Chapter 4

Discussion

Synthetic pyrethroids have been introduced for malaria control in Thailand since 1992. Since that time pyrethroids have been used as a replacement for DDT (the DDT phase-out occurred from 1995-1999) (Department of Communicable Disease Control, 1995-2000). Permethrin and lambdacyhalothrin are used primary for impregnated bed nets, whereas deltamethrin is a major insecticide for an indoor-residual house spray (Department of Communicable Disease Control, 1995-2000). Approximately, one year after bed nets impregnated with permethrin were introduced as a malaria control measure in the northern part of Thailand, evidence of physiological resistance in An. minimus from the northern Thailand was reported (Department of Communicable Disease Control, 1995-2000). However, little was known about the level of physiological resistance, or the factors or mechanisms of resistance. Deltamethrin is another effective compound that should be monitored for development of physiological resistance in vector populations. Deltamethrin is normally applied 1 or 2 times a year in malaria endemic areas of Thailand (Department of Communicable Disease Control, 1995-2000). Continuous indoor-residual spraying of deltamethrin may select for resistance in the vector populations.

Anoppeles minimus is an excellent model for the study of insecticide resistance in Thailand. This species is considered sufficiently endophagous and anthropophilic to come into contact with insecticide residues in houses (Nutsathapana et al., 1986 and Chareonviriyaphap et al., in press). Therefore, careful and complete monitoring of malaria vectors for resistance to deltamethrin should be a primary emphasis of malaria control activities.

Resistance is defined as the acquired ability of an insect population to tolerate doses of insecticide which will kill the majority of individuals in a normal population of the same species (WHO, 1975). Our results indicated that a high level of physiological
resistance to deltamethrin in *An. minimus* can be developed under selective pressure in the laboratory. Resistance was shown by low mortalities of offspring from parents which survived selective pressures in preceding generations. $L_{D_{50}}$ and $L_{D_{90}}$ values of deltamethrin increased greatly over 10 generations of selective pressure. The selection experiment covered 19 generations but, due to low population numbers, no selective pressure could be applied against adults in generations 11, 12 and 13. There were 26- and 23-fold increases in $L_{D_{50}}$ and $L_{D_{90}}$ values, respectively, of deltamethrin in the $F_{10}$ compared to the parent colony ($F_0$). High variation was found in $L_{D_{50}}$ and $L_{D_{90}}$ values during generations $F_1$-$F_4$, immediately after selective pressure, was applied. We have no explanation for unexpected levels of this variation, but perhaps high variation is typical of populations in early stages of selective pressure. $L_{T_{50}}$ and $L_{T_{90}}$ values also increased during the second part of selections ($F_{14}$-$F_{19}$). There were approximately 3-fold increases in $L_{T_{50}}$ and $L_{T_{90}}$ values in $F_{19}$ compared to $F_{14}$. A similar study on rapid development of physiological resistance (in pyrethroids and fenvalerate) was reported on *An. stephensi* (Verma and Rahman, 1986).

Theoretically, a susceptible colony composed of totally susceptible individuals will produce the highest slope for a regression line of dose-response data. With selective pressure from exposures to insecticides, a population will become heterozygous for resistant genotypes. As frequency of resistant genotypes increases, the slope of the regression line will drop off and the line will shift to the right (Brown and Pal, 1971). The slope of regression lines, based on data from this selection experiments, continuously declined overtime. The result is consistent with increasing physiological resistance in the test populations. However, a shift to the right without a change in slope is indicative of increased population vigor, not physiological resistance (Brown and Pal, 1971). There was no shift to the right in regression lines from this dose-response data.

Resistant status can be evaluated according to the WHO protocol; thus, a population is considered resistant if more than 20% of the population survived the diagnostic dose compared to the susceptible colony (WHO, 1981b). In our tests the
percent mortality continually increased from one generation to the next, hence 52% of test specimens survived after 19 generations of almost continuous selective pressure. Cross-resistance to DDT was also observed in the deltamethrin resistant colony. Cross-resistance may have occurred as a result of similar actions of DDT and pyrethroids on the voltage-dependent sodium channel of nerve axons (Brooke et al., 1999; Bloomquist, 1996). There is evidence to support a claim of cross-resistance between DDT and pyrethroids. Previous studies have shown that pyrethroid resistant populations of Aedes aegypti in Thailand are frequently resistant to DDT (Brealey et al., 1984; Prasittisuk and Busvine, 1977). In addition, pyrethroid resistance in An. stephensi larvae was reported for a strain that had developed DDT resistance as a result of selection experiments in Pakistan (Omar et al., 1980). The similar mode of action of DDT and synthetic pyrethroids has led, in some cases, to a cross-resistance mechanism, commonly known as knock down resistance (kdr). Kdr is conferred by single amino acid changes in the sodium-channel insecticide-binding site in the nerve sheath (Oppenorth, 1985). Whether cross-resistance to DDT reported here is conferred by kdr will be conducted in the near further at molecular level.

