Chapter 3

Results

An. minimus species A was placed under selection pressure and each generation was tested for susceptibility to deltamethrin using dose and time mortality relationships (Tables 5 and 6). The lethal dose at 50% mortality (LD_{50}) and lethal dose at 90% mortality (LD_{90}) values for susceptibility to deltamethrin fluctuated widely during the first four generations. The reason for this variation is unclear. Under continuous selective pressure, the LD_{50} and LD_{90} values continuously increased throughout the selection process. There was approximately a 26-fold increase in the LD_{50} value and a 23-fold increase in the LD_{90} value by the F_{10} generation (LD_{50} = 0.00912 and LD_{90}= 0.03120). The increase is based on comparisons with susceptibilities of the parent generation (F_0) (LD_{50} = 0.00035 and LD_{90}= 0.00137) (Table 5). Similarly, LT_{50} and LT_{90} values of deltamethrin also increased under selective pressure from the F_{14} to F_{19} generations. Due to technical difficulties, selective pressure was not applied from F_{11} to F_{13}. There was roughly a 3-fold increase in LT_{50} and LT_{90} when F_{19} was compared to the F_{14} generation (Table 6). This continuous increase in lethal dose and lethal time values suggests that populations under selective pressure became less susceptible to deltamethrin than the parent colony. Hence, development of physiological resistance of successive generation could be resulted.

The slopes of regression lines for test data from each generation were computed and values for slope varied 1.20 to 2.98. The highest value was obtained from the F_5 generation (2.98) and the lowest value was obtained from the F_3 generation. Low χ^2 values demonstrated that the response of mosquitoes to deltamethrin in susceptibility tests completely fit a linear model (*P>0.01*) (Tables 5 and 6 and Figures 8 and 9).

Table 5 Susceptibility data of deltamethrin based on dose/mortality relationships tested against *An. minimus* species A populations.

Sample	No. Tested	LD_{50} (%)	95%CI	LD_{90} (%)	95%CI	Slope(SE)	χ^2
F_0	240	0.00035	0.00031-0.00040	0.00137	0.00113-0.00173	2.20± 0.15	6.06
F_1	360	0.00049	0.00043-0.00057	0.00200	0.00157-0.00275	2.10±0.16	4.62
F_2	240	0.00042	0.00034-0.00054	0.00500	0.00298-0.01115	1.20±0.14	1.11
F_3	360	0.00027	0.00021-0.00033	0.00249	0.00169-0.00440	1.33±0.14	4.28
F_4	360	0.00030	0.00015-0.00154	0.00283	0.00086-0.00401	1.32±0.26	7.27
F_5	360	0.00188	0.00169-0.00210	0.00506	0.00429-0.00621	2.98±0.21	5.63
F_6	239	0.00269	0.00238-0.00306	0.00828	0.00672-0.01090	2.62±0.21	3.88
F_7	120	0.00292	0.00182-0.00517	0.01243	0.00656-0.05066	2.04±0.34	3.62
F_8	360	0.00603	0.00472-0.00782	0.02060	0.01953-0.03078	2.00±0.15	3.69
F_9	360	0.00617	0.00584-0.00741	0.02063	0.01709-0.02642	2.04±0.19	4.97
F_{10}	360	0.00912	0.00621-0.01024	0.03120	0.02417-0.03587	1.85±0.14	2.78

CI = Confidential

Table 6 Susceptibility data of deltamethrin (0.05%) based on time/mortality relationships tested against *An. minimus* species A populations.

Sample	No Tested	LT_{50} (min)	95%CI	LT_{90} (min)	95%CI	Slope (SE)	χ^2
F_{14}	360	19.07	8.38-89.17	75.22	27.43-71.34	2.71±0.45	9.37
F_{15}	240	29.34	18.39-58.19	73.77	43.45-96.15	2.21±0.26	5.94
F_{16}	360	31.97	18.60-41.33	97.82	59.05-120.25	2.17±0.38	2.51
F_{17}	360	46.32	40.32-55.43	179.90	123.57-350.67	1.69±0.32	9.52
F_{18}	360	47.54	41.34-54.34	185.25	154.23-325.32	1.58±0.34	5.12
F_{19}	240	55.32	45.23-78.15	204.12	189.13-398.12	1.42±0.26	4.12

