Literature Review
2. LITERATURE REVIEW

Wright and Pal (1967) reviewed elaborately the work on mosquito genetics. Historically the origin of mosquito genetics as a formal discipline was traced in a paper entitled “Mosquito genetics and cytogenetics” by Kitzmiller (1953). Till the mid 1950s, when the studies on mosquito genetics began, entomology was largely a descriptive enterprise and genetics, as a formal discipline was hardly existed in the entomological domain. The work done with Venerable fruit fly, *Drosophila melanogaster* Meig., laid the foundation for genetic studies in the early part of the twentieth century (Rai, 1973).

The finding of the formal genetics of mosquitoes was based on the isolation, characterization and genetic mapping of morphological mutants. Most of the advances in formal genetics have been made in the species of *Culex* and *Aedes*. In yellow fever vector *Aedes aegypti* L., some of the earliest mutants described were yellow larva (Craig and Gilham, 1959), Sex – ratio distortion (Craig and VandeHey, 1960) and white eye (Bhalla, 1968). More than 80 morphological mutants affecting all parts of the body were characterized. Of these 28 were assigned to three linkage groups in *Ae. aegypti* (Craig and Hickey, 1967). Shetty (1974, 1989) and Shetty and Chowdaiah (1972, 1976, 1977) have listed 46 genetical and morphological variations, 100 gynandromorphs, 150 intersexes and 2 mosaics from six strains of *Culex fatigans* Weid.
Resistance is a widespread phenomenon and resistant populations of nearly all economically important pests can now be found. Resistance in 156 species or 35% of the Dipterans reflects the strong selection pressure that has been applied against mosquitoes throughout the world. Cyclodiene resistance is found in 62% of reported species and DDT resistance in 52% followed closely by organophosphosphate resistance in 47%. Lower percentage has been reported for carbamate and synthetic pyrethroids (Georghiou, 1986; Leibee and Capinera 1995).

WHO (1992) listed 56 Anopheline and 39 Culicine mosquitoes are resistance to insecticides. Insecticide resistance / susceptibility status in mosquitoes to various insecticides have been reported (Sharma and Subba Rao, 1980; WHO, 1980, 1986; Curtis and Pasteur, 1981; Brown, 1986; Shidrawi, 1990; Arteme’ev et. al., 1992; Sergieva and Gracheva, 1992; Baskar and Shetty, 1992; Pushpalatha and Vijayan, 1994; Vijayan and Revanna, 1994; Ghosh et. al., 2002; Singh et. al., 2002; Shetty, 2002a).

The inheritance of resistance to DDT has been studied in 5 anopheline species, An. sundaicus (Davidson, 1958), An. stephensi (Davidson and Jackson, 1961), An. albimanus (Davidson, 1963a), An. quadrimaculatus (Davidson, 1963b) and in Culex pipiens fatigans (Pal and Singh, 1958; Rozeboom and Hobbs, 1960; Thomas, 1962) and Aedes aegypti (Abedi and Brown, 1960; Brown and Abedi, 1962; Coker, 1958; Klassen and Brown, 1964; Qutubuddin, 1958).
The genetics of DDT resistant strains of *Ae. aegypti* from Trinidad, Malaya and Haiti (Coker, 1958; Qutubuddin, 1958), has shown that a single, partially dominant factor was involved in resistance. However the cross resistance spectrum of Malayan strain (Busvine and Coker, 1958), as well as the result of crosses between this strain and susceptible strain (Coker, 1958) suggested that the resistance factor in the Malayan strain might not be identical to that of the Trinidad and Haiti. The definite evidence of mode of inheritance of DDT resistance in *Cx. tarsalis* has not been obtained, however it was shown to be independent of malathion resistance (Plapp et. al., 1961).

An interesting study of the rate of development and regression of resistance to DDT was carried out by Abedi and Brown (1960) on a strain of *Ae. aegypti* from Malaya. The said strain was unusual in possessing a remarkable ability to develop high levels of DDT resistance in contrast to previously studied strains which had gained little or no resistance to DDT under laboratory selection pressure (Shidrawi, 1957; Surtees, 1958).

