CHAPTER 2: REVIEW OF LITERATURE

Mosquitoes, family Culicidae, belong to the order Diptera, the two-winged flies. The family is a large and abundant group which occurs throughout temperate and tropical regions of the world, and well beyond the Arctic Circle. On the basis of the fossil record, it is generally accepted that mosquitoes had evolved by the Jurassic, approximately 210 million years ago (Edwards 1932). This is around the time continental drift began (Wilson 1963), leading to fragmentation and geographic isolation, presumably promoting rapid speciation. The Dipteran family Culicidae, subdivided into three subfamilies, Anophelinae, Culicinae and Toxorhynchitini (Service 1993). Recently the family of Culicidae (Diptera) divided in two subfamily Anophelinae and Culicinae, 11 tribes, 113 genera, and 3526 species in the world fauna. The subfamily Anophelinae has three genera and Culicinae has 108 genera segregated into 11 tribes (Harbach 2007). Recently 3597 species in the world and out of which in India 356 species have been reported (WRBSC, 2013).

2.1 Taxonomy and Classification:

Several authors are working on the taxonomic history and classification of mosquitoes. The discovery at the turn of the nineteenth century that mosquitoes transmit malaria and yellow fever initiated an outburst of interest in the description and classification of these insects. The British Museum (Natural History) employed Fred. V. Theobald in 1899, and as a consequence many new generic names were
introduced in an effort to classify numerous new species into seemingly natural groups. It eventually became apparent that Theobald’s system of classification was neither practical nor natural. Consequently, during the two decades following the publication of Theobald’s Monograph of the Culicidae in 1910, significant changes were made toward a much more conservative system of classification. Particularly noteworthy were the efforts of F.W. Edwards in Europe and Harrison G. Dyar in North America, whose work contributed most significantly to the acceptance of the broad genus-group concepts (Edwards, 1932). That provided the framework on which the “traditional” classification (Stone et al., 1959; Belkin, 1962; Knight & Stone, 1977) of the twentieth century was built.

According to the Edwards (1932), he recognized three tribes within Culicinæ, i.e. Anophelinæ, Toxorhynchitini (as Megarhinini) and Culicini, and divided the last tribe into five groups, i.e. Sabethes, Uranotaenia, Theobaldia, Aedes and Culex. In 1957, Stone removed Dixidae and Chaoboridae from Culicidae and restricted family Culicidae to the Culicinæ of Edwards (1932). This brought about changes in subfamily and tribal designations that were adopted by Stone et al. (1959), in their world catalogue of mosquitoes. This classification recognized subfamilies Anophelinae, Culicinae and Toxorhynchitinae, and two tribes within Culicinae, the Culicini and Sabethini. Belkin (1962), disagreed with this change and retained Edwards’ subfamily structure, but reorganized the classification of Culicinae (“true mosquitoes”) to include 12 tribes instead of three. He retained Anophelini and Toxorhynchitini and recognized ten tribes in place of Edwards’ Culicini. At least some authors (e.g. Belkin et al., 1970) continued to treat Dixids and Chaoborids as subfamilies of Culicidae until Knight and Stone (1977) once again excluded them from the family. This action resulted in the recognition of three subfamilies, i.e. Anophelinae, Culicinae and Toxorhynchitinae, and the division of subfamily Culicinae into the 10 tribes established by Belkin (1962). Mattingly (1969, 1971, 1981), however, was unwilling to accept the division of Culicinae into 10 tribes and consequently followed Stone et al. (1959) in recognizing only two, i.e Culicini and Sabethini. Now recently (Harbach 2007) the family of Culicidae (Diptera) divided in two subfamily, 11 tribes, 113 genera, and 3526 species in the world fauna. The
subfamily Anophelinae has three genera and Culicinae has 108 genera segregated into 11 tribes (Harbach 2007).

In India several authors have been published the identification keys for anopheline mosquitoes by which the most important are those by Christophers (1933) and Puri (1954, adults; 1960, larvae and Nagpal and Sharma 1994). Christophers’ work is providing detailed description of each species, and it is valuable reference work for taxonomists on Anophelines. He described 43 species and 10 varietal forms, of which 40 species and 9 variations are restricted to present-day India. The key by Puri is developed in the form schematic table for rapid identification of the adult Anophelines and has been widely used by taxonomist in India but also in some of the neighbouring countries. Puri also described 43 species and 9 varietal forms, of which 40 species are restricted to present India. Wattal & Kalra (1961), published pictorial keys for different regions of India but these keys included only 32 species of female Anophelines. In 1974, Ramchandra Rao T. published a book on “The Anophelines of India”, after that Bina Pani Das, R. Rajagopal and J. Akiyama in (1990), provides a key for 54 species of Anophelines found in India. In 1994, Nagpal B. N. and Sharma V. P. published pictorial key cum book on Indian Anophelines.

