Chapter 1.

Introduction
1. INTRODUCTION

Malaria continues to be one of the most serious public health problems throughout the tropical world despite decades of international efforts. This disease affects approximately forty percent of the global population in about 100 countries. It affects all the age groups, especially poor, pregnant mothers, infants and children. As per the recent estimate of World Health Organization, total annual incidence is around 300-500 million clinical cases with about 1.7-2.5 million malaria related deaths, of which one million are children below 5 years, over 90% of which are in Sub-Sahara Africa. During the last five years in South East Asia Region (SEAR) the reported cases of malaria is approximately 21 million each year with about 3000-4000 deaths against estimated about 121 million cases and 27000 to 30000 deaths each year. India accounted >85% of reported cases while Myanmar reported more than 50% malaria deaths. Based on the Disability Adjusted Life Years (DALYs) lost, malaria was estimated to cause an annual loss of about US $ 3 billion (WHO, 1999).

Four Plasmodium species cause malaria in humans; these are Plasmodium vivax, Plasmodium falciparum, Plasmodium malariae and Plasmodium ovale. Out of the four human malaria parasites, P. vivax is the most common infection in India followed by P. falciparum. P. malariae is rare and limited in distribution to a few pockets in Orissa and P. ovale is not found in India but occurs in Africa.

Among all the vectors of malaria in India, An. culicifacies is the most important vector of rural and peri-urban malaria in peninsular India. This vector is responsible for 60-70% of total malaria cases per year in the Indian subcontinent (Rao, 1984). In spite of being the most important vector of malaria in India, the studies pertaining to the biology of An. culicifacies were very limited till mid 1970’s. Several attempts to colonize An. culicifacies had failed, and as a result no advances could be made in our understanding of the basic biology of An. culicifacies. After the colonization of An. culicifacies in India (Ansari et al., 1977) studies on the species were intensified in 1980’s with an objective of having a better understanding of their biology. The most notable of these studies was the cytological identification of An. culicifacies as a complex of two sibling species, species
A and species B (Green and Miles, 1980). Subsequently, three more sibling species, species C (Subbarao et al., 1983), species D (Vasantha et al., 1991) and species E (Kar et al., 1999) have been discovered in *An. culicifacies*.

Since the recognition of *An. culicifacies* as a species complex, various studies have been initiated to develop identification techniques and to find out the biological differences among different members of the complex. The different identification techniques established so far are based on the microscopic examination of the polytene chromosomes from ovarian nurse cells of the half gravid females for fixed paracentric inversions (Green and Miles, 1980, Subbarao et al., 1983), Y-chromosome variations in the neurogonial cells of the fourth instar larvae (Vasantha et al., 1982, 1983), the differences in the cuticular hydrocarbon profile (Milligan et al., 1986), electrophoretic variation in lactate dehydrogenase (Ldh) enzyme (Adak et al., 1994b). Further, molecular tools like DNA probes (Gunasekera et al., 1995), allele specific-polymerase chain reaction (Singh et al., 2004) and PCR-restriction fragment length polymorphism (Goswami et al., 2005) have also been developed. Among various biological differences of members of the species complex, the most notable are their distribution pattern (Subbarao et al., 1980, 1987a), response to insecticides (Raghavendra et al., 1991, 1992) host feeding preferences (Joshi et al., 1988) and malaria transmission potential (Subbarao et al., 1980, 1988a, 1992).

Among various biological differences, differential disease transmission potential of species A, species B and species C of *An. culicifacies* complex is probably of greatest epidemiological importance. Since the recognition of sibling species in this complex, many host parasite interaction studies have been carried out in the laboratory involving species A, species B and species C and different *Plasmodium* species. All these studies unequivocally demonstrated that, among all the members of species complex, species A is the most susceptible while species B is the least susceptible to both human (Adak et al., 1999) and rodent *Plasmodium* infection (Kaur et al., 2000).

Natural variability in malaria susceptibility is well known among mosquito species depending on the parasite species and strains. Variability also exists between
individuals and strains within a vector population, as reported in *An. gambiae* Giles (Burgess, 1960), *An. maculipennis* Meigen (Ramsdale and Coluzzi, 1975), *An. albuminus* (Collins *et al.*, 1976) and *An. culicifacies* (Adak *et al.*, 1999, Kaur *et al.*, 2000, Adak *et al.*, 2006). The natural variability in malaria susceptibility because of differential genetic factors of mosquitoes is not yet been clearly understood. However, several recent studies have shown the correlation between different types of mosquito immune responses with differential mosquito susceptibility to various parasitic infections (Richman *et al.*, 1996).

Over the last one decade tremendous progress has been made in understanding the molecular mechanisms concerning the mosquito’s immune response against their natural and unnatural parasites (Beemtsen *et al.*, 2000). The understanding is increasing rapidly with the studies involving pattern recognition peptides, signaling pathways and antimicrobial peptides (Barillas-Mury *et al.*, 2000, Dimopoulos *et al.*, 2001). In parallel with these studies are genetic approaches that are beginning to identify the genes involved in the immune response (Dimopoulos *et al.*, 2000). Several genes/ loci that determine the differences between a susceptible and refractory strain against malaria parasite have been reported (Gorman *et al.*, 1997, Zheng *et al.*, 1997, Blandin *et al.*, 2004, Osta *et al.*, 2004a). The majority of these molecular interaction studies involving different plasmodia have been carried out in the members of *An. gambiae* complex, the most important vector of malaria in Africa.

Since the recognition of *An. culicifacies* as a species complex, various studies have been undertaken regarding the biological differences among the various members of this complex. However, to combat malaria further understanding of the biology of both parasite and mosquito, as well as of their interactions is required. Since most of the studies carried out on malaria vector-parasite interactions are focussed on *An. gambiae*, Indian malaria vector, *An. culicifacies* did not received much attention in this regard. It seems that the immune responses of any member of the *Anopheles culicifacies* complex to any natural or unnatural parasites have not been studied. *P. vivax*-refractory strain of *Anopheles culicifacies* has been isolated from field since 1999. The molecular mechanism underlying refractoriness is still not explored. Therefore, in the present study