The inheritance of Dieldrin resistance in anophelines has been investigated in *An. albimanus* (Rozeboom and Johnson, 1961), *An. gambiae* (Davidson, 1958; Davidson and Hamon, 1962), *An. stephensi* and *An. pharoensis* (Davidson and Mason, 1963). Resistance was found in all cases to be monofactorial and partially dominant with the exception of strains of *An. gambiae* from the Ivory Coast (Davidson and Hamon, 1962), and *Cx. fatigans* in Upper Volta, Cameroon and Liberia (Davidson, 1964) in which resistance was reported to be dominant.
The genetic mode of inheritance of synthetic pyrethroids Deltamethrin and Cypermethrin and Organophosphate insecticides Malathion and Fenitrothion in *An. stephensi* has been established. The mechanism of resistance and dosage mortality ratios of the resistant and susceptible strains of *An. stephensi* clearly showed that the genes for Malathion resistance (*MR*), Deltamethrin resistance (*Dr*), Fenitrothion resistance (*Fnr*) and Cypermethrin resistance (*Cr*) are incompletely dominant and autosomal (Rao and Shetty, 1994; Rajasree and Shetty, 1998a; Ghosh and Shetty, 1999; Priyalakshmi and Shetty, 2000). Malathion resistance in *Cx. tarsalis* was reported to be inherited as an autosomal, dominant character (Plapp *et. al.*, 1961), a single partially dominant gene for the same species has been reported by Matsumura and Brown (1961).

Cross-resistance to DDT and pyrethroids as a consequence of the similar mode of action of DDT and pyrethroids resistance resulting in a phenomenon known as “knock down resistance” (*kdr*) in *An. minimus* reported (Bloomquist, 1996; Brooke *et. al.*, 1999). There is an evidence to support cross-resistance between DDT and pyrethroids in various mosquito species have shown that pyrethroid-resistant populations of *Ae. aegypti* in Thailand are frequently resistant to DDT (Prasittisuk and Busvine, 1977; Brealey *et. al.*, 1984). Pyrethroid resistance in *An. stephensi* has been reported from DDT resistant strains (Omar *et. al.*, 1980; Verma and Rahman, 1986), and more recently, pyrethroid resistance in *An. gambiae* in many West Africa countries has been linked, to a certain extent, by the past intensive use of DDT (Chandre *et. al.*, 2000).
The electrophoretic studies play an important role in understanding the enzymes associated with insecticide resistance in mosquitoes. Rao and Shetty (1996) and Rajasree and Shetty, (1998b) have reported the variation of protein and esterases between the susceptible and the resistant strains of *An. stephensi*. The variation of Acid and alkaline phosphatase, glucose -6 – phosphatase (G6pdh) and Phospho glucomutase (pgm) enzymes associated with insecticide resistant and susceptible stains have been studied (Rajasree, 1998; Ghosh, 2002; Shashikanth, 2003). Similar studies have been carried out in different species of mosquitoes (Raymond, *et. al.* 1996; Vaughan *et. al.*, 1997; Callaghan *et. al.*, 1998; Karunaratne *et. al.*, 1998; Paton, *et. al.*, 2000).

Townson (1971, 1972) described the genetics of certain esterase isozymes in *Ae. aegypti* and Saul *et. al.*, (1976) discussed esterase isozyme variation in strains of *Ae. aegypti*. Garnett and French (1971) reported a total of 13 sites of esterase isozyme in *Cx. p. quinquesacriatus*. Gargan and Barr (1977) studied the linkage relationships of two esterase isozyme in *Cx. pipiens*. Narang and Kitzmiller (1971, 1973) reported genetic analysis of multiple esterase isozyme loci in natural population of *An. punctipennis*. Furthermore, elevated sex-limited esterase in *An. funestus* (Green, 1977) and variability of esterase enzyme in *An. gambiae* group (Miles, 1978) were reported. Similarly, Freyvogel *et. al.*, (1968) reported sexual dimorphism for esterase activity in *An. stephensi*, in which only males showed a zone of enzyme action. Green (1977) also reported sexual dimorphism in *An. funestus*. However, no direct comparison is made between these two species.
In insects, the enzymes such as Acid and Alkaline Phosphatases have been studied in respect to the insecticide resistance (Beckman and Johnson, 1964; Harper and Armstrong, 1972). Studies on the amplification of several enzymes including esterases, acetyl cholinesterase, aldehyde oxidase, Xanthine dehydrogenase, monoxygenases and glutathion-S-transferases in resistant population of various species of mosquitoes (Mouches et al., 1990; Peiris and Hemingway, 1990; Hughes et al., 1992; Karunaratne et al., 1995; Vaughan and Hemingway, 1995; Vaughan et al., 1995; DeSilva et al., 1997; Hemingway and Karunaratne, 1998; Eduardo et al., 1999; Roger and Christine, 1999; Hemingway et al., 2000; Coleman et al., 2002; Enayati et al., 2003).