Obviously, several factors other than frequency of insecticide spraying serve to influence the intensity of selection and development of physiological resistance in a population. The most important factors include frequency of resistance gene in a population, number of genes interacting to produce the resistant character, size of population, and the dominant relationship of the gene (Ferdinand, 2000). The proportion of sprayed houses with undisturbed surfaces and the extent of contamination of breeding places and outdoor resting habitats with agricultural insecticides may influence resistance development in insect population (Anonymous, 1987).

The study provided a baseline for susceptibility and varying levels of deltamethrin resistance in An. minimus in Thailand for the first time. If the level of resistance is maintained, then the resistant colony will be used to study the actions of pyrethroid insecticides and mechanisms of resistance. It has been reported that selection by toxic substances can increase the amount of enzymes that are responsible
for detoxification (Ferrari and Georghiou, 1990). Common insecticide resistance mechanisms in insect pests were reported elsewhere, including 3 possible pyrethroid resistance mechanisms, namely mixed-function oxynases (MFOs), elevated esterases, and reduced sensitivity of sodium channels (Kerkut and Gilbert, 1985; Georghiou, 1986; Nelson et al., 1996; Roberts and Andre, 1994; Scott et al., 1998; Feyereisen, 1999). In addition, an increase in Glutathion-S-transferases (GSTs) was reported in many pyrethroid resistant insects, such as Spodoptera litturata (Lagadic et al., 1993), Tribolium castaneum (Reidy et al., 1990), and Aedes aegypti (Grant and Matsumura, 1988). Identifications of elevated esterase and MFO in the pyrethroid resistant colony were also obtained in this report. An increase in the quantity of enzymes can be associated with gene amplification or overexpression of target genes. This appears to be the cause of protein over production when an organism is under environmental stress (Mouches et al., 1990).

Insect populations may survive the effect of chemical compounds by different physiological mechanisms including reduced target site sensitivity or elevated detoxifying enzymes (Martinez-Torres et al., 1998; Brooke et al., 1999). Many insecticide resistant mechanisms were reported elsewhere, including 4 mechanisms for pyrethroids i.e. MFOs, esterases, reduced sensitivity of sodium channels and GSTs (Oppennoorth, 1985; Georghiou, 1986; Grant and Matsumura et al., 1988; Reidy et al., 1990; Lagadic et al., 1993; Nelson et al., 1996; Roberts and Andre, 1994; Scott et al., 1998; Feyereisen, 1999; Chandre et al., 1999; Brooke et al., 2001). However, GSTs was found to be a minor detoxifying enzyme in pyrethroid resistant An. funestus as suggested by Brooke et al., (2001). Theoretically, esterase, MFOs and GSTs may implicate for detoxification enzymes in pyrethroid resistance insects (Vulule et al., 1999; Brooke et al., 2001).

