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Study on the cytoplasmic incompatibility in some natural population of *Culex pipiens* complex in China and Japan*

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Abstract — The cytoplasmic incompatibility in the field population of *Culex pipiens* complex from China and Japan was studied. The results of the crossing experiments between 11 different strains suggest that some Japanese strains might be possible to be used for control Chinese strains by using male sterile technique, and the heavy infection of wolbachiae in mosquitoes may have some relations with the cytoplasmic incompatibility in the field population of *Cx. pipiens* complex.

Keywords: cytoplasmic incompatibility; natural population; *Culex pipiens*.

1 Introduction

In the transmission of *Wuherelia bancrofti, Culex pipiens* complex is known as the most common and important vector throughout China and Japan (Manson, 1978; Sasa, 1976; Zhong, 1981). Two subspecies of the *Cx. pipiens* complex are considered to be occurring in China: *Cx. p. quinquefasciatus,* a tropical subspecies in southern China: *Cx. p. pallens,* a subspecies in the Temparate Zone. In Japan, in addition to these 2 subspecies, *Cx. p. molestus,* an autogenous race is also occurring.

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In relation to the biological control, cytoplasmic incompatibility of *Cx. pipiens* complex has been studied by several workers, and the results showed that the crosses between members of this mosquito group from different geographical origin may be compatible, partially compatible, or in compatible in one or both directions (Laven, 1951; Barr, 1966; Sasa, 1966; Subbarao, 1977).

Laven (Laven, 1957; 1967) showed that the factors causing incompatibility were maternally transmitted through the cytoplasm. Although the Expert Committee of the World Health Organization (WHO, 1964) has suggested that cytoplasmic incompatibility could be used to produce sterile males for eradication of *Cx. pipiens* by the male sterile technique (Knipling, 1955), there has been no encouraging results in the field (Krishnamurthy, 1962; Barr, 1970; Subbarao, 1974). There is evidence that one of the cytoplasmic factors responsible for incompatibility is a rickettsia-like microorganism, *Wolbachia pipientis*, in the cytoplasm of *Cx. pipiens* complex (Yen, 1973; 1974; Fine, 1977; Suenaga, 1982). Recently, incompatibility in crosses of sympatric strains of *Cx. pipiens* complex and the collection of incompatible egg rafts in the field have been reported from Los Angeles (Barr, 1980) and Nagasaki (Suenaga, 1982). The present field survey and crossing experiments were made in Shanghai from August, 1981 through November, 1982, to make clear the crossing types of natural populations of *Cx. pipiens* complex in China. A part of the results of the field survey in Shanghai has been reported recently (Suenaga, 1981). The crossing experiments between some Chinese strains and Japanese strains were also made in Shanghai and Nagasaki.

This is a report of appearance of unhatched egg rafts of *Cx. pipiens pallens*, though they contained some developed eggs, from the same breeding place in the field in China, and of the results of crossing experiments with some strains from China and Japan.

2 Materials and methods

2.1 Collection of egg rafts from field

An earthen jar of about 10 litres in size and containing wheat bran solution was set up as an ovi-trap for house mosquitoes in a corner of a park in Shanghai from August, 1981 through November, 1982. All egg rafts of *Cx. pipiens* complex which were deposited on the water surface in the jar were picked up every morning and incubated for 2 or 3 days in a polyethylene cup of 90 ml separately in an insectarium at about 27°C for observations of the egg viability. Some of the larvae rafts hatched were reared for several generations separately in the insectarium for crossing experiments. If an egg raft produced autogenous females, it was regarded as the egg raft of *Cx. pipiens molestus*.

