Treatment of *Wolbachia pipientis* Infection with Tetracycline Hydrochloride and the Change of Cytoplasmic Incompatibility in a Nagasaki Strain of *Culex pipiens molestus*

Osamu SUENAGA

Reference Center, Institute of Tropical Medicine, Nagasaki University, 1−12−4 Sakamoto, Nagasaki 852, Japan

Abstract: A wild type strain of *Culex pipiens molestus*, which was originated from one egg raft collected by an ovi-trap in Nagasaki, Japan in 1980 and maintained at an insectarium of 25℃ and 70% RH, was cured by exposing the first instar larvae to 2.5-5%, 5%, 10%, and 20% water solutions of tetracycline hydrochloride for 20-48 hours befor food was added. Only one out of 12 lines of the strain has been cured of the *Wolbachia* infection when it was treated by 5% solution for 24 hours. Although treated and aposymbiotic (or *Wolbachia* free) females produced viable progeny when they mated with aposymbiotic males, no progeny was produced by the females when they were backcrossed with the original males with symbiotes. The newly established aposymbiotic strain has been maintained for over 11 generations.

Key words: *Wolbachia pipientis*, cytoplasmic incompatibility, *Culex pipiens molestus*, tetracycline hydrochloride.

INTRODUCTION

In relation to the biological control of *Culex pipiens* complex, cytoplasmic incompatibility has been studied by several workers and the results showed that the crosses between members of the complex from different geographical origins may be compatible, partially compatible, or incompatible in one or both directions (Laven, 1951; Barr, 1966; Sasa *et al.*, *1966; Subbarao et al.*, 1977; Suenaga, 1982b). Laven (1957, 1967) showed that the factors causing incompatibility were maternally transmitted through the cytoplasm. Although the Expert Committee of World Health Organization (WHO, 1964) has suggested that cytoplasmic incompatibility could be used to produce steril males for eradication of *Cx. pipiens* by the steril male technique (Knipling, 1955), there has been no encouraging results in the field ((Knipling, 1955), there has been no encouraging results in the field) (Krishnamurthy *et al.*, 1962; Barr, 1970; Subbarao *et al.*, 1974). Yen and Barr (1971) proposed a new theory that one of the cytoplasmic factors responsible for incompatibility might be the presence of a

Received for publication, October 4, 1993.
Contribution No.2874 from the Institute of Tropical Medicine, Nagasaki University.

rickettsia-like microorganism, *Wolbachia pipientis*, in the reproductive organs of the mosquitoes, and this hypothesis was confirmed by Yen and Barr (1973, 1974), Fine *et al.* (1977), and Suenaga (1982a, 1992). This paper presents results of curing *Wolbachia* infection in a Nagasaki strain of *Cx. pipiens molestus* by using tetracycline hydrochloride {Achromycin ® V capsules 50 mg, Lederle (Japan), LTD.}

Materials and Methods

A wild type strain of *Cx. pipiens molestus* which originated from one egg raft collected by an ovi-trap in Nagasaki city, Japan in 1980 and maintained at an insectarium of 25℃ and 70% RH was used for the experiments.

The first instar mosquito larvae which hatched within 12 hours at 25℃ were treated by 20%, 10% and 5% tetracycline hydrochloride (TC) solutions, and also treated by 2.5% and then 5% solutions for 20-48 hours at 25℃ or 37℃ room temperature, respectively, as shown in Table 1. These 12 lines (including some duplicate) of experiments were designed based on the previous reports (Yen and Barr, 1973, 1974; Fine *et al.*, 1977; Suenaga, 1982a, 1992). After the treatment, the surviving larvae were put into a polyethylene pan containing tap water and a piece of mouse-feed pellet as food, and aeration was started. Food was suitably added 3 or 4 days later. The pan was covered with plastic plate till the larvae became pupae. All pupae were transferred into a 90 ml plastic cup containing tap water and put in a rearing cage of 20×20×30cm, with a cube sugar on a small plastic plate as adult food. Some of the newly emerged female mosquitoes were examined for wolbachiae, and others were allowed to lay eggs without feeding blood during about one week after their emergence.

All egg rafts obtained from each line were confirmed whether they hatched or not. The hatched larvae were reared by the usual procedure to get the next generation.

Crossing experiments were made at the 3rd and 11th generations after TC treatment. Two colonies, TC treated and original, of the strain were reared at the same time separately from early stage larvae.

Pupae of each colony were separated to males and females individually under a stereomicroscope, and 50 females and 50 males of the 2 colonies were crossed and backcrossed each other in a rearing cage. Some females of each colony were examined for wolbachiae in their ovaries by using Giemsa staining technique (Wright and Wang, 1980). The females crossed or backcrossed laid egg rafts on the surface of tap water in a plastic cup in the cage after few days of crossing, and all egg rafts were examined for developing or hatching conditions after 3 days of oviposition under the stereomicroscope.

