The biology and control of malaria vectors in India

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Despite considerable success of malaria control programme in the past, malaria still continues as a major public health problem. The malaria control in India relies mainly on indoor residual spraying of insecticides, which has become a formidable task due to widespread resistance in malaria vectors, particularly in *Anopheles culicifacies*, responsible for a majority of malaria cases in India. The success of any vector control programme relies on knowledge of vector species and their bionomics, which is complicated due to the fact that among the six recognized primary malaria vectors in India, all except *An. stephensi* are species complexes. There are growing evidences that the members of species complexes differ significantly in biological characteristics that are vital for malaria control point of view such as vectorial potential, host-preference, resting behaviour and response to insecticides. Culicifacies and Fluviatilis complexes are the most studied vector systems from India, which together are responsible for a majority of malaria cases. Vector control has become less effective in recent years due to poor adoption of alternative tools. This review article illustrates an update on recent advances in the field of vector biology, particularly recognition of sibling species, methods for their identification, differential bionomics of members of species complexes and vector control options currently available.

Keywords: *Anopheles*, biology, insecticides, malaria, sibling species, vector.

MALARIA continues to be a major global health problem despite more than 100 years of research since the discovery of malaria parasite in human blood by Laveran in 1880 and establishment of mosquito's role in transmitting malaria by Ross in 1898. According to an estimate by the World Health Organization, 300–500 million people suffer from malaria worldwide each year mainly in Africa and south of Sahara, between 1.1 and 2.7 million people are killed either with malaria alone or in combination with other diseases, and over 2400 million remain at risk[1]. In India around two million malaria cases are being reported annually; but the real picture is grossly underestimated[2]. In the past, there was a dramatic decrease in malaria cases after introduction of DDT in public health programme but the success was quickly reversed mainly due to the development of insecticide resistance in vectors in addition to drug resistance in parasite. An effective vaccine against malaria is yet to come.

The vector control is one of the essential components of any malaria control programme, the success of which relies on the knowledge of the vector species, their bionomics and vector control options suitable for vector species. Unfortunately, the research on vector biology was hampered in the past due to the spectacular success of insecticide in reducing malaria incidence. The research on vector biology was renewed after return of malaria due to various reasons, including development of insecticide resistance in mosquitoes. Since then, there has been considerable increase in knowledge of vector system, especially recognition of sibling species and their bionomics. Currently a new paradigm of vector control by means of introducing malaria-refractory genes into the wild mosquito has emerged. However, for the success of any such genetic control programme, clear understanding of population biology of the vector and of barriers to gene flow is essential.

The malaria vectors

All human malaria is transmitted through anophelines (genus *Anopheles*) only, but not all anophelines are vectors of malaria. To become a vector, one has to be susceptible to malaria sporogony, be anthropophagic and have enough longevity to become infective to human. There are 444 formally named species and 40 unnamed members of species complexes recognized as distinct morphological and/or genetic species of *Anopheles*[3]. In India, 58 *Anopheles* have been described, six of which have been implicated to be main malaria vectors, namely *An. culicifacies, An. dirus, An. fluviatilis, An. minimus, An. sundaicus* and *An. stephensi*. Besides, some are of local importance, viz. *An. philippinensis-nivipes, An. varuna, An. annularis* and *An. jeyporiensis*.

Current knowledge on the vectors and their precise role in malaria transmission is incomplete due to the fact that all the major malaria vectors, except *An. stephensi*, are complexes of more than one biological species which are morphologically indistinguishable and are called as sibling or cryptic species. Studies on their bionomics, distribution, role in malaria transmission have become important

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due to growing evidences that these cryptic species may differ significantly in biological characteristics especially those which are of importance from malaria control point of view, such as, response to insecticides, vectorial competence, host-preference and resting behaviour. The correct identification of malaria vector in vector-control programme is another issue which is critical to the success of the programme. This becomes more important in the case of closely related species and members of species complexes. There are instances where anophelines (particularly members of Myzomia group) have been misidentified in malaria control programme due to the overlap of morphological characters in closely related species[4,5]. Sibling species in most of the vector systems have been recognized through chromosomal studies or biochemical methods. Cyto-taxonomic methods based on ovarian polytene chromosome are often difficult to be carried out, need expertise and are applicable to semi-gravid individuals, which constitute a small proportion of the population. Molecular methods have not been extensively used for recognition of sibling species; however, such tools have been extensively used for species diagnosis. A PCR-based species-diagnostic assay is relatively easy to be performed and is applicable for all stages and sex of mosquitoes, but such a technique, though relatively simple, is available in research laboratories only.

During the past two decades there has been a significant progress in the direction of recognizing new sibling species in vector systems and development of methods for their identification, understanding of their bionomics and establishment of their role in malaria transmission. A brief update of major malaria vector systems has been summarized below:

Culicifacies Complex

Anopheles culicifacies contributes to about 60–65% of all malaria cases of India[6] mainly from rural and peri-urban areas and is widely distributed throughout the country. It is also an important vector in Iran, Afghanistan, Pakistan and Sri Lanka[7]. Culicifacies complex comprised five sibling species provisionally designated as species A, B, C, D[7] and E[8] that can be identified on the basis of fixed paracentric inversions on polytene chromosome X and 2. However, species B and E, being isomorphic for polytene chromosome complements, are differentiated on the basis of Y-chromosome karyotype of son (F_1 male progeny) which is acrocentric in species B and submetacentric in species E[8]. Biochemical techniques based on lactate dehydrogenase allozyme (*ldh*) have been developed which can differentiate species A from B and C[9]. Several papers have appeared on molecular techniques for the identification of members of species complex such as DNA probe[10], PCR–RFLP[11], allele-specific PCR assays (ASPCR)[12,13]. The PCR-RFLP approach based on mitochondrial DNA (cytochrome oxidase II)[11] was, however, reported to be unsuitable for differentiation of species B and E in Sri Lanka[14].