2.2 Methods of laboratory breeding

Mosquito larvae hatched from each egg raft, were removed respectively to a polyethylene pan of 300 × 210 × 48mm³ in size and containing water with an aeration and a piece of laboratory mouse food was supplied as the nutrients for larval growth. Each pan was covered with vinyl chloride plate to prevent the colony from contamination by other mosquito colonies. The pupae from each colony were picked up every day with a pipette, transferred to the cup of 90 ml as same as mentioned above and then placed in a mosquito cage separately. The mosquito cages for keeping adults are made of semitransparent synthetic fiber and wire frame of 200 × 200 × 300 mm³ in size, with an sleeve on one side. A cube sugar as the nutrients and a cup of water for adults were placed separately in each cage. The same cages were also used for crossing experiments. The egg rafts of an autogenous colony were recovered after the females were fed on laboratory mice, each of them was fixed on its back on a piece of wooden plate (150 × 80 × 20 mm³ in size) and removed the abdominal hair by using scissors, while those of an autogenous colony have been able to collect through a number of generations without giving blood meals.

2.3 Methods of crossing

Crossing experiments were made with following 215 strains or colonies from China and Japan (Table 1).

Table 1 Resources of the samples of strains or colonies

Source of sample	No.
Zb (Zhabei, Shanghai, China)	18
Cs (Chuansha, Shanghai, China)	1
Fx (Fuxing Park, Shanghai, China)	185
Ja (Jinan, Shanghai, China)	3
Fz (Fuzhou, Fujian, China)	1
Hx (Hexian, Guangxi, China)	1
Gz (Guangzhou, Guangdong, China)	1
Fs (Fushan, Guangdong, China)	1
A (Nagasaki, Japan)	1
B (Nagasaki, Japan)	1
C (Nagasaki, Japan)	1
D (Naha, Okinawa, Japan)	1
Total	215

In a crossing experiment between two mosquito strains, pupae of each strain were screened through 20 mesh wire screen and separate by sex under a stereomicroscope by using a soft fine brush in a petri dish, and 60 males of one strain were put in a

90 ml polyethylene cup containing water and were removed into an adult cage together with 50 females of the other strain, while 50 females of the former strain were put into another cage together with 60 males of the latter. The inbreeding cages containing 60 males and 50 females were also prepared respectively as the control of the crossing experiments.

2.4 *Wolbachia* examination

For *Wolbachia* examination, the procedure presented by Wright and Wang (Wright, 1980) is follows:

Dissect out the ovaries in Bailar's saline (Breland, 1961), transfer with a fine needle to a clean microscope slide, smear, and dry.

Fix in absolute methanol for at least 10 minutes. Slides may be left in the methanol overnight if desired.

Without rinsing, stain the smear in a freshly mixed 1:50 dilution of Giemsa in phosphate buffer (pH 7.2 ~ 7.4) for 2 h.

Rinse in tap water or distilled water and gently blot or air-dry.

Examine by oil immersion optics with a blue filter.

Deciding whether an ovary smear was positive for wolbachiae was facilitated by using a known positive slide as a control. This was necessary because other microscopic structures may be mistaken for wolbachiae.

3 Results

3.1 Number and hatchability of egg rafts collected in the field

The number and hatchability of egg rafts of *Cx. p. pallens*, which were deposited on the water surface in an earthen jar outdoor at Fuxing Park in Shanghai, are shown in Table 2. Although 2012 or 98.4% out of 2044 egg rafts of *Cx. pipiens* complex had hatched well, 32 egg rafts or 1.6% produced no larvae or fe larvae. The maximum number of egg rafts was observed in August, and the minimum one was in November in 1981 and 1982. From December, 1981 through April, 1982, no egg rafts were collected. The first egg raft in 1982 was collected on May 10. No egg rafts of an autogenous subspecies, *Cx.p. molestus* were collected through the survey period.

The state of egg rafts that hatched normally is shown in Table 3.

The hatchability of 256293 eggs from 2012 egg rafts was 97.1%, and the mean size, or number of eggs, of the egg rafts were 127.4.

The details of the state of egg rafts that produced no larvae of few larvae are shown in Table 4. In the case of egg raft No. 354, for example, the egg raft was collected on August 31, 1981, and its size was 225. Among them 201 eggs unhatched, though they developed, and 24 eggs undeveloped.

