322.3	92.8	2.5	0
1996	0	0	0	0.3	3.3	41.0	158.9	0.5	0
1997	0	0	0	0.1	6.8	5.3	80.8	2.2	0
1998	0	0	0	9.9	115.0	323.2	237.4	3.9	0

Table 5. Relative abundance of *Culex triaeniorhynchus* population in successive years since 1984

Year	At the 3-stations located in suburban areas	
	Total No. collected	Mean percent index
1984	12,875	702.3
1985	3,101	130.1
1986	2,398	100.0
1987	3,184	119.5
1988	2,124	83.7
1989	877	30.8
1990	2,779	115.9
1991	1,871	65.5
1992	3,760	175.4
1993	1,087	45.6
1994	14,516	462.8
1995	6,242	189.8
1996	2,572	90.7
1997	1,768	59.8
1998	8,981	315.5

1995.

The results of relative abundance and MPI calculation for successive years since 1984 are shown in Table 5. and illustrated in

Fig. 2.

Such presentation is useful for indicting annual pattern of mosquito prevalence obtained from the data collected

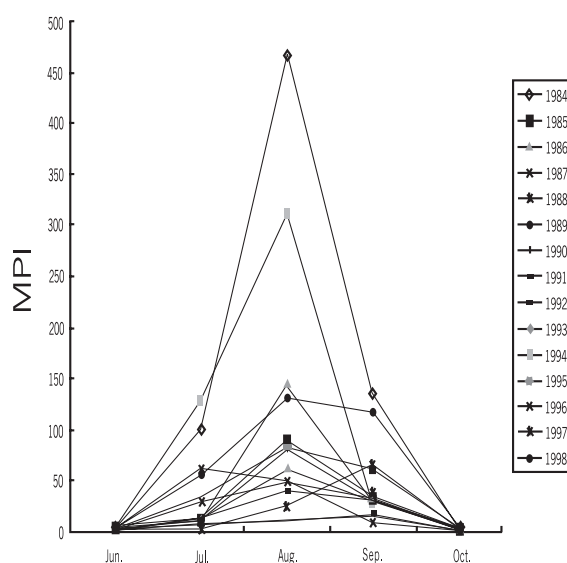


Fig. 2. Annual prevalence of *Culex tritaeniorhynchus*, as shown in mean percent index (MPI) calculated from the data of mosquito collection at 3 stations. Standard year : 1986.

Table 6. Comparison of total and engorged number of *Culex tritaeniorhynchus* collected by light traps at piggery, cow-stall, and house-dwelling during fifteen years, 1984-1998

Year	Piggery		Cow-stall		House-dwelling		Subtotal	
	collected	engorged	collected	engorged	collected	engorged	collected	engorged
1984	7,413	4,056(54.7)*	4,087	1,590(38.9)	1,375	140 (10.2)	12,875	5,786 (44.9)
1985	1,592	598 (37.6)	1,343	394 (29.3)	166	8 (4.8)	3,101	1,000 (32.2)
1986	1,095	547 (50.0)	1,176	485 (41.5)	127	9 (7.1)	2,398	1,041 (43.4)
1987	1,547	707 (45.7)	1,526	651 (42.7)	111	12 (10.8)	3,184	1,370 (43.0)
1988	808	395 (48.9)	1,223	403 (33.0)	93	4 (4.3)	2,124	802 (37.8)
1989	382	156 (40.8)	473	192 (40.6)	22	0 (0.0)	877	348 (39.7)
1990	1,018	319 (31.3)	1,609	484 (30.1)	152	1 (0.7)	2,779	804 (28.9)
1991	619	228 (36.8)	1,187	433 (36.5)	65	9 (13.8)	1,871	670 (35.8)
1992	1,579	515 (32.6)	1,901	1,144 (60.2)	280	41 (14.6)	3,760	1,700 (45.2)
1993	583	235 (40.3)	446	192 (43.0)	58	9 (15.5)	1,087	436 (40.1)
1994	3,283	1,357 (41.3)	11,043	6,109 (55.3)	190	6 (3.2)	14,516	7,472 (51.5)
1995	2,143	1,007 (47.0)	4,063	2,206 (54.2)	36	3 (8.3)	6,242	3,216 (51.5)
1996	1,440	760 (52.8)	1,069	496 (46.4)	63	2 (3.2)	2,572	1,258 (48.9)
1997	924	613 (66.3)	811	505 (62.3)	33	2 (6.1)	1,768	1,120 (63.3)
1998	5,420	1,544 (28.5)	3,390	654 (19.3)	194	0 (0.0)	9,004	2,198 (24.4)