Identification of Culicine can be difficult for study. The publication of Barraud’s (1934) volume in the Fauna of British India series, two important monographs has been published: a revision of genus Culex in Thailand by Bram (1967) and a revision of the subgenus Culex in the Oriental Region by Sirivanakarn (1976). These are not widely available and are perhaps too detailed for the field entomologist. In the paper of R. Reuben, S. C. Tewari, J. Hiriyan and J. Akiyama (1994), they provide simple illustrated keys to the genera of mosquitoes, the subgenera of Culex, and the common Southeast Asian species of Culex S.S. Using this can help to distinguish vector species from the many Culicines which resemble Culex S.S., notably species of the subgenera Lutzia Theobald, Culiciomyia Theobald, and Lophoceraomyia Theobald. However, this key is restricted to 16 common species, mostly of known or potential importance in the natural cycle of JE (Carey et al. 1968, Sirivanakam 1976, Amerasinghe et al. 1988). This key should be alert to the
occasional aberrant specimen that may be a species not included here and should refer to Sirivanakam’s (1976) key for correct identification.

The Aedini is the largest tribe in family Culicidae with approximately 1,240 currently recognized species. The traditional classification of Aedini is based on the concept of recognizing few genera and numerous subgenera (Edwards, 1932; Belkin, 1962). Belkin (1962), viewed the tribe as a natural group but noted that some members showed affinities with all other higher-level taxa of subfamily Culicinae. Belkin (1962) believed that Aedini differentiated in the Indomalayan area of the Old World where the majority of ancestral forms presently exist. The traditional classification of Aedini before the end of the twentieth century included nine genera and 50 subgenera (Knight & Stone, 1977; Knight, 1978; Ward, 1984, 1992).

Sixteen species are recorded under 2 subgenera of *Armigeres*: *Armigeres* (6) and *Leicesteria* (10) from the Indian province. Species resurrected from synonymy under subgenus *Armigeres* are: *subalbatus* (*Coquillett*) and *joloensis* (*Ludlow*) [from *obturbans* (*Walker*)] (Edwards in Barraud 1934 and Stone and Thurman 1958, respectively), Durhami Edwards (from kuchingensis Edwards) [Thunnan, 1958 (1959)]. New records from India are: *joloensis* (*Armigeres*) (Bhattacharya. 2000) and *dolichocephalus* (*Leicester*) (*Leicesteria*) (Rajput and Singh, 1987). In Maharashtra Ramachandra Rao and Rajagopalan worked on same aspects in 1957 from Pune district.

### 2.2 Mosquito Distribution and Diversity study:

The first anopheline survey was done by Challam (1923), from Kamrup district of Assam. Later the surveys were carried out by Christophers (1925), Strickland (1929), Ramsay (1930), Macdonald and Chowdhury (1931), Gupta and Mazumdar (1932), Rice and Savage (1932), Rice and Mohan (1936), P. Sen (1938) survey on *Anopheles sundaicus* in and around Calcutta, Viswanathan et al. (1941), Sarkar et al. (1981) in Assam. In other states the surveys were carried out by Mortimer 1946) in Manipur; Shortt (1924) and Rajagopal (1976) in Meghalaya; Sarkar et al. (1980) in Nagaland; Misra and Dhar (1955) in Tripura; Misra (1956) and Sen et al. (1973) in Arunachal Pradesh. In recent years surveys have been carried out by Malhotra et al. in Nagaland (1982), Manipur (1983) and Mizoram (1982; 1984). The number of anopheline species collected by these authors ranged from 6 to 19 in Assam; 7 to 13 in Manipur; 8 to 13 in Meghalaya; 8 to 10 in Nagaland; 11 in Tripura state; 6 to 13 in Arunachal Pradesh and 14 in Mizoram. Dash A. P., Behura D. K., Roy J. R. (1984), worked on the distribution of Anopheline mosquitoes in Orissa, India. Prakash A, Bhattacharyya D. R, Mohapatra P. K, Mahanta J. (1998) studies on investigation on malaria vectors and mosquito fauna in South Tripura District, Tripura State and same author studied in year 2000 mosquito fauna and malaria vectors in Jairampur, district Changlang, Arunachal Pradesh, India. M. K. Das, B. N. Nagpal et al. (2003), studies on bio-ecology of *An. phillippinensis* in Andaman group of Islands. I Baruah, N. G. Das and S. C. Das (2004), a study carried out on Anopheline fauna and prevalence of malaria incidence in Dimapur, Nagaland. V. Joshi et al. (2005), studies on malaria vector in Thar desert, Rajasthan, India. N. Pemola Devi