Table 2 Number and hatchability of egg rafts of *Culex pipiens* pallens which were deposited on the water surface in an earthen jar outdoors at Fuxing Park in Shanghai from August 1981 through November, 1982

Year	Month	No. of egg rafts			Percentage of egg rafts, %	
		Collected	Hatched	Unhatched*	Hatched	Unhatched
1981	Aug.	386	382	4	98.96	1.04
	Sep.	268	263	5	98.13	1.87
	Oct.	31	30	1	96.77	3.23
	Nov.	5	5	0	100.00	–
	Dec.	No egg rafts were collected				
1982	Jan.					
	Feb.					
	Mar.					
	Apr.					
	May	34	34	0	100.00	–
	June	225	221	4	98.22	1.78
	July	311	308	3	99.04	0.96
	Aug.	341	333	8	97.65	2.35
	Sep.	248	244	4	98.39	1.61
	Oct.	162	160	2	98.77	1.23
	Nov.	33	32	1	96.97	3.03
Total		2044	2012	32	98.43	1.57

* Some of them produced a few larvae, and the details are shown in Table 4

Table 3 The state of egg rafts that hatched normally after 3 days of incubation at 27°C from August 1981 through November, 1982

Year	Month	a No. of egg rafts	No. of eggs Hatched	Developed	Undeveloped	Total	Eggs h tched, %	Mean size of egg raft
	Aug.	382	44 188	415	1 201	45 804	96.5	199.9
	Sep.	263	33 673	346	904	34 923	96.4	132.8
1981	Oct.	30	4 925	92	85	5 102	96.5	170.1
	Nov.	5	1 177	3	13	1 193	98.7	238.6
	Dec.	No egg rafts were collected						
	Jan.							
	Feb.							
	Mar.							
	Apr.							
	May	34	5 479	97	310	5 886	93.1	173.1
1982	June	221	32 016	348	311	32 675	98.0	147.9
	July	308	42 922	334	635	43 891	97.8	142.5
	Aug	333	40 608	541	769	41 918	96.9	125.9
	Sep.	244	23 617	147	514	24 278	97.3	99.5
	Oct.	160	16 472	108	202	16 782	98.2	104.9
	Nov.	32	3 720	94	27	3 841	96.8	120.0
	Total	2012	248 797	2 525	4 971	256 293	97.1	127.4

Table 4 The state of egg rafts that produced no larvae or few larvae after 3 days of incubation at 27°C from August 1981 through November, 1982

Egg raft No.	Date of collection	Size of egg raft	No. of eggs Hatchad	Developed	Undeveloped	Percentage of eggs, % Hatched
51	Aug. 16, 1981	133	0	0	133	0.0
100	Aug. 21, 1981	90	0	0	90	0.0
335	Aug. 31, 1981	189	0	0	189	0.0
354	Aug. 31, 1981	225	0	201	24	0.0
448	Sep. 3, 1981	129	0	38	91	0.0
453	Sep. 3, 1981	82	8	71	3	9.8
454	Sep. 3, 1981	53	3	50	0	5.7
510	Sep. 7, 1981	48	2	44	2	4.2
514	Sep. 7, 1981	133	0	0	133	0.0
698	Oct. 30, 1981	45	0	0	45	0.0
67	June 6, 1982	282	8	0	274	2.8
196	June 29, 1982	140	0	5	135	0.0
223	June 30, 1982	173	3	92	78	1.7
229	June 30, 1982	115	1	0	114	0.9
258	July 1, 1982	130	0	120	10	0.0
365	July 6, 1982	94	2	0	92	2.1
438	July 8, 1982	145	0	15	130	0.0
587	Aug. 2, 1982	136	0	57	79	0.0
589	Aug. 2, 1982	127	0	121	6	0.0
603	Aug. 3, 1982	90	0	83	7	0.0
618	Aug. 4, 1982	111	0	102	9	0.0
659	Aug. 7, 1982	128	0	113	15	0.0
709	Aug. 17, 1982	216	0	129	87	0.0
755	Aug. 20, 1982	90	0	81	9	0.0
880	Aug. 30, 1982	73	0	53	20	0.0
917	Sep. 1, 1982	116	0	96	20	0.0
928	Sep. 1, 1982	149	2	125	22	1.3
951	Sep. 2, 1982	82	0	34	48	0.0
1 117	Sep. 25, 1982	160	0	0	160	0.0
1 156	Oct. 5, 1982	90	0	2	88	0.0
1 209	Oct. 9, 1982	96	0	86	10	0.0
1 306	Nov. 6, 1982	120	0	0	120	0.0