Enzyme-based metabolic mechanisms of insecticide resistance were investigated, comparing a deltamethrin-susceptible parent stock and resistant colonies of *An. minimus* species A using biochemical assays. Development of physiological resistance to deltamethrin in laboratory, resistant-selected generations of *An. minimus* is primarily associated with increased detoxification by over-expression of monooxygenases. The oxidases are the major contributors to pyrethroid resistance and the importance of ‘kdr’ has yet to be convincingly determined. This finding represents the first report from Thailand on such metabolic mechanism of resistance in anophelines (Chareonviriyaphap et al., 2003).

Xanthine dehydrogenase (Xdh) has been studied in respect to the rosy eye mutant in *Drosophila melanogaster* (Geibart et al., 1974; Hughes et al., 1992) and Benedict et al., (1996) reported activity of Xdh with respect to the different
phenotype of *An. albimanus*. The variation of gene structure of Xdh enzyme has been reported (Keith *et al.*, 1987; Komoto *et al.*, 1999). The association of Xdh gene with insecticide resistance in mosquito has been reported (Coleman and Hemingway, 1997).

The studies among protein isozymes pattern in *An. stephensi* were analysed for deltamethrin, fenitrothion, cypermethrin resistant and susceptible strains during the developmental stages were reported by Shetty (2002b).

A clear evidence for resistance based on an altered AChE in insects was first reported in a population of the green rice leaf hopper resistant to carbamate insecticide by Hama and Iwata (1971) and OP resistance by Iwata and Hama (1972). Evidence has been obtained for an AChE insensitive by inhibition by paraoxon and propoxur in *An. albimanus* (Ayad and Georghiou, 1975).

The *Cx. pipiens* mosquitoes, subjected to insecticide treatments with temephos was found that the resistance level had not developed beyond a 14-fold level and relatively low resistance was attributed to the presence of several identified resistance genes such as the insensitive target (Ace-R) and overproduced esterases (*A₁*, *A₄*, and *B₄*) (Raymond and Marquine, 1994).

The role of oxidative metabolism as a major mechanism of resistance for all insecticide classes and their role in detoxification enzyme systems, and the various reactions including hydroxylation of DDT, the epoxidation of cyclodienes.
the aromatic hydroxylation of the carbamates, carbaryl and propoxur and oxidation of phosphorothioates have been studied (Feyereisen, 1999). In An. albimanus seventeen P450 enzymes have been discovered (Scott et al., 1994).

Common insecticide resistance mechanisms in insect pests against pyrethroids include P450 mediated monooxygenases, elevated non-specific esterases, and reduced sensitivity of sodium ion channels along nerve axons (Oppenoorth, 1985; Georghiou, 1986; Nelson et al., 1996; Roberts and Andre, 1994; Scott et al., 1998; Feyereisen, 1999). Moreover, increased levels of glutathione S-transferases (GSTs) have been associated with conferring pyrethroid inhibition in many insect species (Lagadic et al., 1993; Reidy et al., 1990), including Ae. aegypti (Grant and Mastumura, 1988), An. gambiae (Ranson et al., 2001) and An. dirus B (Prapanthadara et al., 1998). More recently, elevated GSTs have been found to bind to molecules of many pyrethroid insecticides compromising effectiveness and toxicity by a sequestering mechanism (Kostaropoulos et al., 2001).

Insecticide resistance mechanisms (as opposed to insecticide avoidance behaviors important in the control of malaria vectors) have a biochemical basis. The two major forms of biochemical resistance are target-site resistance, which occurs when the insecticide no longer binds to its target, and detoxification enzyme-based resistance, which occurs when enhanced levels or modified activities of esterases, oxidases, or glutathione S-transferases (GST) prevent the insecticide from reaching its site of action. An additional mechanism based on
thermal stress response has been proposed, but its importance has not been assessed. Four primary insecticide resistant mechanisms have been reported associated with pyrethroid resistance, i.e., over-expression and increased production of monooxygenases (sometimes referred to as mixed function oxidases), non-specific esterases, GSTs and reduced sensitivity of sodium ion channels on the nerve membrane (‘kdr’ knockdown resistance), the target site for DDT and pyrethroids (Oppenoorth, 1985; Georghiou, 1986; Grant and Mastumura, 1988; Nelson et. al., 1996; Chandre et. al., 1999).