The same authors are worked on *Aedes* such as T. Ramchandra Rao (1967), studied on distribution, density and seasonal prevalence of *Aedes aegypti* in the Indian subcontinent and Southeast Asia. A. P. Pandya (1979), studies on *Aedes aegypti* and *Aedes vittatus* in Surat city, Gujrat. Survey of mosquito fauna of Northeastern region of India in 1986 carried out by B. N. Nagpal and V. P. Sharma. Mosquito collections were made from 22 localities of Assam, Meghalaya, Arunachal Pradesh and Mizoram, were observed into 61 species of 8 genera viz. *Anopheles, Aedes, Armigeres, Coquillettidia, Culex, Malaya, Mansonia* and *Toxorhynchite*. S. C. Tewari and J. Hiriyan in 1991 described new species of *Aedes portonovoensis* from south India. In 1992, S. C. Tewari and J. Hiriyan described another two new species of *Aedes reubenae* and *Aedes agastya*. Raminder Kaur in 1992, work on mosquito types present in the Delhi and enlist the 65 species of the 8 tribe *Aedes, Culex, Armigeres, Heizmannia, orthopodomyia, Tripteroides, Uranotaenia* and *Toxorhynchites*. S. C. Tewari and J. Hiriyan (1995) *Aedes niveus* described and illustrated in detail from Andman and Nicobar Island, India. Rakesh Katyal, Kaushal Kumar and Kuldip Singh Gill (1997), a survey was undertaken to ascertain the *Aedes aegypti* indices in rural areas of Faridabad and Gurgaon district of Hariyana State, India. Shriram A. N. and Sehgai S. C. (1999), worked on distribution and larval ecology of *Aedes aegypti* in Port Blair, Andaman and Nicobar Islands of India. S. K. Sharma and K. K. Hamzakoya (2001), worked on geographical spread of *Anopheles stephensi* and *Aedes aegypti* in the Arabian sea islands of Lakshadweep, India. Yadurappa Satishkumar et al. (2004), survey was carried out In Rajiv Gandhi national park in Karnataka state, India. A total 60 species were reported belonging to 10 genera. P. V. M. Mahadev, P. V. Fulmali and A. C. Mishra (2004), studied on geographic distribution and prevalence of *Aedes aegypti* in the Goa, India. R. S. Sharma, S. M. Kaul and Jotna Sokhay (2005), studies on
the seasonal fluctuation of dengue fever vector, *Aedes aegypti* in Delhi, India. P. Dutta and J. Mahanta (2006), survey conducted in seven states in north-eastern region of India revealed that the region is very rich in mosquito fauna, especially *Aedes* and related species. Mihir K. Pramanik, Gautam Aditya and Srimanta K. Raut (2007), a survey of three year was carried out on the prevalence of immature stages of the vector mosquito *Aedes aegypti* in the city of Kolkata, India. Bennet Angel and Vinod Joshi (2008), worked on distribution and seasonality of vertically transmitted dengue viruses in Aedes mosquitoes in arid and semi-arid areas of Rajasthan, India and 2009 same author worked on distribution of dengue virus types in *Aedes aegypti* in dengue endemic districts of Rajasthan, India.

Jagdish Kaur and Jagbir S. Kirti (2003), work on a total number of mosquito 21 species referable to five genera *Anopheles, Aedes, Culex, Armigers* and *Mansonina* have been reported from Haryana state from India. N. Pemola Devi and R. K. Jauhari (2004) studied on altitude distribution of mosquitoes in mountainous area of Garhwal region Part -1 and reported 34 species in five genera *Anopheles, Aedes, Culex, Armigers* and *Uranotaenia*. N. Pemola Devi and R. K. Jauhari (2005), study on habitat biodiversity of mosquito in Garhwal, Uttaranchal, India and 45 species of mosquito under 3 different genera. Gautam Aditya, Mihir K. Pramanik and Goutam K. Saha (2006), carried out survey on larval habitats and species composition of mosquitoes in Darjeeling Himalayas, India and reported 6 mosquito species belonging to four genera *Anopheles, Aedes, Culex, Armigers* and *Toxorhynchites* were noted with significant difference in temporal variation in their relative and absolute numbers. N. Pemola Devi and R. K. Jauhari (2007), reported 34 species of mosquito of 5 genera and studied on mosquito species associated with in some western Himalayas phytogeographic zones in the Garhwal region of India.