Results and Discussion

Effects of TC solution on the mosquitoes and wolbachiae are shown in Table 1. Among 12 TC treatment experiments, in only one case (No. 12) when the mosquito larvae were treated by 5% TC solution for 24 hours at 25℃, their survival rate to the pupal stage was 44.0%, and no wolbachiae were found in the mosquitoes of this colony. However, in other 11 cases of colonies, experiments have failed to get progeny, because in one case (No. 6), all treated larvae died; in 2 cases (Nos. 1 and 2), though most of larvae developed and emerged, the female adults laid no eggs; in 3 cases (Nos. 4, 7 and 8) though some larvae emerged and females laid egg rafts, all eggs did not hatch; and in 5 cases (Nos. 3, 5, 9, 10 and 11), though some larvae matured and females laid egg rafts, it is difficult to get the next generation because of reduced hatch rate.

Table 1. Effect of tetracycline hydrochloride solution on a Nagasaki strain of *Culex pipiens molestus*

No.	Strain	Concentration of T.C.(%)	Hours for treatment	Room temp.(℃)	No. of larvae		Survival rate(%)	Effect on mosquitoes
					treated	survived		
1	1m F61	20.0	24	25	1,000	870	87.0	No egg rafts
2	1m F62	10.0	24	25	1,000	750	75.0	No egg rafts
3	1m F72	10.0	20	37	300	209	69.7	Reduced hatch
4	1m F75	10.0	24	37	1,000	17	1.7	Unhatch
5	1m F75	10.0	24	37	1,000	650	65.0	Reduced hatch
6	1m F60	2.5 / 5.0	18 / 20	25	1,000	0	0.0	All larvae died
7	1m F64	5.0	20	25	1,000	196	19.6	Unhatch
8	1m F64	5.0	24	25	1,000	555	55.5	Unhatch
9	1m F72	5.0	20	37	2,000	37	1.9	Reduced hatch
10	1m F72	5.0	20	37	500	139	27.8	Reduced hatch
11	1m F76	5.0	48	25	3,000	520	17.3	Reduced hatch
12	1m F76	5.0	24	25	2,000	880	44.0	Hatch*

* *Wolbachia* eliminated

Table 2. Results of crossing experiments in the 3rd generation of a Nagasaki strain of *Culex pipiens molestus* after tetracycline hydrochloride treatment

Cross (F×M)	No. of egg rafts examined	No. of eggs examined	% of eggs		
			hatched	developed	undeveloped
1m F78×1m F78	10	456	96.2	0.9	2.9
1m F78×1map F3	8	381	90.8	1.3	7.9
1map F3×1m F78*	10	447	0.4	31.6	68.0
1map F3×1map F3	7	333	87.2	4.2	8.6

ap: Aposymbiotic strain * Incompatible cross

The results of crossing experiments between the 3rd generation of aposymbiotic or wolbachiae free strain (1map F3) and 78th generation of original strain (1m F78) are shown in Table 2. Although the aposymbiotic females produced viable progeny when mated with treated males, the females produced no progeny when backcrossed with the males of original strain with symbiotes. In contrast to aposymbiotic females, the females of original strain with wolbachiae produced viable progeny when crossed with both of the males of the same strain and the aposymbiotic strain. Though only 2 eggs (0.4%) hatched, it is considered to parthenogenesis.

Table 3 shows the results of crossing experiments between the 11th generation of aposymbiotic strain (1map F11) and the 88th generation of original strain (1m F88). It is clear from this table that among 4 crossing combinations, though each strain was compatible itself and also the females of original strain were compatible with the aposimbiotic males, only aposymbiotic females produced no progeny when they backcrossed with the males of original strain, and the results were almost the same as those of the former experiments with 1map F3.

The results of these two experiments indicate that the crossing type of the original strain with wolbachiae have changed after cure of the microorganism infection and TC treatment rendered them apposymbiotic. All these results are fully consistent with those obtained by Yen and Barr (1973), Fine *et al.* (1977), and Suenaga (1982a).

However, the elimination of wolbachiae in *Cx. pipiens* complex by using TC is very difficult sometimes. For example, in the case No. 8 of the present experiment, though the experimental condition is the same to No. 12, I have failed to get the progeny because the all egg rafts unhatched, and in the other case of *Cx. pipiens quinquefasciatus* from Thailand, successful concentration of TC for *Wolbachia* elimination was 10% (Suenaga, 1992). To produce steril males for eradication of *Cx. pipiens* complex, the experiments should be extent to the other members of this mosquito group.