Many evidences indicated that MFOs involved in many insecticide resistance mechanisms and is clearly associated with pyrethroid resistance (Hemingway and Ranson, 2000). Recent studies revealed that elevated MFOs were responsible for detoxification of pyrethroids in many insects including An. pseudopunctipennis
(Ocampo et al., 2000) and An. funestus in Africa (Brooke et al., 2001). In this study, increase in specific activity of MFOs in selected colonies was accompanied by changes in bioassay results obtained via WHO test methods (Chareonviriyaphap et al., 2002). There was a 5-fold increase in specific activity of MFOs in deltamethrin resistant colony of F_{19} compared to the control colony (F_{0}). Many studies suggested that previous exposure to DDT used in Public Health Control Program can produce an increase in MFOs that may reduce susceptibility level of pyrethroids (Prasittisuk and Busvine, 1977; Brogdon and Mc Allister, 1998). This is true for this study. An. minimus species A was collected from Rong Kwang District, Prae Province in 1993 (Chareonviriyaphap per comm). DDT was applied as an IRS twice a year in this area to protect human from the bite of mosquitoes since 1950. In addition, DDT and some organocholine related compounds were previously used for agricultural pests and termite protection. Illegal use of these compounds for termite control and agricultural practice in this area is a quite common event in Thailand. The selected colony of An. minimus in this study demonstrated decreasing of DDT susceptibility level from F_{8} (90% mortality) toward the end of selection of F_{19} (60% mortality), indicating an occurrence of cross-resistance to DDT. This resistant could be originated from the previous DDT usage in this area. Cross-resistance to DDT may have occurred as a consequence of similar mode of actions of DDT and pyrethroids on sodium channel of nerve axons known as “kdr”. Kdr is conferred by the change of one amino acid in the sodium-channel insecticide-binding site in the nerve sheath. Whether cross-resistance to DDT reported here is conferred by kdr should await further studies at molecular level (Bloomquist, 1996; Brooke et al., 1999). However, there are evidences to support a claim of cross-resistance between DDT and pyrethroids. Previous studies have shown that pyrethroid resistant populations of Ae. aegypti in Thailand are frequently resistant to DDT (Brealey et al., 1984; Prasittisuk and Busvine, 1977). In addition, pyrethroid resistance in An. stephensi larvae was reported on a strain that had developed DDT resistance in selection experiments in Pakistan (Omar et al., 1980). Recently, pyrethroid resistance in An.
*gambiae* in many West Africa countries caused, to a certain extent, the intensive use of DDT (Chandre et al., 2000).

Most pyrethroid compounds contain an ester linkage that is susceptible to hydrolysis by esterase (Oppennoorth, 1985). Previous use of organophosphate and carbamate may induce esterase mechanisms to confer cross-resistance to pyrethroids as an evidence of *An. albimanus* from Guatemala (Brogdon and Barber, 1990). Elevated esterase levels have been documented in many pyrethroid resistant insects (Abdel-Aal and Soderlund, 1980; Riskallah, 1983; Beach et al., 1989; Rodriguez et al., 1997; Penilla et al., 1998). Recently, there were evidences of pyrethroid resistance associated with elevated esterases in *An. gambiae* from Africa (Vulue et al., 1999; Chandre et al., 1999; Ranson et al., 2001). In this study, there was on approximately 2-4-fold increase in hydrolysis of alpha naphthylpropionate to naphthol in homogenates from selected colonies when compared to the control colony (completely susceptible to deltamethrin). However, in the case of beta esterase, extremely low specific activity in F_{18} was observed. The reason for this discrepancy was unknown. But, it could be due to that beta structure of the esterase was not responsible in the true resistant mechanism of detoxifying esterase.

GSTs was reported to play a significant role in detoxification and resistance of DDT (Ranson et al., 1997; Prapanthadara et al., 1998). This enzyme was elevated in many DDT resistant insects including *An. dirus* species B from Thailand and *An. gambiae* from Africa (Prapanthadara et al., 1998; Ranson et al., 2001). In addition to a main mechanism to DDT, GSTs was inhibited by varieties of synthetic pyrethroids and bound to molecule of many pyrethroid compounds in a sequestering mechanism (Prapanthadara et al., 1998; Kostaropoulos et al., 2001). However, this enzyme was not associated with pyrethroid/DDT resistance in an *An. minimus* resistant population in this study. An increase in the quantity of enzymes is invariably associated with gene amplification or over expression of the target genes. Increasing of enzyme activity appeared to be the mechanism causing protein over production when an organism is under environmental stress (Mouches et al., 1990).
In malaria endemic areas, there is a need for comparative studies on susceptible and refractory populations, in as many known vectors as possible. This is especially true if there has been continuous intradomicillary spraying with deltamethrin and other insecticides. Additionally, such studies should be representative of different geographical conditions and be conducted with greater frequency than in the past. Detection of incipient or operationally and unacceptably high levels of physiological resistance will help public health workers to take appropriate steps to counter the reductions in effectiveness of control efforts that may lead to insecticide resistance. Furthermore, cross-resistance or resistance as a result of agricultural uses of insecticides may evolve and have an adverse impact on the switching of an alternative insecticide for disease control. Resistant colonies established in this study will attempt to identify genes coding for deltamethrin resistance. Metabolic detoxification of pyrethroids implicated with increased monooxygenase production in mosquitoes will also be the matter of further research.