In Maharashtra state very little work done by researchers on mosquito. In 1934, the monograph of Barraud P. J. “The Fauna of British India Including Ceylon and Burma” in which mentioned that the work was carried out in Poona (recently known as Pune), Maharashtra state. Geevarghese G., H. N Kaul and V. Dhanda (1975), studied on observations on the reestablishment of *Aedes aegypti* population in Poona (Pune) city and suburban’s, Maharashtra State, India. Geevarghese G., V. Dhanda, P. N. Ranga Rao and R. B. Deobhankar (1977) field trials for the control of *Aedes aegypti*
with abate in Poona city and suburban’s. Kulkarni S. M., K. Banerjee, P. K. Deshmukh, M. A. Ilkal, N. C Venkateshar (1977), studied on dengue epidemic in Amalner town, Jalgaon district, Maharashtra, India. Shetty P. S. and G. Geervaghese (1977), survey carried out on tree-hole breeding of Aedes aegypti in Poona city-a short note. Mahadev P. V. M, V. Dhanda, P. S. Shetty (1978), studied on distribution and larval habitat of Aedes aegypti (L.) in Maharashtra State. Prasad Rao, C. G. Khasnis, F. M. Rodrigue, N.P. Gupta., C. B. Shah., P. Y. Guru and B. D. Pinto (1981), worked on investigation of the dengue epidemic in Amalner town of Maharashtra. Panicker K. N., M. Geethabai et al. (1982), studied on well breeding behavior of Aedes aegypti in Poona city. Mahadev P.V. M. et al. (1983), worked on a case study of Aedes aegypti prevalence by settlement type in Dehu town group of Maharashtra state. H. N. Kaul and M. D. Gokhale (1984), carried out survey on Aedes aegypti mosquito in Dehu town group Pune district, Maharashtra. T. V. Sathe and B. E. Girhe (2001), study on biodiversity of mosquitoes in Kolhapur district, Maharashtra, India. During the study period nine species of mosquitoes are resulted belonging to the genera Anopheles (3), Culex (3) and Aedes (3) have been reported. In addition three unknown species have also been recorded from the district. B. E. Girhe & T, V. Sathe (2001), a new species, Aedes sangiti sp. (Diptera: Culicidae) have been described for the first time from Maharashtra, India. Laxmikant V. Shinde and Hema Makne (2011), studied on density of urban malaria vector An. stephensi in Parbhani, (M.S.) India. Laxmikant Shinde, Kisan Thete et al., (2011), studied vector mosquito diversity in association with environmental factors from Parbhani, (M.S.) India. Their findings are mosquito distribution is very sensitive to environmental factors. Results show that maximum population and density of Anopheles, moderate of Culex and very less population of Aedes spp. E. L. Jaid, S. V. Nikam, B.V. More and J. C. Bhandari (2011), survey on diversity of mosquitoes in Jalna urban, Maharashtra state, India. But their results are not satisfactory because no any species of Mansonia (Genus) are confirmed after molecular study in Jalna district. R. K. Singh et al. (2012), study on susceptibility of malaria vectors to insecticides in Gadchiroli district (Maharashtra), India. Kisan Thete and Laxmikant Shinde (2012), reported survey of container breeding mosquito larvae in Jalna city (M.S.) India and reported four different species Aedes aegypti, Aedes albopictus, Culex quinquefasciatus and Culex vishnui from study area. Laxmikant
Shinde and Kisan Thete, reported first time record of *Aedes scatophagoides* (*Theobald, 1934*) from Latur (M.S.); deccan plateau of India during August 2010.

### 2.3 Climate and Mosquitoes:

Climate has been established as an important determinant in the distribution of vectors and pathogens. In fact, the physical environment is an important modifier of local climate. Climate variability and the breeding activity of *Anopheles* are considered one of the important environmental contributors to malaria transmission. There is a paucity of literature on climatic variables and malaria transmission from India (Akhtar R. et al. 1996 and Singh N, Sharma V. P. 2002). Gupta R. 1996, reported the correlation of rainfall with upsurge of malaria in Rajasthan. Martin Beniston (2002) wrote review article on climate change, possible impact on human health. Bhattacharya S., Sharma C., Dhiman R. C., Mitra A. P. (2006), work on climate change and malaria in India. William K. Reisen et al. 2007, climate variation affects all of these processes at multiple scales. Like patterns in the occurrence of vector-borne disease outbreaks (Gubler et al. 2001). It is unequivocal that climate change is happening and is likely to expand the geographical distribution of several vector-borne diseases, including malaria and dengue etc. to higher altitudes and latitudes. India is endemic for six major vector-borne diseases (VBD) namely malaria, dengue, chikungunya, filariasis, Japanese encephalitis and visceral leishmaniasis. Impact of climate change on dengue also reveals increase in transmission with 2°C rises in temperature in northern India. Reemergence of kala-azar in northern parts of India and reappearance of chikungunya mainly in southern states of India has also been discussed (Ramesh C. Dhiman et al. 2010).

### 2.4 Molecular Systematic study:

The study of biodiversity is an important aspect to understand the intricate evolutionary trends of life. Linnaean classification of animals and plants remains as an important step made toward this goal, and with that commenced the systematic based on morphological characteristics, which is being followed for more than two centuries. However, only ≈10% of extant species on earth (10-15 million) is known to science so far (Besansky et al. 2003, Pennisi 2003).
Molecular systematic is one of the best tools for the correct identification of animals. The researchers are developed different techniques in molecular study. The presence of good quality polytene chromosomes in the species belonging to order Diptera, proved to be one of the most productive tools towards meeting the requirements of karyosystematics. First upon Sorsa (1969), Lefevre (1976) and Saura and Sorsa (1979) gave detailed description of their structure and behavior by studying these chromosomes in insects like *Drosophila*, *Sciara*, *Rhyncosciara* and *Chironomus*. The normal banding patterns of different species and sibling species were compared by putting their maps together so as to know the extent of genetic relationships among them. To this technique, induced hybridization and the study of chromosomal complements of the hybrids were also added (Kreutzer and Kitzmiller, 1972; Hartberg, 1972; Subbarao *et al*., 1988). Taylor *et al*., (1993), worked on polytene chromosome for estimates of effective population size of *An. arbiensis*. Toure *et al*., (1998), worked on polytene chromosome variation, chromosomal forms of *An. gambiae* complex. Surendra *et al*., (2000), studied of Y-chromosome differentiation of sympatric species of *An. culicifacies* complex. Kamau *et al*., (2002), work carried out on population structure of *An. funestus* using polytene chromosomes. For a number of years the polytene chromosome examination remained only method in the identification of species with confusing morphotaxonomic characters.