* The number in the parentheses means engorged percentage.

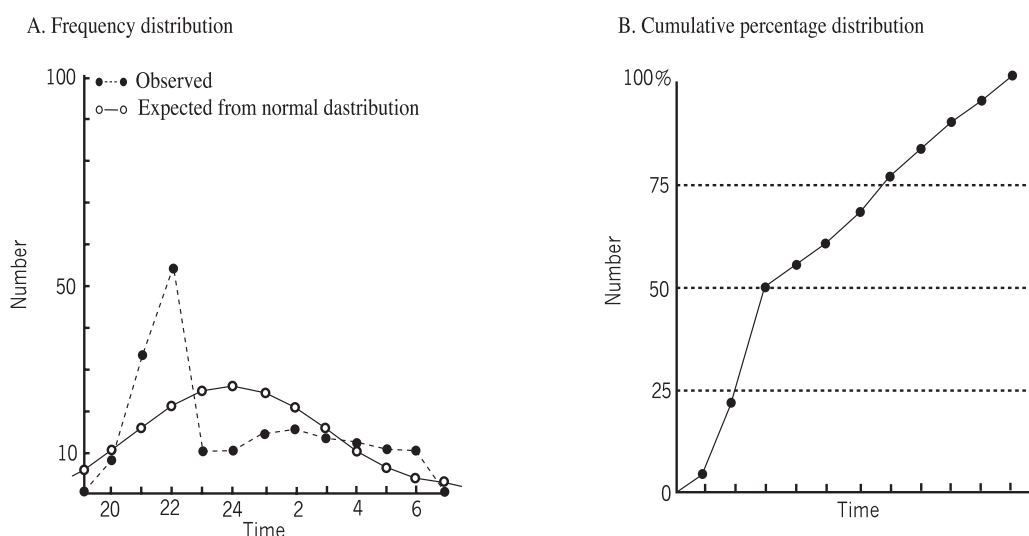
at several stations. It was found that the total number of *Cx. tritaeniorhynchus* progressively decreased during the 5-year

intervals. A marked decrease in MPI was obtained in 1989, 1993 and 1997.

Table 6 shows the numbers and

Table 7. Monthly fluctuation of total and engorged number of *Culex tritaeniorhynchus* collected by light trap (1984–1998)