All three of these major groups of enzymes have been implicated in promoting detoxification of pyrethroids in resistance insects (Brogdon and McAllister, 1998; Vulule et. al., 1999). In general quantitative increases in these enzymes, associated with gene amplification or over-expression of target genes can result in protein overproduction in insects under selection pressure, thus conferring insecticide resistance (Mouches et. al., 1990). Hemingway and Ranson (2000) have reported the association of monooxygenases with pyrethroid resistance in mosquitoes.

Many authors have reported the effects of various insecticides on the fecundity, hatchability and fertility in few species of mosquitoes. Rao and Shetty (1992) have reported reduced fecundity, egg hatchability and sex ratio distortion towards male for Malathion, Fenthion and Methyl parathion, Priyalakshmi et. al., (1999) for Fenitrothion, Cypermethrin and Deltamethrin. Gaaboub and Darwood (1973) reported in Culex. pipiens for DDT and Malathion with respect to decrease in fecundity, but there was no reduced hatchability. Similarly, the resistant strain
was less fecund to temephos in *Cx. quinquefasciatus* (Ferrari and Gerghiou, 1981). Verma (1986) reported decrease in fecundity in *Cx. quinquefasciatus*, *Ae. aegypti* and *An. stephensi* for pyrethroids. The reduced fecundity for high and low dosages in *Ae. aegypti* for d-allethrin and d-phenothenrin was reported by Weide Liu et al., (1986).

Mortality of eggs, followed by delayed toxic effect of deltamethrin in larvae, pupae and adults has been reported in *An. stephensi* (Sahagal and Pillai, 1993). Penetration of the chemical into the egg was believed to be responsible for the mortality of eggs during embryogenesis (Grosscurt, 1977; Broadbent and Pree, 1984).

As the mosquitoes are developing resistance to newer insecticides, alternative strategies should be developed. Genetic or autocidal control is one such method. Knipling (1955) suggested the possibility of Insect eradication by the application of Sterile Insect Technique (SIT). Whitten and Foster (1975) elaborated the pest control through genetic manipulation. Radiation induced chromosomal translocation and inherited semisterility have been extensively studied in mosquitoes for the vector control (Aslamkhan and Aaqil, 1970; Sharma et al., 1978; Heemert et. al., 1983; Shetty, 1987, 1993; Gayathri and Shetty, 1992).

The chromosomal translocation in the genetic control programme of *Cx. p. quinquefasciatus* (Shetty, 1993) and *An. fluviatilis* James (Shetty, 1983) have been considered from our laboratory. The mating competitiveness of the translocated
lines of *An. stephensi* has been studied for the sterile male release method and were found to be more competitive than the normal males in the laboratory condition (Shetty and Gayathri, 1989).

The different species of mosquitoes have been developed for the preferential elimination of females during the early developmental stages for the vector control, which include; *An. gambiae* Giles (Curits et. al., 1976), *An. albimanus* Weid. (Kaiser et. al., 1978), *An. stephensi* (Robinson, 1986), *An. arabiensis* Patton (Curtis, 1978) and *Cx. quinquefasciatus* (Shetty, 1987).

The review article of Genetic control of insect vectors of diseases (Shetty, 1997, 2002a) and Genetics of mosquitoes (Rai, 1999) emphasis the relevance of different genetic control techniques including the sterile male release method, genetic sexing system, refractoriness and cytoplasmic incompatibility in mosquito control. Refractory strain/s of *An. qudrimaculatus* for the rodent malaria *Plasmodium yoelii*, bird malaria *P. gallinaceum* and simian malaria *P. cynomolgi* have been isolated in pure stocks and the genetic basis for the same has bee established (Shetty et al., 1996). Recently, transgenic refractory strain of *An. stephensi* having synthetic gene (AgCP[SM1]4) was tested successfully for the impaired transmission of malaria parasite (Ito et al., 2002).