In spite of the polytene chromosome maps have become indispensable as standard references for locating genes, puffs, and inversion breakpoints of unique DNA sequences useful in the molecular taxonomy of mosquitoes. It is for this reason Chaudhry *et al*., (2005), presented a line map and a photomap of the salivary polytene chromosomes of *Anopheles (Cellia) subpictus* Grassi, an important emerging vector of malaria in India. The need of accurate identification is crucial as time and money may be wasted in studying and controlling species of no medical importance (Beebe and Cooper, 2000). The correct identification of species involved in disease transmission is vital for development of public health strategies and assessment of effectiveness implemented strategies (e.g., vaccination or vector control efforts). Primary
disease vectors may shift in response to variation in local environments including changes driven by human activity. Majority of the malaria vectors in the genus *Anopheles* exist in the form of species complexes whose members are morphologically similar but reproductively isolated. As a case study Subbarao and Sharma (1997) concentrated on the Indian malaria vector *Anopheles culicifacies* Giles sensu lato (Diptera: Culicidae).

In the recent years with the introduction of the multiple technique system of classification the role of different parameters of study have become indispensable for the investigations on the insects of public health importance. The most recent addition to these areas is the study of nucleotide variations by the application of the technique of *in vitro* amplification of nuclear and mitochondrial DNA through polymerase chain reaction (PCR) protocols (Fritz et al., 1994; Sucharit and Komalamisra, 1997; Foley et al., 1998; Xu et al., 1998; Favia and Louis, 1999; Van Bortel et al., 2000; Linton et al., 2001, 2005; Chaudhry and Neetu, 2004; Marrelli et al., 2005; Chaudhry and Sharma, 2006; Chaudhry and Kohli, 2007; Kohli and Chaudhry, 2007).

When electrophoretic studies were realized out on the isozyme polymorphism, Tomita et al., 1996; Coosemans et al., 1998, worked on the *Anopheles gambiae* complex in a rural village and a city in South-western Burkina Faso. Both the species could be separated on the basis of isozyme like octanol dehydrogenase (*Odh*) and mannose phosphate isomerase (*Mpi*) profiles. Results showed *Plasmodium falciparum* infected *An. gambiae* differed from the non infected ones in their allelic and genotypic frequencies at *Mpi* and acid phosphatase (*Acp*). Some researchers are worked on allozymes for mosquito systematic. Cianchi et al., (1983), worked on Variability and chromosomal forms of *An. gambiae* s.s using allozymes. Adak et al., (1994), studied on differentiation between species groups of *An. culicifacies* complex. Lanzaro et al., (1995), work carried out on variability and comparison to STRs of *An. gambiae* s.s. using allozyme. Sucharit et al., (1988), worked on complex Variability between populations, species differentiation of *An. minimus* using the allozymes. Trindade and Scarpassa, (2002), worked on *An. (Nyssorhynchus)* subgenus for genetic distance between members of the subgenus
Advances in molecular biology techniques have provided the basis for uncovering virtually unlimited numbers of DNA markers. The utility of DNA based markers is generally determined by the technology that is used to reveal DNA-based polymorphism. Currently, the restriction fragment length polymorphism (RFLP) assay has been the choice for many species to measure genetic diversity and construct a genetic linkage map.

Maximum work of RFLP carried out on differentiation of Anopheles complexes. McLain and Collins (1989), restriction mapping and map comparison between species (IGS) An. gambiae s.l. Favia et al. (1997), worked on RFLP-based molecular form-level identification method (IGS) of An. gambiae s. s. Conn et al. (1999), worked on population genetics (mtDNA genome) of An. darling. Kumar (1999), methods for RFLP mapping of An. gambiae s.s. Torre et al. (2001), RFLP (IGS) and cytology comparison of chromosomal form designations della of An. gambiae s.s. Cooper et al. (2002), species distributions as defined by RFLP identifications (ITS2) for An. punctulatus group. O. P. Singh, Geeta Goswami et al. (2004), studied on an allele specific polymerase chain reaction assay (PCR-RFLP) for the differentiation of members of the Anopheles culicifacies complex from India. Geeta Goswami, O. P. Singh et al. (2006), studied in India for identification of all members of the Aanopheles culicifacies complex using PCR-RFLP. A polymerase chain reaction (PCR) assay based on the D3 domain (D3-PCR) of 28S rDNA and a polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) assay involving ITS2 of rDNA are available for the discrimination of the members of the An. culicifacies complex.

An RFLP assay which detects DNA polymorphism through restriction enzyme digestion, coupled with DNA hybridization, is in general, time consuming and laborious. Over the last decade, polymerase chain reaction (PCR) technology has become a widespread research technique and has led to the development of several novel genetic assay based on selective amplification of DNA (Erlich H. A. et al., 1989).