Year	Jun.		Jul.		Aug.		Sep.		Oct.	
	Coll.	Engorg.	Coll.	Engorg.	Coll.	Engorg.	Coll.	Engorg.	Coll.	Engorg.
1984	50	6 (12.0)	998	381 (38.2)	8,812	3,798 (43.1)	3,012	1,599 (53.1)	3	2 (66.7)
1985	1	0	260	98 (37.7)	1,872	632 (33.8)	922	261 (28.3)	46	9 (19.6)
1986	2	1 (50.0)	269	91 (33.8)	1,366	640 (46.9)	761	309 (40.6)	0	0
1987	16	0	1,679	582 (34.7)	1,288	710 (55.1)	199	78 (39.2)	2	0
1988	9	1 (11.1)	604	267 (44.2)	438	102 (23.3)	1,055	430 (40.8)	18	2 (11.1)
1989	7	0	151	55 (36.4)	291	109 (37.5)	410	182 (44.4)	18	2 (11.1)
1990	7	0	264	59 (22.3)	1,698	383 (22.6)	810	362 (44.7)	0	0
1991	9	0	314	116 (36.9)	982	223 (22.7)	544	322 (59.2)	22	9 (40.9)
1992	1	0	577	211 (36.6)	1,969	879 (44.6)	1,203	570 (47.4)	10	5 (50.0)
1993	10	7 (70.0)	118	23 (19.5)	553	228 (41.2)	406	178 (43.8)	0	0
1994	33	30 (90.9)	3,410	1,656 (48.6)	10,341	5,461 (52.8)	718	327 (45.5)	14	4 (28.6)
1995	2	0	262	168 (64.1)	4,834	2,557 (52.8)	1,114	478 (42.9)	30	12 (40.0)
1996	3	0	40	7 (17.5)	615	271 (52.9)	1,907	978 (51.3)	7	2 (28.6)
1997	1	0	102	63 (61.8)	663	317 (47.8)	969	721 (74.4)	33	19 (57.6)
1998	138	58 (42.0)	1,380	397 (28.8)	3,878	844 (21.8)	3,561	893 (25.1)	47	6 (12.8)

**Fig. 3.** Total numbers of *Culex tritaeniorhynchus* collected by light trap at hourly interval at Kyungsan county.

percentages of engorged female *Cx. tritaeniorhynchus* collected by light traps at three localities during fifteen years, ranging from 1984 to 1998. The overall

rates of engorgement, as calculated by dividing the number engorged with the total, which reflect the blood-sucking activity of *Cx. tritaeniorhynchus* in 1986

Table 8. Frequency distribution and cumulative percentage of *Culex tritaeniorhynchus* collected by light trap at hourly intervals at Kyongsan county (1991-1993)*

Time of collection	Cumulative			Z	P	(f-F) ²	
	Freq**	No	%			F	F
1700-1800	0	0		-1.85	0.0322	6.0858	6.0858
1800-1900	0	0		-1.52	0.0321	6.0669	6.0609
1900-2000	8	8		-1.18	0.0547	10.3383	0.5289
2000-2100	33	41	21.69	-0.84	0.0814	15.3846	20.1696
2100-2200	54	95	50.26	-0.50	0.1081	20.4309	55.1558
2200-2300	10	105	55.56	-0.16	0.1279	24.1731	8.3099
2300-2400	10	115	60.85	0.18	0.1350	25.1731	9.4342
2400-0100	14	129	68.25	0.52	0.1271	24.0219	4.1811
0100-0200	15	144	76.19	0.86	0.1066	20.1474	1.3120
0200-0300	13	157	83.07	1.20	0.0798	15.0822	0.2875
0300-0400	12	169	89.42	1.54	0.0533	10.0737	0.3683
0400-0500	10	179	94.71	1.87	0.0311	5.8779	2.8908
0500-0600	10	189	100.00	2.21	0.0171	2.2319	14.1735
0600-	0				0.0136	2.5704	2.5704
Total	189				1.000	189.000	131.5704

Note : Mean and standard deviation of the table were calculated from the frequency distribution by the class interval of an hour. Mean $\pm$ SD = 11:46 $\pm$ 2.95 hours; * Date reported by Joo and Kang [12]; ** Freq: Frequency.

were in the order of 50.0% on the piggery, 41.2% on the cow-stall, and 7.1% on human dwelling. The general patterns of engorgement rates in the other years were similar to those in 1986, but the overall rates of engorgement during the periods from 1992 to 1995 were dissimilar to those in 1986.

In Table 7, the monthly fluctuations in the blood-sucking rate of *Cx. tritaeniorhynchus* are listed according to the year studied. The blood sucking rates varied greatly by different months every year. The rate was 17.5 to 64.1% in July, 21.8 to 55.1% in August and 25.1 to 74.4% in September. Although the rate decreased in October, it was kept at 11.1-66.7%.