CI = confidential

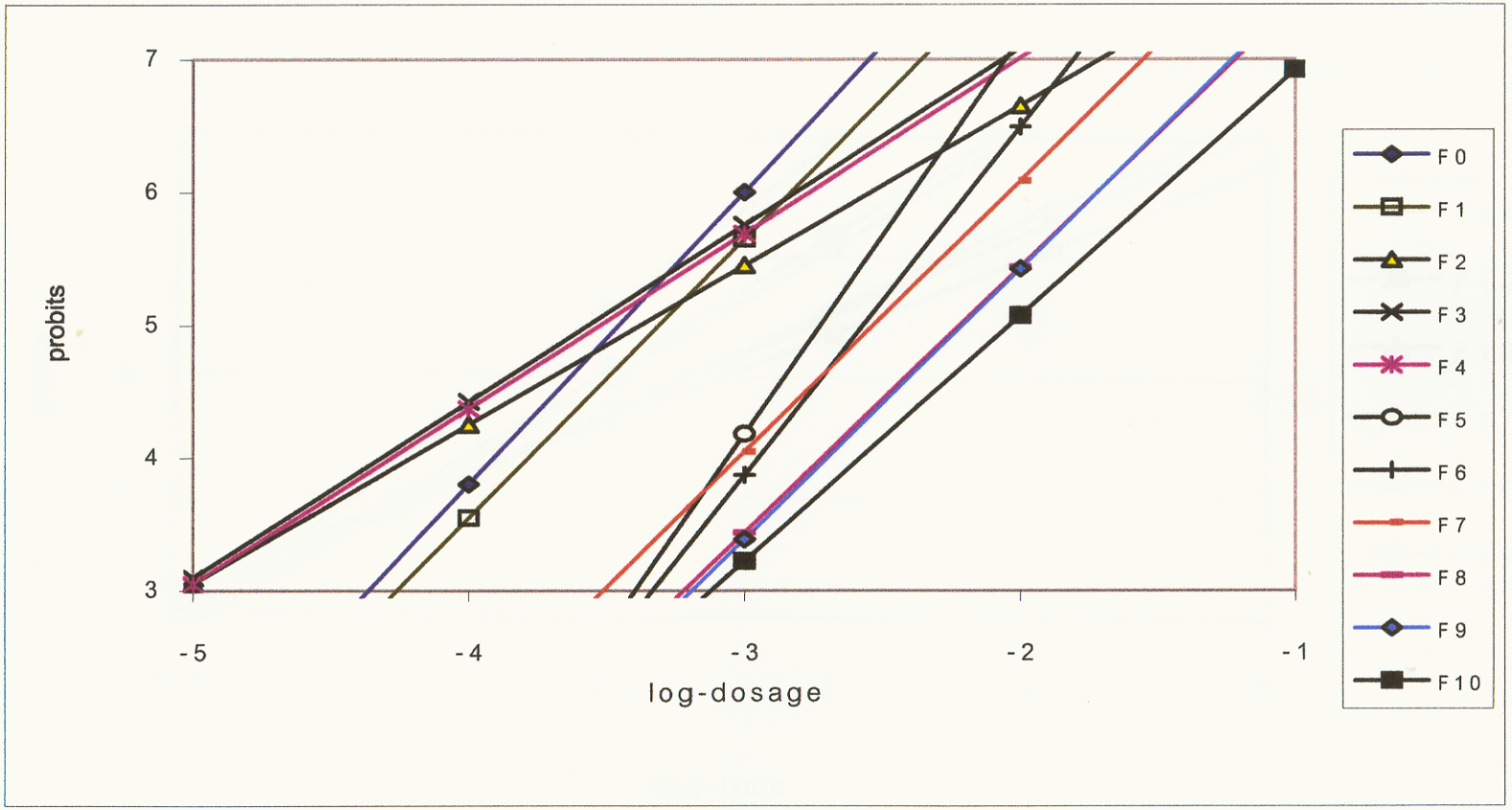

Figure 8 Log-dosage probit lines for deltamethrin selected (F_0-F_{10}) adults of *An. minimus* species A populations