The discovery that PCR with random primers can be used to amplify a set of randomly distributed loci in any genome facilitated the development of genetic markers for a variety of purposes. The success of RAPD analysis is the gain of a large number of genetic markers that require small amounts of DNA without the
requirement for cloning, sequencing or any other form of the molecular characterization of the genome of the species in question (Williams et al. 1990, Welsh J. et al. 1990). The introduction of the technique of polymerase chain reaction (PCR), especially the randomly amplified polymorphic DNA (RAPD-PCR) has actually revolutionized the genomic characterization of species and their populations as it provides more accurate results of genetic relatedness (Neetu and Chaudhry, 2005).

Highly conserved morphology but marked differences in potential vectorial capacity require the development of polymerase chain reaction (PCR) based tests that unambiguously distinguish among the different species. A polymerase chain reaction (PCR)-based method is described for the identification and differentiation of mosquito species and populations. The method, described first by Williams et al. (1990), employs single 10 base-long primers of arbitrary DNA sequence and results in the amplification of random segments of DNA known as random amplified polymorphic DNA (RAPD). RAPD of mosquito DNA results in the amplification of a series of DNA fragments of varying length. Richard C. Wilkerson et al. (1995), studied Random amplified polymorphic DNA polymerase chain reaction (RAPD-PCR) analysis was applied to samples from wide spread populations of the poorly characterized Anopheles (Nyssorhynchus) alhitarsis Lynch-Arribitzaga species complex, and 4 genetically differentiated species were distinguished. S. R. Naddaf Dezfooli et al. (2002), use of RAPD-PCR and ITS2 PCR assays for differentiation of populations and putative sibling species of Anopheles fluviatilis (Diptera: Culicidae) in Iran. Anopheles fluviatilis complex is known to be a vector of malaria in Iran. RAPD-PCR assay was used to differentiate geographic populations of this species. Results of RAPD-PCR and ITS2 analysis suggest that this taxon in Iran comprises of only one species with a low genetic variation among geographic populations.

In spite of the RAPD-PCR technique, the PCR method is rapid, reliable, extremely sensitive and relatively tolerant to degraded DNA from poorly preserved specimens. DNA prepared from as little as a single leg of a mosquito is more than ample for the PCR, allowing the rest of the specimen to be used for other analyses, such as parasite detection. The method is based on the principle of allele-specific PCR
(ASPCR), whereby DNA templates, differing by as little as a single base pair, can be distinguished by the inability of Thermus aquaticus (Taq) DNA polymerase to extend primers that are mismatched to their template DNA. This technique widely used by several workers in the field of mosquito molecular study. Ugozzoli and Wallace (1991), studied on identification of *An. dirus* complex using the DNA polymerase chain reaction (PCR) method.

The molecular technique, ribosomal RNA genes (rDNA) have been used to distinguish between other mosquito species. Scott et al., (1993), worked on the *An. gambiae* complex and Beebe and Saul (1995), the *An. punctulatus* complex. rDNA genes are arranged in tandem and there are hundreds of copies per genome, making them particularly easy to amplify. C. Walton et al. (1999), selected the second internal transcribed spacer, known as ITS2, which separates the 5.8S and 28S rDNA subunits. Although it may be involved in RNA splicing, this sequence has a high rate of evolution (Schlotterer et al., 1994) making it likely to vary between even closely related species. The method utilizes allele-specific amplification to detect fixed differences between the species in the DNA sequence of the ribosomal DNA internal transcribed spacer 2. The method was tested on 179 mosquitoes of the *An. dirus* complex from many parts of Thailand (C. Walton et al., 1999). G. N. Fritz et al. (2004), describe the first multiplex PCR for identifying four species in the subgenus *Nyssorhynchus* that are vectors of human Plasmodium spp. Four species specific primers, together with a universal primer that anneals to the 5.8S rDNA region, produce amplicons of the internal transcribed spacer two (ITS2) with base pair sizes of 131, 308, 371, and 441 for *An. triannulatus, An. trinkae, An. strodei*, and *An. rangeli*, respectively. Anil Prakash et al. (2006), also worked on the molecular characterization and species identification of the *Anopheles dirus* and *Anopheles minimus* complexes in north-east India using r-DNA, ITS2.

The mitochondrial genome is frequently utilized in phylogenetic and population genetic studies. The sequence and genome organisation of the *An. gambiae* mitochondrial genome was published nearly a decade ago (Beard et al., 1993). This information was quickly utilised in field and laboratory based studies where both coding (i.e. NADH dehydrogenase subunit 5 (ND5) and cytochrome Oxidase subunits I and II (COI and COII)) and non-coding (16S and 12S RNAs)
regions are frequent targets for these analyses. Numerous investigations have utilised mitochondrial DNA to derive the phylogenies of *An. gambiae s.l.* and to address questions of genetic structuring among members of this species complex (Caccone et al., 1996; Besansky et al., 1997; Lehmann et al., 1997; Thelwell et al., 2000). Mitochondrial DNA has been similarly utilised in investigations of a wide variety of *Anophelines* and *Anopheline* species complexes. Foley et al. (1998), used the COII gene to derive the phylogeny of the Australasian *Anophelines*. De Merida et al. (1999) also utilized mtDNA (ND5) and SSCP analysis to examine the population structure of *An. albimanus*. Fairley et al. (2000) used a COI gene fragment to look at genetic structuring and gene flow among populations of *Anopheles punctipennis* in Vermont.

