CHAPTER 02

REVIEW OF LITERATURE
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Historically, the origin of mosquito genetics as a formal discipline is traced to a seminal paper entitled 'Mosquito genetics and cytogenetics' by Kitzmiller (1953). This is a long half century after the 'rediscovery' of Mendelism and the 'birth' of genetics in 1900. The work done with the fruit fly, *Drosophila melanogaster* during the early part of the twentieth century laid the infrastructure for genetic studies in mosquitoes (Rai, 1999). *Culex pipiens* L., was the first mosquito on which genetical work was carried out (Laven, 1967). The development of genetic studies on *Anopheline* mosquitoes have been closely linked to their medical importance (Coluzzi and Kitzmiller, 1975).

The founding of the formal genetics of mosquitoes was based on isolation, characterization and genetic mapping of morphological mutants of the yellow-fever mosquito, *Aedes aegypti* (Craig and VandeHey, 1962; VandeHey and Craig, 1962). Thereafter, considerable number of morphological variants has been recorded including in *Anopheline* mosquitoes. More than 60 variants, occurring in different species were listed by Kitzmiller and Mason (1967) and around 50 variants in *An. stephensi* by Aslamkhan et al., (1972). Shetty (1974, 1989) and Shetty and Chowdaiah (1972; 1975a,b; 1976; 1977) have listed 46 genetical and morphological variations, 100 gynandromorphs, 150 intersexes and 2 mosaics from six strains of *Culex fatigans* Weid.

Some of the earliest larval morphological mutants studied in *An. stephensi* includes autosomal recessive green (Suguna, 1981; Subbarao and Adak, 1981; Gayathri and Shetty, 1993 and Shetty et al., 1995), autosomal codominant stripe (Sakai et al., 1974), autosomal greenish brown (Sharma et al., 1979), diamond palpus (Sakai et al., 1981), black (Suguna, 1981a), black scale (Rathor et al., 1984), golden yellow (Adak et al., 1990), yellow (Shetty et al., 1994), brown (Shetty et al., 1995), greyish brown (Madhyastha and Shetty, 1999), greyish black (Shetty et al., 2007a), dark larva (Hariprasad and Shetty, 2007) and greenish black (Shetty and Ghosh, 2005).
Apart from morphological mutants, a few eye colour mutants have also been reported in *An. stephensi* which includes sex-linked recessive white eye (Aslamkhan, 1973a,b; Sharma *et al*., 1977; Shetty *et al*., 1994); sex-linked red eye (Sharma *et al*., 1979); sex-linked rosy eye (Aslamkhan and Gul, 1979); autosomal maroon eye (Mahmood and Sakai, 1982); sex-linked chestnut eye (Rathor *et al*., 1983); scarlet, pigment less and red spotted mutants (Parvez *et al*., 1985); cremish white eye (Adak *et al*., 1999); autosomal ruby eye (Madhyastha and Shetty, 2002) and autosomal sienna eye (Hariprasad and Shetty, 2009).

Allelic studies have been studied in *An. stephensi*, between green larvae and brown larvae (Shetty *et al*., 1995), grey larvae and greenish black larvae (Shetty and Ghosh, 2005). Hariprasad & Shetty (2010) have shown that the larval body colour mutants namely dark, grey, greenish black in *An. stephensi* belongs to an allelic series. Allelic studies were also reported in a few other species of mosquitoes. Shetty and Chowdaiah (1976) carried out allelic test in *Cx. quinquefasciatus* Say, among golden yellow, greyish brown, green and brown larva. Allelic studies have been also reported in *An. quadrimaculatus* (Mitchell and Seawright, 1984a,b), *An. gambiae* (Kitzmiller and Mason, 1967; Cooper *et al*., 1983), *Cx. pipiens* (Huff, 1929; Laven, 1957) and in *Ae. aegypti* (Craig and Gillham, 1959).

One important aspect of formal genetics is the correlation of genetic loci with the known chromosomal elements. There are three pairs of chromosomes in *Anophelines*, therefore three groups of linked genes may be expected (Kitzmiller and Manson, 1967). The enzyme markers, morphological mutants, inversion polymorphisms, insecticide resistant genes have been used to construct linkage maps in mosquitoes by several workers (DiDeco *et al*., 1978; Akthar *et al*., 1982; Sakai *et al*., 1983). Linkage studies involving genes for sex-determination, stripe and dieldrin-resistance was reported by French and Kitzmiller (1964) in *An. quadrimaculatus*. Madhyastha and Shetty (2002) have shown that the genes for ruby eye colour mutant and greyish brown larval body colour are on separate chromosomes in *An. stephensi*. Linkage studies in *An. stephensi* between sienna eye colour gene and greyish brown larval body colour gene have been reported by Hariprasad.
and Shetty (2009). Induced chromosomal aberrations were found to be associated with detectable genetic and cytogenetic alterations which could be used to assign linkage groups to their respective chromosomes (Rabbani and Seawright, 1976; Sakai et. al., 1983).

