Journal of the American Mosquito Control Association, 16(1):9–12, 2000

RESISTANCE AND RESPONSE TO SELECTION TO DELTAMETHRIN IN *ANOPHELES SINENSIS* FROM ZHEJIANG, CHINA

JINFU WANG

College of Life Sciences, Zhejiang University, No. 232, Wen San Road, Hangzhou, Zhejiang 310012, People's Republic of China

ABSTRACT. Resistance levels to deltamethrin were measured in 5 natural populations of *Anopheles sinensis*. The median lethal concentrations (LC_{50}s) of deltamethrin in these populations were higher than those in susceptible strains originating from the same populations, especially in the Wenzhou population, which had a resistance ratio (RR_{50}) of 11 relative to its susceptible strain. Resistant strains were selected with deltamethrin for 12 generations. Resistance levels in resistant strains were 130 to 190-fold higher than in susceptible strains, and 10 to 40-fold higher than in natural populations. Response of selection (R) in the resistant strain from the Wenzhou population was less than 0.1, and those in resistant strains from other natural populations were more than 0.1. This suggests that a resistant strain from a natural population with higher resistance has a lower increase in RR than a resistant strain from a natural population with low resistance under identical insecticide selection. These results are discussed in relation to mosquito control strategies.

KEY WORDS. *Anopheles sinensis,* deltamethrin, resistance, response of selection

INTRODUCTION

Anopheles sinensis Wiedemann, an important vector of malaria, is widely distributed in rural areas of Zhejiang, China. Malaria vector control was started using organophosphate insecticides in the middle 1960s. The disease has been absent from most of areas in Zhejiang since the early 1980s, but is still present in a few areas, such as in Wenzhou, Zhejiang. In 1991, mosquito-curtains soaked with deltamethrin were used to control *An. sinensis* in the rural area of Wenzhou. This is the 1st report of mosquito control with pyrethroids in Zhejiang, although pyrethroids have been used in agriculture since the early 1980s. This work was carried out for 2 years and resulted in a sharp decrease of malaria in this area (Yao 1992).

To coordinate research on mosquito-curtain soaking in Wenzhou, we carried out a study of deltamethrin resistance in mosquitoes. This study was undertaken to determine the resistance level in the Wenzhou population of *An. sinensis* by comparing the lethal concentration of pyrethroids to its susceptible strain. Because pyrethroid resistance is now widespread in many crop pest species in Zhejiang, possible resistance of mosquitoes to deltamethrin in other rural areas of Zhejiang was also determined. A resistant strain from each natural population was selected with deltamethrin, and the increases in their ratios of resistance were analyzed.

MATERIALS AND METHODS

Mosquitoes: Adult *Anopheles sinensis* were collected from breeding sites in 5 rural areas (Wenzhou, Jinhua, Ningbo, Hangzhou, and Jiaxing) during the summer of 1995–96. Each adult population collected was held in a screen cage (29×22×23 cm) and fed 15% glucose solution. A silk fabric laid on moistened cotton in a culture plate was supplied for oviposition. Larvae from each population were reared in an enamel washbowl containing 2,000 ml of water. Emerging adults were again held in the screen cages as described above. Larvae and adults were held at 27 ± 1°C with 80 ± 10% relative humidity, and a photoperiod of 12:12 h (light: dark). When needed, females were bloodfed on mice. A portion of the 4th-stage larvae from each natural population was used for bioassays, with the 4th-stage larva of its susceptible strain as the control. Another portion of the 4th-stage larvae was selected with deltamethrin.

A resistant strain from each natural population was selected with deltamethrin for each generation at a dose resulting in 60–70% mortality. Each resistant strain was selected for 12 generations and the lethal concentration of the 4th-stage larvae was measured in each generation.

Susceptible strains of *An. sinensis* with the same genetic backgrounds as their respective natural populations were provided by the epidemic prevention stations in Wenzhou, Jinhua, Ningbo, Hangzhou, and Jiaxing. These strains had been held under relaxation of insecticide selection for more than 10 years (Wang et al. 1996a).

Insecticides: Deltamethrin (98% powder) supplied by the Chemical Laboratory, Zhejiang Academy of Agricultural Sciences, was diluted to 1 and 0.1% stock solutions with acetone.