The similar molecular techniques were applied on nonanopheline species of mosquitoes. Phool Chand Kanojia et al. (2009), studied on four populations of *Culex tritaeniorhynchus* (Giles) (Diptera: Culicidae), collected from Bellary, Cuddalore, Pune, and the microbial containment complex laboratory culture in India were analyzed for morphological and allozyme variation. Their findings suggested that transition of morphological characters and allozyme variations in *Cx. tritaeniorhynchus* populations seem to be consequences of influence and selection by the environmental conditions.

Dina M. Fonseca et al. (2001), work carried out on genetic diversity using RAPD-PCR assay and sequences of ND4 of mtDNA by comparing samples from populations spanning the range of this mosquito in Japan (six samples) and the United States (nine samples) as well as specimens intercepted in New Zealand.

The ribosomal DNA sequence divergence in the internal transcribed spacer regions (ITS 1 and ITS 2) was examined for the populations of *Aedes simpsoni* complex which includes some of the most important vectors of Yellow fever in Africa showing variation in their anthropophilic or non-anthropophilic tendencies in their host preference for blood meal (Kenge et al., 2003). Bhargavi et al. (2005), work carried out the sequence analysis of ITS 2 region of seven species of the genus *Culex* belonging to five different geographical locations in India. Maria E. Morales et al. (2005), worked on differential identification of *Ascogregarina* species (Apicomplexa: Lecudinidae) in
Aedes aegypti and Aedes albopictus (Diptera: Culicidae) by polymerase chain reaction. They report 2 polymerase chain reactions (PCR)-based methods for distinguishing morphologically similar gregarine species based on amplification of variable regions of the internal transcribed spacer region (ITS) of ribosomal DNA. Lydia A. Hill et al. (2008) used ITS1 for rapid identification of Aedes albopictus, Aedes scutellaris and Aedes aegypti life stages using Real-time polymerase chain reaction assay from Northern Australia. Dhananjeyan K. J. et al. (2010), used internal transcribed spacer (ITS1 and 2) for molecular identification of mosquito vectors using genomic DNA isolated from eggshells, larval and pupal exuvium. The PCR amplification of ITS1 region distinctly differentiated the three species of the Cx. vishnui subgroup viz., Cx. tritaeniorhynchus, Cx. pseudovishnui and Cx. vishnui by producing 536bp, 344bp and 246bp fragments respectively. The PCR amplification of ITS2 region produced 2 distinct fragments of ~330bp and ~520bp for Ae. aegypti and Ae. albopictus respectively. This is suggestive of the usefulness of exuviae as a reliable source of genomic DNA in molecular taxonomy of mosquitoes.

Monika Sharma and S. Chaudhry (2010), work pertaining to the molecular cytogenetics of malaria vector species of mosquitoes by the application of PCR technique has been carried out. The main objectives of the study included the sequence characterization of nuclear rDNA internal transcribed spacers 1 and 2 (ITS1, ITS2) and mitochondrial DNA CO-II gene as potential molecular markers for studying genetic relatedness and phylogenetic kinship among six important species of genus Anopheles of subgenus Cellia viz: An. stephensi, An. culicifacies, An. maculatus, An. subpictus, An. annularis and An. splendidus. The total length of each DNA band with respect to the number of nucleotides was calculated along with GC:AT content, ratio of substitutions due to transitions and transversions (ts/tv), insertions/ deletions and identification of tandem and nontandem repeat sequences. Rashmi Kohli, S. Chaudhry et al. (2011), studied on sequence characterization of nuclear rDNA internal transcribed spacers ITS 2 and mitochondrial DNA CO II gene and 16 S gene fragment as potential molecular markers for studying genetic relatedness and phylogenetic kinship among five important species of genus Culex.

Sequence analysis of the mitochondrial 16S rRNA gene has been used for molecular taxonomy in many insects. Shouche and Patole (2000) have
analysed a 450 base pair hyper variable region of the mitochondrial 16S rRNA gene in three major genera of mosquitoes, *Aedes*, *Anopheles* and *Culex*. The sequence was found to be unusually A+T rich and in substitutions the rate of transversions was higher than the transition rate and phylogenetic tree was constructed with these sequences. An interesting feature of the sequences was a stretch of Ts that distinguished *Aedes* from *Culex* on one hand and *Anopheles* on the other. On similar lines work was also done in two populations of *Culex quinquefasciatus* and *Armigeres subalbatus* (Chaudhry and Kohli, 2007; Kohli and Chaudhry, 2007).