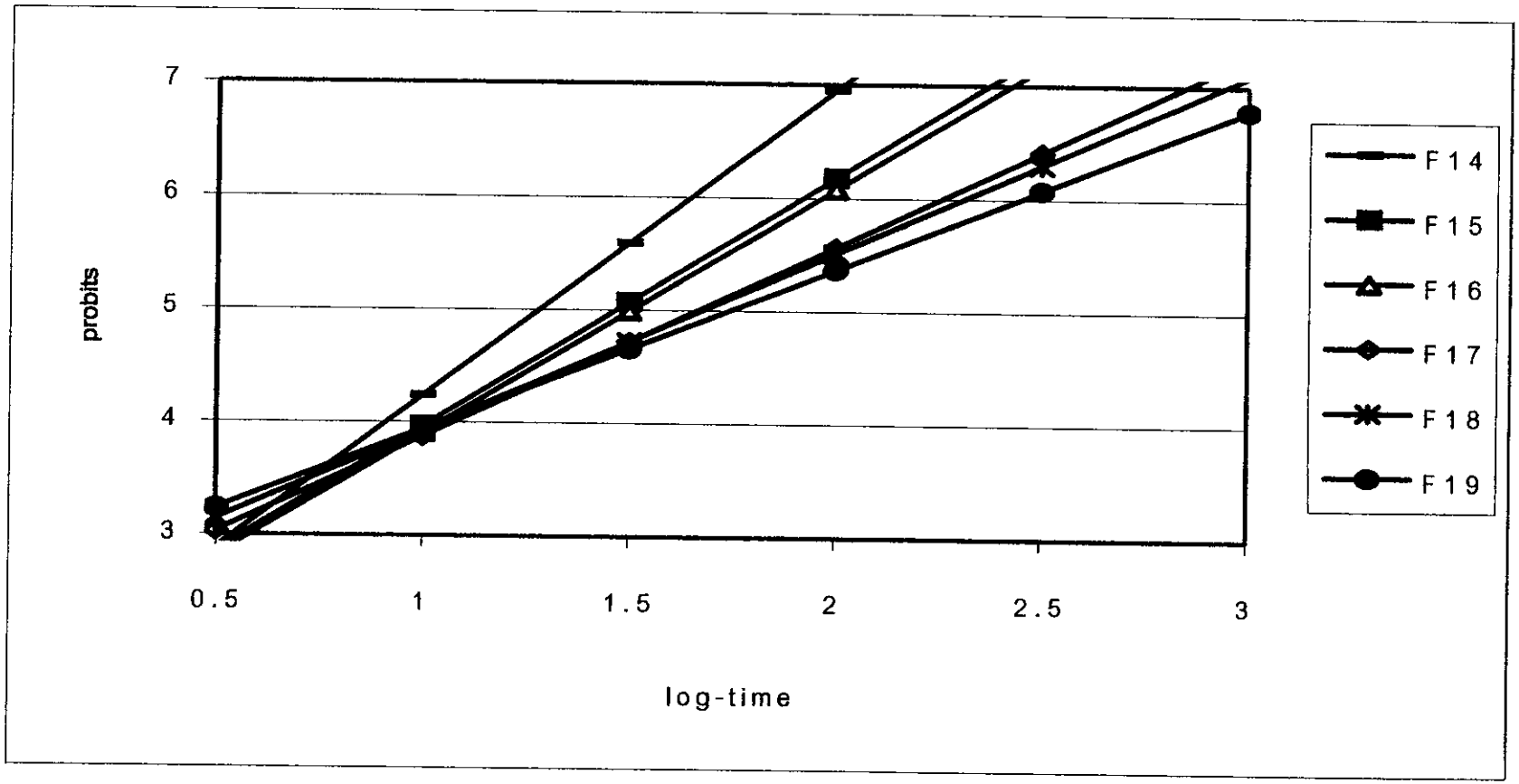

Figure 9 Log-time probit lines for deltamethrin selected (F_{14}-F_{19}) adults of *An. minimus* species A populations.

Results of susceptibility tests at the single diagnostic dose of deltamethrin (0.05%) for different generations of both populations under selection pressure and populations not under selection pressure are presented in Table 7. The ability of mosquitoes to survive the diagnostic dose after 24 h is indicative of resistance in the population; as defined by percent mortality in the test population. Our result showed that F_1-F_5 generations were completely susceptible to deltamethrin as evidenced by 100% mortality (Table 7). Partial survival (5%) was first observed in the F_6 generation of the population under selection pressure. Percent mortality of the treatment colony continually decline toward the end of the study. The highest mortality was observed in the F_{19} generation (48% mortality). The continuous decline in percent mortality of the treatment colony was indicative of increasing resistance to deltamethrin. Treatment populations were low in some generations (F_8, F_9 and F_{11}) as a consequence no diagnostic susceptibility tests could be performed.

Table 7 Mortality from WHO diagnostic test kit for 0.05% deltamethrin against 2 test populations of *An. minimus* species A, 24 h-post exposure.

Sam	Control colony		Selected colony		Sam	Control colony		Selected colony	
-ple	% Test	%Dead	%Test	%Dead	-ple	%Test	%Dead	%Test	%Dead
F_1	100	100	100	100	F_{11}	NA	NA	NA	NA
F_2	100	100	100	100	F_{12}	100	100	100	80
F_3	100	100	100	100	F_{13}	100	100	100	76
F_4	100	100	100	100	F_{14}	100	100	100	76
F_5	100	100	100	100	F_{15}	100	100	100	71
F_6	100	100	100	95	F_{16}	100	100	100	64
F_7	100	100	100	90	F_{17}	100	100	100	64
F_8	100	100	NA	NA	F_{18}	100	100	100	50
F_9	100	100	NA	NA	F_{19}	100	100	100	48
F_{10}	100	100	100	85	F_{20}	NA	NA	NA	NA

NA = Not Applicable

Anopheles minimus populations under selective pressure were also tested against diagnostic dosages of 4% DDT to check for cross-resistance. Percent mortality from DDT in the treatment colony declined in populations under deltamethrin selective pressure. Percent mortalities in tests with treatment populations against DDT were 90% in the F_8 and 60% in generation F_{19} (Table 8). The control colony remained completely susceptible to DDT (100% mortality).