The total number of *Cx. tritaeniorhynchus* in each hour for all the successive

collections, their cumulative number and cumulative percentage distributions are shown in Table 8 and illustrated in Fig. 3. As for the trend of nocturnal activity of *Cx. tritaeniorhynchus*, they became very active on darkening, and their activity gradually decreasing towards midnight, but slightly increasing towards dawn. The cumulative percentage distribution of the number of mosquitoes collected at hourly intervals derived from the grand total was on a rough sigmoid curve. From this figure, it is obvious that about 50.0 % of *Cx. tritaeniorhynchus* attacking pigs in a night can be collected before 23:00 p.m. or about 70.0% can be collected with seven hours' work from 20:00 p.m. to 03:00 a.m. Also, these results indicate that comparative studies of the mosquito

Table 9. Age structures of immature stages of *Culex tritaeniorhynchus* in the study area (1990)**

Date	Total No. in the study area at the median age of each stage ($\times 10^3$)*				
	L ₁	L ₂	L ₃	L ₄	Pupae
Jul. 27	64	37	53	18	18
Aug. 2	67	50	44	36	18
Aug. 10	10	375	284	157	24
Aug. 19		380	289	172	35
Aug. 23		15	25	17	3
Aug. 30	48	19		12	7
Sep. 6	46	14	11	12	6
Sep. 13	25	18	49	14	12
Sep. 20	22	11	19	11	11
Total	282	919	774	449	134

* Average No. per dip $\times 186 \times$ total area with water in m^2 /mean period of the stage at the then temperature. Ten day average temperature was used here. ** Data reported by Baik and Joo [5].

population between different localities or by seasons are useful to save the labour of a collector.

The grand total of the number of mosquitoes collected in Kyongsan county in 3 nights was 189, and the mean time calculated from this data was 11:46 with standard deviation of 2.95 hours. The theoretical number per each hour expected from normal distribution were compared with the numbers observed, and the value of chi-square was 131.5338 with the degrees of freedom of 11 thus the probability being less than 0.005.

The results suggest that the biting rhythm of *Cx. tritaeniorhynchus* observed under this method takes a pattern significantly different from a normal frequency distribution.

Table 9 summarizes the age structure of immature stages of *Cx. tritaeniorhynchus* and the seasonal prevalence of

the total number of larvae plus pupae in the survey areas. The highest larval density in cultivated fields was 876×10^3 on August 19, 1990. After the last part of September, the densities markedly decreased and the larvae and pupae of *Cx. tritaeniorhynchus* were rarely found until rice plants were harvested. In fact, the general pattern of the seasonal prevalence in the surveyed areas was determined largely by the prevalence in cultivated fields. The total number reached at its yearly peak during the mid-August, 1990.

Table 10 shows Organophosphate resistance levels of larval *Cx. tritaeniorhynchus* reared for one generation in the laboratory, and the levels were compared with that of susceptible laboratory strain. The larval *Cx. tritaeniorhynchus* showed a high resistance against 5 organophosphorus compounds. Among the compounds tested LC₅₀ of temephos, diazinon, malathion and

Table 10. Resistance levels and resistance ratios of larval *Culex tritaeniorhynchus* exposed to 5 organophosphorus compounds for 24 hours

Compound	1987			1990	
	LC ₅₀	R.R.*	S.S.**	LC ₅₀	R.R.
Diazinon	17.0	1,133	0.015	38.0	2,533
Malathion	20.8	5,200	0.0042	18.6	4,650
Fenitrothion	9.5	13,571	0.0007	10.8	15,429
Fenthion	3.3	2,357	0.0014	3.2	2,286
Temephos	61.8	79,231	0.0007	64.0	91,429

* Resistance ratio, Data reported by Department of Medical Entomology, NIH, Japan; **Data reported by Baik and Joo (1991).

fenitrothion against *Cx. tritaeniorhynchus* were 64.0 ppm, 38.0 ppm, 18.6 ppm and 10.8 ppm, respectively. The LC₅₀ to diazinon and malathion were about 2,500 and 5,000 times higher than that of susceptible strain. Of the mosquitoes tested in 1990, the resistance levels and resistance ratios to diazinon were increased, but the ratios to malathion and fenthion were slightly decreased. No fluctuation was found in resistance levels toward fenitrothion and temephos.