The mitochondrial DNA is also being used as an important molecular marker as it has its own advantages as a source of detecting genomic variations at the molecular level. In addition to its applications in taxonomy, its use is also a matter of choice depending upon the research programmes of an individual worker or a group involved in the area of molecular entomology or mosquito genomics. In most metazoans the mitochondrial genome is a small circular molecule with 16-17 kb containing 36-37 genes with a densely packed set of 13 protein coding genes, 22 tRNA genes and two rRNA genes (Boore, 1999). These genes are enlisted here as small ribosomal subunit RNA (rrnS, 12S), large ribosomal subunit RNA (rrnL, 16S), cytochrome oxidase subunits I-III (cox1,2,3), cytochrome b apoenzyme (cob), NADH dehydrogenase subunits 1-6, 4L (nad 1-6, 4L), ATP synthase subunits 6 and 8 (atp 6, 8) and transfer RNAs.
The first report of complete mitochondrial genome sequence belonging to the flour beetle *Tribolium castaneum* of the order Coleoptera in which 15,881bp long *Tribolium* mitochondrial genome was found to encode 13 putative proteins, two ribosomal RNAs and 22 tRNAs. Surprisingly there was found to be very near arrangement identical to that in *Drosophila melanogaster* which is considered an ancestor of insects and crustaceans (Boore *et al*., 1998; Hwang *et al*., 2001). Given in the family Culicidae mtDNA sequences have been successfully used in distinguishing sibling species among the genera *Anopheles, Culex, Aedes* and *Armigeres*.

By contrast with the many studies on nuclear genes, little taxonomic work has targeted haploid mitochondrial DNA sequences in mosquitoes and less yet has examined sequence diversity in the CO1 gene (Rey *et al*., 2001; Fairley *et al*., 2000, 2002; Sallum *et al*., 2002), despite its established potential for the diagnosis of biological diversity (Hebert *et al*., 2003a, 2003b).

Paul D. N. Hebert *et al.* (2003a) convinced that the sole prospect for a sustainable identification capability lies in the construction of systems that employ DNA sequences as taxon ‘barcodes’. They established that the mitochondrial gene Cytochrome c oxidase I (COI) can serve as the core of a global bioidentification system for animals. They demonstrate that COI profiles, derived from the low-density sampling of higher taxonomic categories, ordinarily assign newly analysed taxa to the appropriate phylum or order. Second, demonstrate that species-level assignments can be obtained by creating comprehensive COI profiles. The COI identification system will provide a reliable, cost-effective and accessible solution to the current problem of species identification. While another (2003b), work has suggested that a DNA-based identification system, founded on the mitochondrial gene, Cytochrome oxidase subunit 1 (COI), can aid the resolution of this diversity.

Nigel W. Beebe *et al.* (2005), studied on genetic diversity of the dengue vector *Aedes aegypti* in Australia and implications for future surveillance and mainland incursion monitoring. They are work on mitochondrial DNA for COI haplotype study of *Aedes aegypti* for identifying the origins from Australia. Toni Zitko *et al.* (2011), work carried out on to investigate the genetic variability of *Ae. albopictus* the East-
Adriatic coast and islands of Croatia and Montenegro and using two mitochondrial molecular markers: Cytochrome Oxidase I (COI) and NADH dehydrogenase 5 (ND5).

Cywinska et al. (2006), worked on a short fragment of mtDNA from the cytochrome c oxidase 1 (CO1) region was used to provide the first CO1 barcodes for 37 species of Canadian mosquitoes (Diptera: Culicidae) from the provinces Ontario and New Brunswick. Sequence variation was analysed in a 617-bp fragment from the 5’ end of the CO1 region. Sequences of each mosquito species formed barcode clusters with tight cohesion that were usually clearly distinct from those of allied species. Gang Wang et al. (2012), identifying the main mosquito species in China based on DNA barcoding. They construct that DNA barcodes of the cytochrome c oxidase subunit 1, the COI gene, for the more common mosquito species in China, including the major disease vectors. A total of 404 mosquito specimens were collected and assigned to 15 genera and 122 species and subspecies on the basis of morphological characteristics. Individuals of the same species grouped closely together in a Neighborhood-Joining tree based on COI sequence similarity, regardless of collection site.

N. Pradeep Kumar et al. (2007), India, carried out work on species identification of mosquitoes (Diptera: Culicidae) through DNA barcode. The efficacy of this tool for mosquitoes remains unexplored. Hence, a study was undertaken by Kumar to construct DNA barcodes for several species of mosquitoes prevalent in India, which included major vector species. In total, 111 specimens of mosquitoes belonging to 15 genera, morphologically identified to be 63 species, were used. This number also included multiple specimens for 22 species. DNA barcode approach based on DNA sequences of mitochondrial cytochrome oxidase (COI) gene sequences could identify 62 species among these, in confirmation with the conventional taxonomy.

(2011) and Laxmikant Shinde (2012) these are the some researchers are work on molecular systematic of mosquito from India.

From Maharashtra state only few researchers are working on molecular systematic on mosquitoes such as Shouche and Patole (2000), Phool Chand Kanojia, Mandar S. Paingankar, Avinash A. Patil, Mangesh D. Gokhale and Dileep N. Deobagkar (2010) and Laxmikant V. Shinde (unpublished data, 2012). But no one can work on relation of environmental factor on genetic diversity of mosquitoes. All are working on only diversity, taxonomy and distribution of mosquitoes.