With regard to 32 egg rafts tabulated, as a whole, in seven cases (Nos. 51, 100, 335, 514, 698, 1117 and 1306) all eggs did not develop, in eight cases (Nos. 453, 454, 510, 67, 223, 229, 365 and 928) the eggs partly hatched (0.9% − 9.8%), but most of the eggs unhatched, and in 17 cases (Nos. 354, 448, 196, 258, 438, 587, 589, 603, 618, 659, 709, 755, 880, 917, 951, 1156 and 1209) the eggs partly developed, but all of them did not hatch.

3.2 Crossing experiments

The results of the crossing experiments between 207 Shanghai strains (Zb 18, Cs 1, Fx 185 and Ja 3) showed that these strains are only one crossing type except one, Fx No. 526. This strain started originally from one egg raft, which was collected by the ovi-trap at Fuxing Park on July 27, 1982. The males of this strain was incompatible with females of other Fx strains, though the crossing type of females of this strain is unknown, because no egg rafts were collected after the crossing. The strain has regrettably become extinct before its crossing type is confirmed at the following generations.

The hatchabilities of egg rafts obtained from crossing experiments between 11 different strains of *Cx. pipiens* complex from China and Japan showing in Fig. 1 are seen in Table 5, and the crossing relationships of them are shown in Table 6. As shown in these tables, the results of the crossing experiments are as follwos: All Chinese strains examined were compatible each other except the females of a Fuzhou strain were incompatible with the males of Zhabei and Hexian strains; a Nagasaki strain (A) of *Cx. pipiens molestus* and an Okinawa strain (D) of Cx. quinouefasciatus

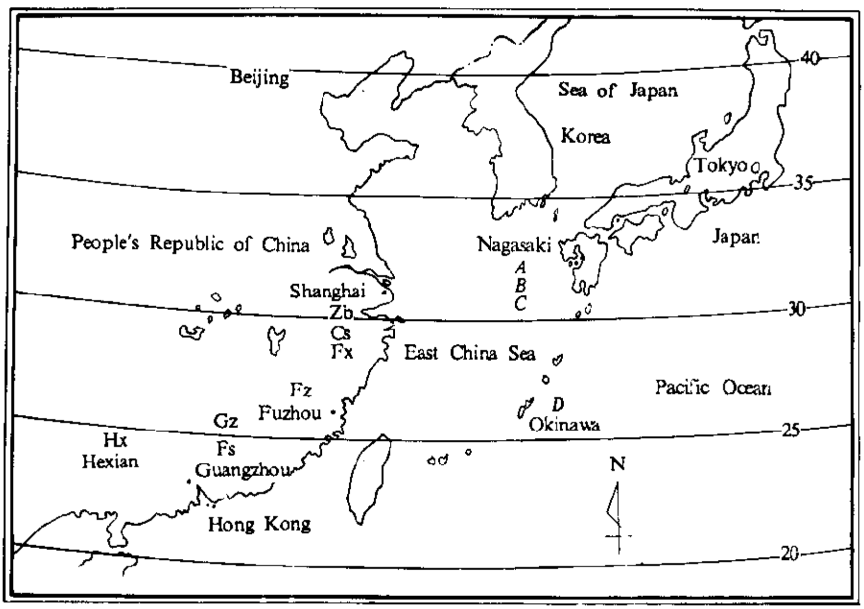

Fig. 1 A map of China and Japan showing the origins of *Culex pipiens* complex strains which were used for crossing experiments in 1981 and 1982

Table 5 Hatching rate of egg rafts obtained from crossing experiments between different strains of *Culex pipiens* complex from China and Japan