Larval bioassay: Resistance of larvae to deltamethrin was measured in a total volume of 200 ml using the standard World Health Organization (WHO) test method (WHO 1975, Liu 1985). Sets of 25 larvae were placed in enamel bowls containing 200 ml reactive volume. For the range-finding test, the concentration gradient points were 2–2.5, and each regular test contained 6–7 concentrations (i.e., the regular test for the Wenzhou natural population contained 7 concentrations from 0.04 to

Table 1. Log-dosage probit mortality data analysis for deltamethrin tested against 5 natural populations (N) and susceptible strains (S)[1].

Population	Strain	LC_{50}[2]	LC_{99}	RR_{50}	RR_{99}	χ^2
Wenzhou	N	0.3327 ± 0.0912	3.7204	10.91	20.20	16.72*[3]
	S	0.0305 ± 0.0012	0.1842			1.55
Jinhua	N	0.1672 ± 0.0614	2.9073	6.58	11.62	13.24*
	S	0.0254 ± 0.0009	0.2501			1.37
Ningbo	N	0.1355 ± 0.0317	3.0556	4.22	14.79	10.37*
	S	0.0321 ± 0.0011	0.2066			1.21
Hangzhou	N	0.1037 ± 0.0725	2.8254	4.96	12.92	10.04*
	S	0.0209 ± 0.0017	0.2187			2.09
Jiaxing	N	0.1447 ± 0.0574	2.9526	4.54	14.81	11.73*
	S	0.0319 ± 0.0008	0.1994			1.14

[1] LC_{50}, median lethal concentration; LC_{99}, 99% lethal concentration; RR_{50}, resistance ratio to deltamethrin at LC_{50} relative to susceptible strains; RR_{99}, resistance ratio to deltamethrin at LC_{99} relative to susceptible strains.

[2] χ^2 values for the fit-goodness of data to straight lines are given. Includes standard error for the LC_{50}.

[3] * indicates a significant deviation from a linear response.

2.56 ppm with the gradient point = 2) with 3 replicates per concentration. Mortality 20 min after treatment was recorded. Relative concentrations of acetone were used as controls.

Statistical analysis: Mortality data were analyzed using the log-probit program of Raymond (1993), based on Finney (1971). This program tests for the linearity of mortality curves, provides lethal concentrations (LCs), tests whether mortality lines are parallel, and computes resistance ratios (RR). Response of selection (R) in each resistant strain was calculated with the equation suggested by Tabashnik and McGaughey (1994) as follows:

$$R = \log(RR_{50F12})/n,$$

where RR_{50F12} was the resistance ratio at the median lethal concentration (LC_{50}) in the 12th generation of each resistant strain relative to its natural population, and n was the number of generations under insecticide selection. This parameter indicates the increase in RR in each resistant strain under insecticide selection. Resistance differences among mosquito populations were analyzed with Student's t-test (Hu and Zhang 1985).

RESULTS

Sensitivity status of 5 natural populations

Table 1 gives the results of log-dosage probit mortality analysis of 4th-stage larvae in 5 natural populations and their susceptible strains. All susceptible strains were homogeneous in their response to deltamethrin, with the data points for all natural populations deviating significantly from a straight line. Parallelism of the mortality lines obtained with 5 susceptible strains was not rejected at the 5% confidence level; they were not significantly ($P > 0.05$) different for susceptibility to deltamethrin. All natural populations were significantly ($P < 0.05$) more resistant than their susceptible strains to deltamethrin. The calculated RRs between paired susceptible strains and natural populations indicated that the Wenzhou population had the highest resistance level among these natural populations ($RR_{50} \approx 11$, $RR_{99} \approx 20$), and other natural populations showed <10-fold resistance at the LC_{50} (Jinhua: 6.58, Ningbo: 4.22, Hangzhou: 4.96, and Jiaxing: 4.54) and <15-fold resistance at the LC_{99} (Jinhua: 11.62, Ningbo: 14.79, Hangzhou: 12.92, and Jiaxing: 14.81). The Wenzhou population was significantly ($P < 0.05$) more resistant than all other natural populations.