Insecticide resistance is an important man-made example of natural selection, and the factors governing the origin and spread of resistance-associated mutations are both of academic and of applied importance (french-Constant et. al., 2004). In 1947, first cases of DDT resistance were reported in *Aedes tritaeniorhynchus* and *Aedes sollicitans* (Brown, 1986). The finding in 1951 in Greece that DDT was no longer effective against *Anopheles sacharovi* marked a turning-point in the history of vector control (Zulueta, 1959). This was the first case of insecticide resistance in an *Anopheleline* mosquito (Zulueta, 1959). In *An. stephensi* the first evidence of insecticide resistance was reported in 1955 from eastern coast of Saudi Arabia (WHO, 2002).

In India, Rajagopalan et. al., (1956) first reported larval resistance to DDT in Erode town of Madras state. Since then, more than 100 mosquito species are reported as resistant to various class of insecticides. In Anophelines, altogether 51 species have been reported to be resistant to one or more insecticides: 34 are resistant to DDT; 47 to dieldrin and 30 to both DDT and dieldrin. Organophosphate resistance has been recorded in 10 species and resistance to carbamate in 4 species (WHO, 1992).

As organophosphates were introduced to replace organochlorine insecticides, cases of resistance to these insecticides also appeared, particularly in species of *Culex* and *Aedes* (Georghiou, 1965a,b). Resistance to an organophosphate insecticide temephos was reported in agricultural zones of Spain by Grandes and Sagrado (1988), in the Dominican Republic by Mekuria et. al., (1991) and also in India (Baruah, 2004). Development of temephos resistance in Indian strains of *An. stephensi* was described by Chitra and Pillai (1984). Shetty et. al. (2006a; 2006b; 2007b; 2007c; 2008; 2011) has described resistance status of *An. stephensi* and *Ae. aegypti* collected from different areas of Bangalore to various insecticides namely
DDT (organochlorine); carbofuran (carbamate); fenthion, temephos, malathion (organophosphate); alphamethrin, bifenthrin (pyrethroids) and neem (botanical insecticide). Tikar et al. (2011) have reported An. stephensi from various locations of Arid and Semi-Arid zone of India, resistant to chlorpyrifos and fenthion. Alou (2010) reported carbamate resistance in An. gambiae from West Africa. Propoxur resistance in Thailand was demonstrated by Jirakanjanakit et al. (2007) in two species of Aedes. Vinay et al. (unpublished data) have reported increased propoxur resistance in An. stephensi collected from different areas of Bangalore.

Genetic analysis showed that insecticide resistance in the organisms often resulted from gene mutations (Georgiou, 1969; Hemingway, 1982). The genetic bases of insecticide resistance to various insecticides have been studied in eight Anopheline and two Culicine species (Davidson and Manson, 1963). These include DDT resistance (Halliday and Georgiou, 1985), organophosphate and carbamate resistance in Cx. quinquefasciatus Say (Hemingway, 1982; Shetty, 1987a).

In An. stephensi, genetic basis of several insecticides resistance has been studied for malathion, fenthion and methyl parathion (Rao and Shetty, 1994); deltamethrin (Rajashree and Shetty, 1998a); fenitrothion (Ghosh and Shetty, 1999); cypermethrin (Priyalakshmi and Shetty, 2000); DDT (Chandrakala and Shetty, 2004); chlorpyrifos (Chandrakala and Shetty, 2006a); cyfluthrin (Chandrakala and Shetty, 2006b); Bifenthrin (Zin et al., 2009b). In Ae. aegypti, Myin et al. (2009) has described the inheritance mode of alphamethrin, a synthetic pyrethroid. Further, genetics of carbofuran, neem and alphamethrin resistance have been established in An. stephensi from Centre for Applied Genetics Laboratory (Shetty et al., unpublished data).