Female	Male										
	Zb	Cs	Fx	Fz	Hx	Gz	Fs	A	B	C	D
Zb (*Cx. p. pallens)*	96.2	85.7	97.2	94.3	80.5	82.2	91.1	0.0	96.7	94.1	81.5
Cx (*Cx. p. pallens)*	74.1	93.4	91.2	86.9	65.4	82.2	74.5	0.0	98.9	0.0	12.7
Fx (*Cx. p. pallens)*	88.3	92.5	95.3	98.6	95.1	95.7	95.9	0.2	79.8	0.0	0.2
Fz (*Cx. p. quinquefasciatus)*	0.0	98.2	84.4	94.4	0.0	97.7	98.4	0.0	75.2	0.0	0.0
Hx (*x. p. quinquefasciatus)*	54.1	57.6	86.5	98.5	85.8	81.9	93.4	0.0	69.0	0.3	0.0
Gz (*Cx. p. quinquefasciatus)*	70.2	73.6	98.6	92.1	89.6	83.8	96.3	0.0	99.6	0.2	0.0
Fs (*Cx. p. quinquefasciatus)*	87.7	80.9	97.4	96.6	84.1	94.9	97.9	0.0	65.0	0.0	0.0
A (*Cx. p. molestus)*	0.0	88.1	59.0	0.0	0.0	0.0	0.0	88.0	0.2	76.3	63.6
B (*Cx. p. pallens)*	88.6	74.7	86.6	98.3	94.2	96.0	94.4	0.0	98.4	0.1	23.5
C (*Cx. p. pallens)*	95.8	97.6	91.3	88.3	0.0	69.7	94.8	0.0	82.0	96.3	23.4
D (*Cx. p. quinquefasciatus)*	0.0	87.2	0.0	0.0	0.0	0.4	0.0	6.0	91.9	96.0	88.0

Notes: The abbreviations are the same as those in Table 1.

were incompatible with Fuzhou (Fz), Hexian (Hx), Guangzhou (Gz) and Fushan (Fs) strains of *Cx. P. quinquefasciatus;* a Nagasaki strain (B) of *Cx. p. pallens* was compatible with all of 7 Chinese strains in both directions; the females of the other Nagasaki strain (C) of *Cx. p. pallens* were compatible with the males of 6 Chinese strains except Hexian (Hx), but the males of the same strain were incompatible with 6 Chinese strains except Zhabei (Zb).

Table 6 Crossing relationships between 11 strains of *Culex pipiens* complex from China and Japan

Female	Male										
	Zb	Cs	Fx	Fz	Hx	Gz	Fs	A	B	C	D
China											
Zb (*Cx. p. pallens)*	C	C	C	C	C	C	C	I	C	C	C
Cx (*Cx. p. pallens)*	C	C	C	C	C	C	C	I	C	I	P
Fx (*Cx. p. pallens)*	C	C	C	C	C	C	C	I	C	I	I
Fz (*Cx. p. quinquefasciatus)*	I	C	C	C	I	C	C	I	C	I	I
Hx (*Cx. p. quinquefasciatus)*	C	C	C	C	C	C	C	I	C	I	I
Gz (*Cx. p. quinquefasciatus)*	C	C	C	C	C	C	C	I	C	I	I
Fs (*Cx. p. quinquefasciatus)*	C	C	C	C	C	C	C	I	C	I	I
Japan											
A (*Cx. p. molestus)*	I	C	C	I	I	I	I	C	I	C	C
B (*Cx. p. pallens)*	C	C	C	C	C	C	C	I	C	I	P
C (*Cx. p. pallens)*	C	C	C	C	C	C	C	I	C	C	P
D (*Cx. p. quinquefasciatus)*	I	C	I	I	I	I	I	P	C	C	C

C: compatibility; I: incompatibility; P: Partial compatibility

3.3 *Wolbachia* infection

All of 15 Chinese strains examined and used from crossing experiments have been heavily infected with *Wolbachia pipientis* respectively.

4 Discussion

The purpose of the present investigation was to know the crossing types of natural populations of *Cx.pipiens* complex in China, and was to know the crossing relationships between some strains of *Cx.pipiens* complex in China and Japan. The appearance of unhatched egg rafts, though they contained some developed eggs, in the field suggests that the egg rafts were laid by females which mated with incompatible males in the field. This phenomenon may prove the presence of two or more crossing types of this subspecies in area.