Table 11 lists the biological and life table characteristics of Kyongsan stain *Cx. tritaeniorhynchus*, and the present results compared were with those of Nagasaki and Taipei stains described by Reisen *et al.*[11]. The life expectancy of *Cx. tritaeniorhynchus* in the present study was 28.33 days for males and 59.81 days for females, and was generally longer than Nagasaki and Taiwan strains reported by Reisen *et al.*[11]. The net reproductive rate was found to be 7.81% living female offspring/female per generation, and generation time was 25.6 days.

Discussion

The epidemio-entomological studies on the subject of JE and its vector mosquitoes have been carried out by many investigators in South-east-Asia, including China, Indonesia, Malaysia, Korea, Japan and Thailand. As a result, it has become clear that the infection cycle of JE virus is associated with *Cx. tritaeniorhynchus*, and the amplifier domestic pigs. The fact that patients with JE are among the residents in Korea have been known for a long time, and a disease called "Summer encephalitis" or "Encephalitis lethargica" before 1935 is now accepted to be nothing but Japanese encephalitis.

In the survey of the vector mosquitoes in Korea with a consideration of their importance as JE vector, Shin *et al.*[13] found that *Cx. tritaeniorhynchus* was the most prevalent species, and next in order were *An. sinensis* and *Ae. vexans nipponii*, with *Cx. pipiens pallens* taking 4th place, and reported that the population

Table 11. Comparison of biological and life table characteristics of Kyungsan strain of *Culex tritaeniorhynchus* (1990)*

	Kyungsan, Korea	Nagasaki, Japan	Taipei, Taiwan
Generation in Laboratory	1	128	120
Siphonal index	8.41	7.40	7.61
Adult			
wing length (mm) Male	2.70	3.11	2.71
Female	3.28	3.46	2.92
Fecundity			
No. raft/cage	14.0	71.32	58.0
No. eggs/raft	188.9	172.8	142.8
Fertility			
No. larvae/raft	143.0	121.0	130.5
Hatching (%)	75.7	70.2	91.4
Life table statistics			
e_1 ♂	28.33	14.14	15.08
e_2 ♀	59.81	21.10	19.22
R_0	7.81	21.37	84.97
T_0	15.50	18.02	13.62
G	25.60	28.31	24.01

e_1 : Mean life expectancy at emergence in days; R_0 : Net reproductive rate: living females per female per generation ; G: Generation time in days; T_0 : Age of mean cohort reproduction; * Data reported by Baik and Joo [5].

density of *Cx. tritaeniorhynchus* by month were 0.1% in June, 8.2% in July, 55.2% in August, 36.6% in September and 0.02% in October. Similar results in vector mosquitoes have also been obtained by Self *et al.*[14], Pae *et al.*[15], Frommer *et al.*[16] and Lee *et al.*[17] in some U.S. army Compounds, and by Joo and Wada[4], Baik and Joo[5], Joo and Kang[12] in Kyongbuk Province, Korea. In the general patterns of seasonal prevalence, *Cx. pipiens pallens* was found to be active through out almost the entire season, showing irregular curves with

several peaks, while *Cx. tritaeniorhynchus* first appeared in mid-June and were trapped in large numbers from mid-August to early September, showing a sharply pointed one peak curve. As for *An. sinensis*, the trend of seasonal distribution was similar to that of *Cx. tritaeniorhynchus*, but the earliest dates of appearance and disappearance differed from those of *Cx. tritaeniorhynchus*.