The pioneer work of Frizzi in Italy, working with the salivary chromosomes of the European maculipennis complex, opened up the field for studies in cytogenetics of mosquitoes (Kitsmiller, 1963). Rishiieos (1959) reported polytene chromosomes from the salivary gland cells of An. stephensi. Later, Sharma et al. (1969) prepared a standard polytene chromosome map from
the larval salivary gland cells. Coluzzi et. al. (1970) and Gayathri and Shetty (1989) have reported photomap of the polytene chromosome from the ovarian nurse cells of *An. stephensi*. Several authors have compared the polytene chromosomes of salivary gland and ovarian nurse cells (Redfern, 1981; Gayathri and Shetty, 1989). Sharakhova et. al. (2006) developed a new cytogenetic photomap for *An. stephensi*, to facilitate physical genome mapping.

Polytene chromosomes of *Anopheles* mosquitoes are the favorable material for study of inversion polymorphism. Chromosomal polymorphisms (especially inversions and translocations) have been recognized as a major driving force in local adaptation, speciation processes, and evolution of sex chromosomes (King 1993; Noor et. al. 2001; Rieseberg 2001; Hoffmann et. al. 2004; van Doorn and Kirkpatrick 2007). Earlier studies by Italian workers, led by D’Alessandro, Frizzi and Mariani revealed possible correlation of inversions with insecticide resistance (Kitzmiller, 1963). Holstein (1957) indicated that the inversion frequencies were positively correlated with increased insecticidal selection pressure in *An. gambiae*. Ghosh and Shetty (2004) have demonstrated the presence of specific heterozygous inversion(s) in *An. stephensi* resistant to fenitrothion. Such specific heterozygous inversions were also reported in *An. stephensi* resistant to alphamethrin, a synthetic pyrethroid insecticide (Hariprasad and Shetty, *unpublished data*).

Radiation induced chromosomal translocation and inherited semi-sterility has been extensively studied in mosquitoes for the vector control (Sharma et. al., 1978; Heemert et. al., 1983; Shetty, 1983, 1987a, 1993; Gayathri and Shetty, 1992a; Madhyastha and Shetty, 2005; Shetty and Ghosh, 2006). The mating competitiveness of the translocated lines of *An. stephensi* has been studied for the sterile male release method (Shetty and Gayathri, 1989).

The relationship between the resistance alleles and the detoxifying enzymes themselves was first clarified by Oppenooth and Van Asperen (1960). The malathion resistance allele in *Culex tarsalis* was found by Matoumura and Brown (1961). Insecticide-resistance thus basically involves gene-altered enzymes (Oppenooth, 1965). It has been reported that selection by toxic

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substances can increase the amount of enzymes that are responsible for
detoxification (Ferrari and Georgiou, 1990). Common insecticide resistance
mechanism in insect pests were reported elsewhere, including three possible
pyrethroids resistance mechanism, namely mixed-function oxidases (MFOs),
elevated esterases and reduced sensitivity of sodium channels (Georgiou,
1986; Roberts and Andre, 1994; Nelson et. al., 1996; Feyereisen, 1999).

The electrophoretic studies play a pivotal role in understanding the enzymes
associated with insecticide resistance in mosquitoes. Narang and Narang
(1975) have emphasized the importance of using electrophoretic techniques
for isolating badly needed genetic markers in mosquito populations, as well
as assessing cryptic variability in natural populations. Several investigations
have employed electrophoretic techniques for genetic analysis and
characterization of mosquitoes, especially species of medical importance
(Saul et. al., 1976; Kreutzer, 1979; Steiner and Joselyn, 1979; Mathew and
Craig, 1980; Shetty, 1987b).

Esterases are among the first enzymes studied electrophoretically in
Drosophila. Rao and Shetty (1996) and Rajashree and Shetty (1998b) have
reported the variation of proteins and esterases between the susceptible and
resistant strains of An. stephensi. In recent years there have been many
reports of electrophoretic studies of esterases (EST) isozymes in mosquitoes.
Townson (1971) described the genetics of certain EST isozymes in Aedes
aegypti and Saul et al. (1976) discussed EST variation in strains of the same
species. Gargan and Barr (1977) studied the linkage relationships of two
EST loci in Cx. pipiens. In a study on Cx. tritaniormhynchus, Iqbal et. al. (1973)
reported genetic analysis of multiple EST loci in natural populations of
Anopheles punctipennis. Green (1977) reported a sex-limited EST in
Anopheles funestus, and Miles (1978) reported a survey of enzyme variability
in members of the An. gambiae group.