Mixed populations of different crossing types and incompatible egg rafts of *Cx. pipiens* complex have been found in Los Angeles (Barr, 1980) and Nagasaki (Suenaga, 1982). Therefore this is the third report of cytoplasmic incompatibility in natural population of this mosquito group.

Although no egg rafts of *Cx. p.* molestus were found in Shanghai through the survey period, and no body recorded the presence of this autogenous subspecies in China, it may be found somewhere in this country near future, because this subspecies had been found all over Japan except Amami Oshima and Okinawa areas or southern islands of Japan.

The results of the crossing experiments between 11 Chinese and Japanese strains (Table 6) suggest that some Japanese strains (A,C and D) might be used for control Chinese strains of *Cx.pipiens* complex by using male sterile technique. Although the incompatibility seems to be a useful way of producing sterile males of *Cx pipiens* complex, there have been no encouraging results in the field with mosquitoes (Morlan, 1962; Weidhaas, 1962; Krishnamurthy, 1962). Knipling(Knipling, 1955) emphasized that the sterile male technique is best suited for controlling the pests that occur in small populations and the pests of which the males are highly mobile. Barr(Barr, 1966) also pointed out that the possibility of using this technique focuses attention on gaps in our knowledge of mosquitoes, especially their mating behavior. We need much more knowledge about these points to know whether the sterile male technique is useful or not for the control of *Cx. pipiens* complex in the field.

The heavy infections of wolbsachiae in mosquitoes may have some relations with the cytoplasmic incompatibility in the field population of *Cx,pipiens* complex.

References

Barr AR. Proc Calif Mosq Control Assoc, 1966; 33:32

Barr AR. Proc Calif Mosq Control Assoc, 1969; 37:19

Barr AR. Nature, 1980; 283(5742):71

Breland OP. Ann Entomol Soc Amer, 1961; 54:360

Fine PEM, Ellis DS, Curtis CF. Trand Roy Soc Trop Med Hyg, 1977; 71 (5):382

Knipling EF. J Econ Ent, 1955; 48(4):459

Krishnamurthy BS, Ray SN, Joshi GC. Ind J Malariol, 1962; 16:365

Laven H. Evolution, 1951; 5:370

Laven H. Z Indukt Abstamm-u Vererbungslehre, 1957; 88:478

Laven H. Genetics of insect vectors of disease (Ed by Wight, Pal), 1967:251

Manson, P. Chinese Customs Med Rep, 1978; 3(14):1

Morlan HB, McCray EMJr, Kilpatrick JW. Mosq News, 1962; 22:295

Sasa M, Shirosaka A, Kurihara T. Japan J Exp Med, 1966; 36(2):187

Sasa M. Human Filariasis: A global survey of epidemiology and control. Tokyo: University of Tokyo Press, 1976:443

Subbarao SK, Curtis CF, Singh KRP, Krishnamurthy BS. J Com Dis, 1974; 6(2):80

Subbarao SK, Krishnamurthy BS, Curtis CF, Singh KRP, Adak T, Chandrahas RK, Indian Med Res, 1977; 65 Suppl):21

Suenaga O. Trop Med, 1982; 24(2):79

Osamu Suenaga, Liu Weide, Miao Jianwu, Xu Wei. Contributions Shanghai Inst Entomol, 1983; 3:29

Weidhaas DE, Schmidt CH, Seabrook EE. Mosq News, 1962; 22:383

World Health Organization. Genetics of vectors and insecticide resistance, WHO Tech Rpt Ser, 1964; 268:40

Wright JD, Wang B. J Invertebr Pathol, 1980; 35:200

Yen JH, Barr AR. J Inverteb Pathol, 1973; 22(2):242

Yen JH, Barr AR. In: The use of genetics in insect control (Ed. by R Pal, MJ Whitten). Chap 6, North Holland Elsevier, 1974:97

Zhoung H, He L, Cao W. Chinese Med J, 1981; 94(9):567

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