Monthly fluctuation in number of *Cx. tritaeniorhynchus* and other species have earlier been reported however this is the first report on a complete year's collection

with one night per week schedules. It seems that the highest population of *Cx. tritaeniorhynchus* was clearly observed during the period from late July to mid-September, when the temperature was between 16.6 and 34.2° C and humidity 57-89%. The earliest dates of appearance and disappearance in the present survey was the period from early June to late June and the period from late September to early November. The main factors contributing to the population dynamics of *Cx. tritaeniorhynchus* each year seemed to be due to the changes in the rice culture system and/or rural environment such as water management of rice fields, high temperature and small precipitation, extensive uses of chemical insecticides in rice farming, livestock and natural enemies, and the reduction of rice fields by urbanization. Such consideration was also recognized by Mogi[7,18] Maeda *et al.* [6], Baik and Joo[5], and Joo and Kang[19].

The seasonal prevalence of *Cx. tritaeniorhynchus* has been shown usually in the number of *Cx. tritaeniorhynchus* collected by a light trap, but the numbers were found to daily fluctuate. Therefore, the total or average numbers seem to be unsuited for comparison of the abundance of *Cx. tritaeniorhynchus*. Consequently, MPI, being calculated from the totals of vector mosquito collected at stations in comparison with those in a standard year, was used for the comparison of the annual abundance of vector mosquitoes. The results shown in Table 4 and Table 5 indicate that a marked decrease in MPI was obtained in 1989, 1993 and 1997,

accompanied with a decrease in the number of mosquitoes. These data are highly valuable, however were not used for further analysis of the JE epidemic. However these data showed remarkable decrease of *Cx. tritaeniorhynchus* in 5-year intervals, and this decreasing trend in of mosquito number number is likely to be the case not only in Kyongbuk Province, but also all over Korea, It is highly probable that the decrease of *Cx. tritaeniorhynchus* is an important factor in the reduction of human JE cases in recent years. Although the main reasons for the decrease in population density of *Cx. tritaeniorhynchus* can not easily be explained, it has been suggested to be due to intermittent irrigation or early planting of rice plants, which made unsuitable conditions for breeding mosquito larvae, extensive used of chemical insecticides, and use of herbicides such as CNP (p-nitro-phenyl 2,4,6-trichlorophenyl) and NIP (nitrofen) for rice plant cultivations. Furthermore, such factors as the decrease in rice field areas due to urbanization, improved water management, aerial application of agricultural chemicals, and modernized livestock breeding, have contributed to the reduction of larval vector populations.

As for the characteristics in the chronological biting rhythm of the vector mosquito species, Joo and Wada[4] reported that the hourly distribution of the female *Cx. tritaeniorhynchus* observed by the human-baited collections usually had two peaks, one between 21:00 and 22:00, and another between 24:00-01:00

on August 3-4, 1984. In a mosquito survey by light trap and human-baited traps through the night at hourly intervals, Joo and Kang[19] reported that *An. sinensis* appeared to be active throughout the whole night, but was more active during darkness after sunset to mid-night, and the temperature at that time was between 26.9 and 30.1°C and the humidity 67-76%.

In the present study, the nocturnal periodical activity of the female *Cx. tritaeniorhynchus* was not always the same by collection method on the same night. Although the environmental factors such as temperature, light, humidity and wind-borne stimuli, are important in determining the attraction of mosquitoes, the present data can hardly be explained by the hourly changes of the factors because the meteorological conditions are considered nearly the same at least on the same night at the sites where the collection is made. Further works along this line are needed. Previous reports [5,13,19] together with the present observations clearly indicated that *Cx. tritaeniorhynchus* with nocturnal biting habits had two peaks in their activities, one soon after the sunset and another during a few hours' period from 2-3 o'clock, although the relative numbers varied by the collections and the highest peak appeared at different time to the night.