In insects, enzymes such as acid phosphatase, alkaline phosphatase and
xanthine dehydrogenase have been studied in respect to the insecticide
resistance (Beckman and Johnson, 1964; Harper and Armstrong, 1972;

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Benedict et al., 1996). Studies on amplification of several enzymes including alpha esterases, beta esterases, acetylcholine esterases, aldehyde oxidase, xanthine dehydrogenase, monoxygenase, glutathione S-transferases in resistant population is reported in several mosquito species (Lambremont, 1959; Mouches et al., 1990; Hughes et al., 1992; Vaughan and Hemingway, 1995; Malcolm et al., 1998; Hemingway and Karunaratne, 1998; Edurdo et al., 1999; Peiris and Hemingway, 1990; Roger and Christine, 1999; Enayati et al., 2003). Clear evidence has been obtained for an acetylcholinesterase insensitive by inhibition by paraoxon and propoxur in An. albimanus (Ayad and Georgiou, 1975); temephos in Cx. pipiens (Raymond and Marquine, 1994).

Insecticidal effects on insects can generally be either direct toxic effects which cause mortality and/or sublethal effects (Lee, 2000). A number of physiological characteristics have been observed on various occasions in insecticide and susceptible strains of insects (Arnaud et al., 2002). These concerns alterations in reproductive potential, vigor or duration of life stages. The efficacy of deltamethrin against the life stages of Ae. aegypti, as one of the most potent insecticides has been well documented (Sahagal and Pillai, 1993; Kumar et al., 2002). Earlier, Abedi (1960) had reported suppressed fecundity, egg hatch, or other qualities associated with fitness in Ae. aegypti selected by DDT. Reyes-Villanueva et al. (1992) has documented the sublethal effects of various concentration of abate (temephos) on life parameters namely, pupae weight, adult weight, ingested blood weight and longevity. Studies have shown that sub-lethal exposure of insecticides may compromise the target insect’s fitness, its ability to reproduce or affect its longevity (Reyes-Villanueva et al., 1990; 1992; Robert and Olson, 1989; Vasuki, 1999; Silva et al., 2004). Effect of sublethal concentrations of DDT, Abate (temephos) and Sevin on biological parameters have also been reported in Culex sps. (Sadek et al., 1974).

In An. stephensi, effects of sublethal exposure to various insecticides namely fenithion, methyl parathion, malathion, fonitrothion, deltamethrin, cypermethrin, bifenthrin, neem and alphamethrin on reproductive fitness
have been reported (Hemmingway, 1982; Priyalakshmi et al., 1999; Rao and Shetty, 1992; Zin and Shetty, 2008).

Roberts et al. (1974) studied circadian rhythms in Ae. aegypti sensitivity to toxicants. Circadian changes of insecticide sensitivity were also reported in both larvae and adults of three strains of above said species (Bainbridge, 1983). Eclosion rhythm pattern have been reported in Ae. taeniorhynchus and An. gambiae (Nayar, 1967a; 1967b; Reiter and Jones, 2009). Reports on circadian rhythms in An. stephensi are scantily available.

Earlier, Knipling (1955) suggested the possibilities of insect control or eradication of natural population of screw-worm flies through the use of Sterile Insect Technique (SIT). The SIT technique has resulted in the eradication of tsetse fly, Melon fly etc. (Reichard, 2002; Koyama et al., 2004). Whitten and Foster (1975) elaborated the pest control through genetic manipulation. Large number of chromosomal translocation have been induced and isolated in An. fluviatilis (Shetty, 1980; 1983), An. stephensi (Gayathri and Shetty, 1992a) and Cx. p. quinquefasciatus (Shetty, 1993). The mating competitiveness of the translocated lines has been studied for the sterile male release method and was found to be more competitive than the normal males in the laboratory conditions (Shetty, 1984; Shetty and Gayathri, 1989).

Genetic sexing mechanism utilizing conditional lethal (eg. Insecticide resistant gene) and radiation induced translocations have been carried out in An. arabiensis (Curtis, 1978); In An. gambiae, the dieldrin resistant gene was translocated to the Y-chromosome via radiation induced translocation (Curtis et al., 1976). Similarly, propoxur resistant gene was used for genetic sexing in An. albimanus (Seawright et al., 1978); In An. culicifacies, dieldrin resistant gene translocated to Y-chromosome was demonstrated by Baker et al. (1981); malathion resistant gene was used for sexing system in Cx. tarsalis (Mac Donald and Asman, 1982). In Cx. quinquefasciatus, sexing system using malathion resistant gene as conditional lethal was synthesized for preferential elimination of females during early larval stages (Shetty, 1987a).