Development of resistance under deltamethrin selection

Development of resistance to deltamethrin for each resistant strain under insecticide selection is shown in Fig. 1. Under selection for 12 generations, LC_{50}s in resistant strains were more than 4.0 ppm (4.07 ppm for the strain from the Wenzhou population, 4.34 ppm for the strain from the Jinhua population, 5.01 for the strain from the Ningbo population, 4.01 ppm for the strain from the Hangzhou population, and 4.73 ppm for the strain from the Jiaxing populations). Compared with the susceptible strain, resistance levels in these resistant strains increased approximately 130-, 170-, 150-, 190-, and 150-fold, respectively. These resistant strains did not differ significantly ($P > 0.05$) in resistance to deltamethrin.

Resistance rations (RR_{50}) in resistant strains, relative to natural populations, are shown in Table 2. Under deltamethrin selection for 12 generations at 60–70% mortality, resistance levels increased 12- to ~40-fold over natural populations (12.25-fold for the stain from the Wenzhou population, 25.95-fold for the Jinhua population, 36.99-fold for the Ningbo population, 38.67-fold for the Hangzhou population, and 32.70-fold for the Jiaxing population). The RR for the resistant strain from the Wenzhou population was significantly ($P < 0.05$) lower than that of all other resistant strains. Response of selection (R) was 0.0907 for the resistant strain

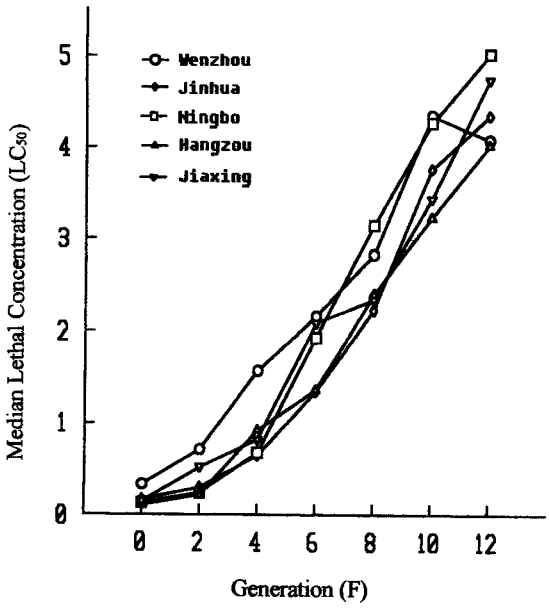

Fig. 1. Median lethal concentrations (LC_{50}s) of each generation for 5 strains under deltamethrin selection at the dose of 60–70% mortality.

from the Wenzhou population, 0.1178 for the Jinhua population, 0.1307 for the Ningbo population, 0.1323 for the Hangzhou population, and 0.1262 for the Jiaxing population. The R value in the resistant strain from the Wenzhou population was significantly ($P < 0.05$) less than that other resistant strains.

DISCUSSION

In Zhejiang, organophosphate insecticides have been used to control mosquitoes for more than 20 years and organophosphate resistance is now widespread in most mosquito species (Wang et al. 1996b). Therefore, replacement of organophosphate insecticides with other classes of insecticides, such as pyrethroids, is an important means of increasing mosquito control. Soaking mosquito-curtains with deltamethrin in Wenzhou played an important role in controlling the *An. sinensis* population, and the incidence of malaria in this area decreased greatly (Yao 1992). However, as use of insecticides continues, susceptible individuals in populations decrease and resistant individuals in populations increase, resulting in a resistant population. The population of *An. sinensis* in the area of Wenzhou, which used mosquito-curtains soaked with deltamethrin for the purpose of controlling mosquitoes, had a high frequency of resistance genes. Resistance levels of other natural populations were higher than those of their susceptible strains, but lower than that of the Wenzhou population. From the similarity of sensitivity between susceptible strains and similarity of

Table 2. Change in resistance ratios ($RR_{50} = LC_{50Fi}/LC_{50F0}$[1]) to deltamethrin at the median lethal concentration (LC_{50}) under selection pressure with deltamethrin relative to the natural population (F_0 generation) for each resistant strains.