Cx. tritaeniorhynchus blood success observed in this study was sometimes a reflection of abundance of this species. Abundance of *Cx. tritaeniorhynchus*

during 1984, 1987, 1992, 1994, and 1998 was greater than other years observed, and the same relationship held true for the number of engorged female mosquitoes collected in these years. However, monthly engorgement success was not always linked to overall mosquito abundance. In 1992, *Cx. tritaeniorhynchus* was most abundant in August, but engorgement of female *Cx. tritaeniorhynchus* had the highest rate in September. The results of a monthly fluctuation of engorged number of *Cx. tritaeniorhynchus* during the period from 1984 to 1998 indicated that blood feeding behaviour was significantly associated with rainfall, which raises the relative humidity, and relative humidity strongly influences mosquito flight and subsequent host-seeking behaviour. Such relationship between adult abundance and rainfall has also been recognized by Day and Curtis [20] and Provost[21] for *Cx. nigripalpus*, by Olson *et al.*[22] for *Cx. tritaeniorhynchus* and *Cx. gelidus*, and by Russel[23] for *Cx. annulirostris*.

The immature stages of *Cx. tritaeniorhynchus* occur in a variety of habitats among which the most important is the rice paddies in Korea. The ecology of this species in various rural and suburban areas in Korea and other parts of the world have been studied by many investigators. Some of their results indicate that the seasonal fluctuation in the larval population densities in paddy water is markedly different from paddies to paddies in the same area and/or from year to year in the same paddies. In the present survey, the

total number of larval *Cx. tritaeniorhynchus* in rice paddies was the highest in mid-August, and its number progressively decreased in early September by insecticide sprays. After late September, densities markedly decreased, and larvae plus pupae of *Cx. tritaeniorhynchus* were rarely found until rice plants were harvested. The major factors to contribute to reproduction of *Cx. tritaeniorhynchus* were temperature, precipitation, water management of rice fields, availability of hosts, chemical insecticides and herbicides applied to rice fields, and natural enemies. Of those, the first two factors were responsible for the yearly change of population size, while the changes of mean population level was attributable to other four factors, which are controlled or influenced by human beings.

There is evidence that the widespread use of insecticides in agriculture has been responsible for the selection of resistant strain of vector mosquitoes in various rural and suburban areas in Korea and other parts of the world. Studies on insecticide resistance of vector mosquitoes in Korea have been carried out by many investigators. Some of their results indicates that the intensive use of insecticides and fungicides for the control of agricultural and/or medical pests, which transmits communicable diseases during the summer season, by government officials and by residents have greatly influenced the populations and resistance of the mosquitoes and arthropods of medical importance. In the present study, the larval *Cx. tritaeniorhynchus* showed

high resistance to diazinon, malathion, temephos and fenitrothion with LC_{50} values of 38.0 ppm, 18.6 ppm, 64.0 ppm and 10.8 ppm, respectively. Less resistance to fenthion was discovered in *Cx. tritaeniorhynchus* collected in Kyongsan county. Higher insecticide resistance to *Cx. tritaeniorhynchus* in this study suggests that it is probably due to different opportunity of contact to insecticides and fungicides.

Geographic variations among the life table characteristics for various species of mosquitoes have been studied by many investigators. As a result, a considerable amount of field and laboratory data can be found, related to the population dynamics, mating and biting rhythms, migratory habits of many species of mosquitoes. Reisen *et al.*[11] studied the geographic variation in the horizontal life table characteristics of 9 strains of *Cx. tritaeniorhynchus* from southern and far eastern Asia. Also, Aslam *et al.*[24] reported the influence of physiological age on the biting rhythm of *Cx. tritaeniorhynchus*.

In this study, the life expectancy at emergence of *Cx. tritaeniorhynchus* males was generally longer than Nagasaki and Taiwan strains of *Cx. tritaeniorhynchus* reported by Reisen *et al.*[11], however similar to that of *Cx. pipiens quinquefasciatus* reported by Walter and Hacker[25] and of *Ae. aegypti* reported by Crovello and Hacker[26]. Finally, *Cx. tritaeniorhynchus* female life expectancy was in general similar to that of *Cx. pipiens quinquefasciatus* reported by Walter and Hacker[25] and Gomez *et al.*[27], and of *Ae. aegypti* reported by Crovello and Hacker[26].

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