Table 8 Mortality from WHO diagnostic test kit for 4% DDT against *An. minimus* species A test populations 24 h-post exposure.

Test Colonies	Control colony		Selected colony	
	% Test	% Dead	% Test	% Dead
F_0	100	100	100	100
F_4	100	100	100	100
F_8	100	100	100	90
F_{12}	100	100	100	80
F_{16}	100	100	100	70
F_{19}	100	100	100	60

Elevated esterase and MFOs assays were performed on resistant and control colonies of *An. minimus* (Table 9). There were no significant differences, as determined by an ANOVA, in the total protein content among the control and the other three selected colonies (F_8, F_{12} and F_{18}) ($P>0.05$). All enzyme activities were calculated based on 1mg protein. Alpha and beta-esterase activities were found greatly fluctuated among all population tested whereas MFOs activity was consistently increased throughout the selection period. The activities of alpha-esterase began to increase in F_8 (0.1016± 0.0136), dropped off in F_{12} (0.0704±0.0160) and re-elevated in F_{18} (0.1422±0.0338) whereas, they were significant from the control colony (0.0363±0.0125) ($P<0.05$). For the beta-esterase, activity was found significantly lower in F_{18} (0.0484±0.0165) as

compared to F_8 (0.1079±0.0519) and F_{12} (0.1017±0.0482) despite consistent continuation of insecticide selection (*P< 0.05*). No significant difference in activities of beta-esterase was observed between F_0 (0.0430±0.0241) and F_{18} (0.0484±0.0165) (*P> 0.05*).

Table 9 Comparison of the specific activities of α, β non-specific esterases and monooxygenases (MFOs) from *An. minimus* species A populations (sample size=20).

Test popula-Tion	Total protein Mean (±SD) mg protein/ml (per mosquito)	α Esterase Mean (±SD) m-mole α naphthol/ min/mg protein	β Esterase Mean (±SD) m-mole β naphthol/ min/mg protein	MFOs Mean (±SD) m-mole-product /min/mg protein
F_0	0.7878±0.1836 a	0.0363±0.0125 a	0.0430±0.0241 a	4.2550±0.2261 a
F_8	0.7442±0.0785 a	0.1016±0.0136 c	0.1079±0.0519 b	4.6950±0.2097 a
F_{12}	0.7405±0.1247 a	0.0704±0.0160 b	0.1017±0.0482 b	5.7700±0.2239 b
F_{18}	0.7696±0.1263 a	0.1422±0.0338 d	0.0484±0.0165 a	21.9000±0.8534 c

No significant difference at 0.05 level in the same letter (within the column)

For MFOs, the results revealed that higher specific activity of MFO was found in a selected colony than those from a control colony. Specific activity was found considerably increased toward the end of selection; activities were 4.255±0.2261, 4.695±0.2097, 5.770±0.2239 and 21.90±0.8534 in F_0, F_8, F_{12} and F_{18}, respectively (Table

9). There was approximately a 5 fold increase in specific activities of MFOs in the F_{18} compared to the control colony (F_0) and 3.8 folds increased in F_{18} compared to F_{12}.

In case of GSTs, the intensity of enzyme was determined and measured based on the optimal densities (OD). The protein content were 0.5887±0.1977, 0.8329±0.0962, 0.4959±0.2050, 0.8385±0.1230 in F_0, F_8, F_{12} and F_{18}, respectively (Table 10). The OD values decreased from F_0 (0.0279±0.0121) to F_8 (0.0156±0.0088), but increased in F_{12} (0.0254±0.0111) and F_{18} (0.0366±0.0139). The OD at 414 suggested that there was significance in the intensity of GSTs among control and resistant colonies ($P< 0.05$) (Table 10). The result suggested that esterase and MFOs may be involve in the development of physiological resistance to deltamethrin in *An. minimus* species A from Thailand.

Table 10 Optical density (OD) of glutathion S-tranferases towards 1-chloro-2,4-dinitrobenzene (CDNB) from *An. minimus* species A populations.

Test population	Mean ± SD mg protein/ml (per mosquito)	OD value at 414 nm	Number of test (mosquitoes)
F_0	0.5887±0.1977 a	0.0279±0.0121 b	20
F_8	0.8329±0.0962 b	0.0156±0.0088 a	20
F_{12}	0.4959±0.2050 a	0.0254±0.0111 b	20
F_{18}	0.8385±0.1230 b	0.0366±0.0139 c	20

No significant differences at 0.05 level in the same letter (within the column).