Generation (F)	RR_{50}				
	Wenzhou	Jinhua	Ningbo	Hangzhou	Jiaxing
0	1	1	1	1	1
2	2.14	1.78	1.75	2.06	3.59
4	4.73	3.87	4.98	8.92	5.68
6	6.49	8.04	14.24	13.10	14.45
8	8.48	13.33	23.17	23.07	16.14
10	13.05	22.46	31.43	31.05	23.67
12	12.25	25.95	36.99	38.67	32.70

[1] $LC_{50Fi} = LC_{50}$ for the F_i (i = 0, 2, . . ., 12) generation; $LC_{50F0} = LC_{50}$ for the F_0 generation (the natural population).

resistance between resistant strains, it could be inferred that these 5 populations should have a similar potential of resistance to deltamethrin and that differences in resistance between the Wenzhou natural population and other natural populations may arise from differences in local control measures. Resistance to deltamethrin in other natural populations may be produced by use of pyrethroids in agriculture and personal protection because pyrethroids have not been used intensively for the purpose of controlling mosquitoes in these areas. This hypothesis should be further tested.

Nerve insensitivity plays an important role in the resistance of insects to pyrethroids (Omer et al. 1980, Bloomquist and Miller 1985, Shono 1985, Zhai 1995). Pyrethroid-resistant strains of mosquitoes were selected under laboratory conditions and were detected by cross-resistance to DDT (WHO 1981, Chen 1990). Deltamethrin-resistant strains with high RRs (>100, relative to susceptible strains) have been selected in my laboratory. Under the same selection pressure, the response to selection in the resistant strain from the Wenzhou population was less sensitive than that of other resistant strains. This may have occurred because the resistance level of the Wenzhou natural population was higher than that of other natural populations. However, resistance levels of the resistant strains were not significantly different.

To decrease resistance of mosquitoes, different classes of insecticides should be rotated in those natural populations with a high resistance to a given class of insecticides. In Wenzhou, pyrethroids should be replaced with other classes of insecticides or used with synergists so as to increase the effect of pyrethroids. For other natural populations, pyrethroids can be used, but the development of pyrethroid resistance in these natural populations should be monitored yearly.

REFERENCES CITED

Bloomquist JR, Miller TA. 1985. A simple bioassay for detecting and characterizing insecticide resistance. *Pestic Sci* 16:611–614.

Chen WM. 1990. Studies on selection of dipterex-resistant strain of *Culex pipiens* with deltamethrin. *Acta Entomol Sin* 33:14–20.

Finney DJ. 1971. *Probit analysis* Cambridge, United Kingdom: Cambridge Univ. Press.

Hu BM, Zhang QD. 1985. *Statistical analysis* Hangzhou, People's Republic of China: Science and Technology Press.

Liu JF. 1985. Measuring technique for the resistance of mosquitoes. In: Liu WD, ed. *The resistance of mosquitoes in China* Shanghai, People's Republic of China: Science Publisher. p. 134–137.

Omer SM, Georghiou GP, Irving SN. 1980. DDT/Pyrethroid resistance interrelationship in *Anopheles stephensi*. *Mosq News* 40:200–209.

Raymond M. 1993. *PROBIT CNRS-UMII. License L93019* St. George d'Orques, France.

Shono T. 1985. Pyrethroid resistance of the Kdr-type-mechanism. *J Pestic Sci* 10:141–146.

Tabashnik BE, McGaughey WH. 1994. Resistance risk assessment for single multiple insecticides: response of indianmeal moth to *Bacillus thuringensis*. *J Econ Entomol* 87:834–841.

Wang JF, Lu SH, Chen JX. 1996a. Study on the distribution probability of resistance factor in *Anopheles sinensis* to two pyrethroids. *Chin J Vector Biol Control* 7:166–169.

Whang JF, Lu SH, Shen HY, Chen JX. 1996b. Analysis on the probability of high esterase activity and the tendency of organophosphate resistance in *Culex pipiens pallens* populations. *Acta Parasitol Med Entomol Sin* 3: 223–230.

WHO [World Health Organization]. 1975. *Instruction for determining the susceptibility or resistance of mosquito larvae to insecticides* WHO/VBC/75.583 Geneva, Switzerland: World Health Organization.

WHO [World Health Organization]. 1981. Resistance of vector of disease to pesticides. *WHO Tech Rep Ser* 655: 82.

Yao LN. 1992. Effects of mosquito-curtain soaking with deltamethrin on *Anopheles sinensis* in Cangnan. *Zhejiang J Prevent Dis* 4:17–18.

Zhai QH. 1995. Some aspects of progress in insect molecular biology: molecular mechanisms of insecticide resistance. *Acta Entomol Sin* 38:493–501.