Table 3. Results of crossing experiments in the 11th generation of a Nagasaki strain of *Culex pipiens molestus* after tetracycline hydrochloride treatment

Cross (F×M)	No. of egg rafts examined	No. of eggs examined	% of eggs		
			hatched	developed	undeveloped
1m F88×1m F88	10	766	87.9	3.6	8.5
1m F88×1map F11	10	772	89.9	4.4	5.7
1map F11×1m F88*	10	760	0.0	0.1	99.9
1map F11×1map F11	10	747	93.0	1.9	5.1

ap: Aposymbiotic strain * Incompatible cross

ACKNOWLEDGEMENTS

I am grateful to Dr. Yoshito Wada, Professor of the Department of Medical Entomology, Institute of Tropical Medicine, Nagasaki University, for his kindness in reading the original manuscript. Tetracycline hidrochroride (Achromycine®) used for the present experiments was supplied from the Nagasaki Branch of Lederle (Japan), LTD.

REFERENCES

1) Barr, A.R. (1966): Cytoplasmic incompatibility as a means of eradication of *Culex pipiens* L. Proc. Calif. Mosq. Control Assoc., 33, 32−35.

2) Barr, A.R. (1970): Partial compatibility and its effect on eradication by the incompatible male method. Proc. Calif. Mosq. Control Assoc., 1969, 37, 19−24.

3) Fine, P.E.M., Ellis, D.S. & Curtis, C.F. (1977): Further studies of *Wolbachia* and cytoplasmic incompatibility in *Culex pipiens*. Transact. Roy. Soc. Trop. Med. Hyg., 71(5), 382.

4) Knipling, E.F. (1955): Possibilities of insect control of eradication through the use of sexually steril males. J. Econ. Ent., 48(4), 459−462.

5) Krishnamurthy, B.S., Ray, S.N. & Joshi, G.C. (1962): A note on preliminary field studies of the use of irradiated males for reduction of *C. fatigans* Weid. populations. Ind. J. Malariol., 16, 365−373.

6) Laven, H. (1951): Crossing experiments with *Culex* strain. Evolution, 5, 370−375.

7) Laven, H (1957): Vererbung durch Kerngene und das Problem der ausserkaryotischen Vererbung bei *Culex pipiens* Ⅱ. Ausserkaryotischen Vererbung. Z. indukt. Abstamm. -u. Vererbungslehre, 88, 478−516.

8) Laven, H. (1967): Speciation and evolution in *Culex pipiens*. pp. 251−275. *In* J. W. Wright & R. Pal(ed.). Genetics of Insect Vectors of Disease. Elsevier Publ. Comp., Amsterdam.

9) Sasa, M., Shirasaka, A. & Kurihara, T. (1966): Crossing experiments between *fatigans, pallens* and *molestus* colonies of the mosquito *Culex pipiens* s.l. from Japan and Southern Asia, with special reference to hatchability of hybrid eggs. Japan. J. Exp. Med., 36(2), 187−210.

10) Subbarao, S.K., Curits, C.F., Singh, K.R.P. & Krishnamurthy, B.S. (1974): Variation in cytoplasmic crossing type in population of *Culex pipiens fatigans* Wied. from the Delhi area. J. Commun. Dis., 6(2), 80−82.

11) Subbarao, S.K., Krishnamurthy, B.S., Curtis, C.F., Singh, K.R.P., Adak, T. & Chandrahas, R.K. (1977): Further studies on variation of cytoplasmic incompatibility in the *Culex pipiens* complex. Indian Med. Res., 65 (Suppl.), 21−33.

12) Suenaga, O. (1982a): Treatment of *Wolbachia pipientis* in a strain of *Culex pipiens* complex. Trop. Med., 24(1), 9−15.

13) Suenaga, O. (1982b): Cytoplasmic incompatibility in a natural population of *Culex pipiens* complex in Nagasaki, Japan. Trop. Med., 24(2), 79−85.

14) Suenaga, O. (1992): Treatment of *Wolbachia pipientis* infection with Tetracycline Hydrochloride and the change of cytoplasmic incompatibility in a strain of *Culex pipiens quinquefasciatus* from Bangkok, Thailand. Trop. Med., 34(1), 15−19.

15) WHO (1964): Genetics of vectors and insecticide resistance. Report of a WHO Scientific Group. WHO Tech. Rpt. Ser. No. 268, 40 pp.

16) Wright, J.D. & Wang, B.-T. (1980): Observation on wolbachiae in mosquitoes. J. Invertebr. Pathol., 35, 200−208.

17) Yen, J.H. & Barr,, A.R. (1971): New hypothesis of the cause of cytoplasmic incompatibility in *Culex pipiens* L. Nature, 232(5313), 657−658.

18) Yen, J.H. & Barr, A.R. (1973): The etiological agent of cytoplasmic incompatibility in *Culex pipiens*. J. Invertebr. Pathol., 22, 242−250.

19) Yen, J.H. & Barr, A.R. (1974): Incompatibility in *Culex pipiens*. pp.97−118. *In*. R. Pal. & M.J. Whitten (ed.). Use of Genetics in Insect Control. Elsevier Pabl. Comp., Amsterdam.