All the members of *An. culicifacies* are predominantly zoophilic[7] except species E[15] and rest indoor, mainly in cattle sheds. Beside its low anthropophagy, it acts as a major malaria vector due to the fact that it is generally found in high density. The preferred breeding sites are streams, rice fields, seepage water, borrow pits, irrigation channels, rain water collections, etc. Due to the vast breeding areas occupied by this vector mainly during monsoon season, anti-larval methods of their control are difficult to employ.

The members of Culicifacies Complex differ in distribution pattern[7], response to insecticides[16–18], vectorial status[19,20] and susceptibility to malaria sporogony[21,22]. Species B, which is regarded as a non-vector[7], is a widely distributed species and found exclusively in eastern India, while in other areas it is found in sympatricity with species C, D or E[15] . Species E, which is regarded as a vector, has been reported from Rameshwaram Island[8] and Sri Lanka[23] only, and is found in sympatricity with species B. In an epidemiological study in Uttar Pradesh, India it was found that the malaria incidence was low or absent in species B-predominant areas, while it was high in species A-predominant areas[24]. Laboratory feeding experiments of different members of Culicifacies Complex revealed that species A is highly susceptible to malaria sporogony followed by species C and B[21,22]. It has also been reported that species B has inherent ability of killing malaria parasite in the midgut during early sporogony by the process of encapsulation. The ability to encapsulate malaria parasites in the midgut varies in different strains and a strain of species B has been isolated which is completely refractory to *P. vivax* sporogony and partially refractory to *P. falciparum*[25]. The members of the Culicifacies Complex also differ in response to insecticides. There are evidences that species A is more susceptible to DDT[16] and malathion as compared to species B[17]. Species B developed resistance to malathion at a faster rate as compared to the sympatric species B[18].

Being a major vector in India, control of *An. culicifacies* is a major concern for vector-control programme in India. Unfortunately this vector has developed resistance against all commonly used insecticides.

Fluviatilis Complex

The *An. fluviatilis s.l.* has been reported to extend from Yemen to Formosa (Taiwan)[26] but its presence beyond east of northeastern states of India is doubtful[5]. Earlier reports of its presence in Thailand and China have been regarded as misidentification of *A. minimus* due to overlapping morphological characters[5,27]. Similarly, earlier report of the presence of *An. fluviatilis* (species U) from Assam, India[7] is doubtful. Recently, using DNA data it was found that the morphologically identified *An. fluvi-*

atilis from Assam are in fact morphological variants of sympatric species *An. minimus s.s.* (Singh, O. P. *et al.*, unpublished data).

Anopheles fluviatilis is now recognized as a species complex comprising at least three sibling species – species S, T and U[28]. Possible existence of two additional taxa within the *An. fluviatilis* complex, one in Iran and another in India, provisionally designated *An. fluviatilis* forms V and X, respectively, was suggested based on variant ITS2 sequences[29]. However, it has been established that species X is synonymous to species S by Singh *et al.*[30]. Sibling species of Fluviatilis Complex was recognized on the basis of fixed inversions in polytene chromosome 2. However, the differentiation of species S and T is not possible by chromosomal method in areas where q^1 inversion, a marker for differentiation between species S and T, is polymorphic. It has been proposed that, in such areas, the biological characteristics (such as host preference) may be taken as criteria to distinguish species S and T in addition to inversion genotypes[7]. A molecular method based on 28S rDNA has also been developed for the differentiation of all members of morphologically identified *An. fluviatilis*[31].

All the three species of the Fluviatilis Complex are found in India. Species T is most widely distributed in India and also found in Iran[32]. Species S seems to be forest species and is predominantly found in Orissa, India, where it is the main malaria vector.

Contrasting differences in biological characteristics have been reported among members of the Fluviatilis Complex. Species S has been recognized as highly efficient malaria vector and is predominantly anthropophagic, whereas species T and U are regarded as non-vectors and are almost exclusively zoophagic[7,33]. It was noted that species T which does not act as vector in nature is highly susceptible to malaria sporogony in laboratory[34]. This is probably because of its zoophagic nature making it a non-vector, but with potential to act as vector where man : cattle ratio is high.

Minimus Complex

According to Harrison[5], the distribution of *An. minimus s.l.* extends from Uttar Pradesh down to the northeastern tip of Andhra Pradesh in India, across the Indochina–Malay peninsular countries down to the Thai–Malay border and north across the People's Republic of China (up to 30"N latitude) to Taiwan and the Ryukyu islands. This species has however been reported to have disappeared from the Terai region of Uttar Pradesh (now in Uttaranchal)[35] following the introduction of DDT. In Singhbhum hills of east-central India, where this species has been absent for nearly 45 years, has now reappeared[36].

A. minimus is now recognized as species complex comprising at least three sibling species A, C and E[3]. Species A has now formally been recognized as *An. minimus s.s.*[37] So far no chromosomal method is available for the identification of members of *An. minimus*. However, *An. minimus s.s.* and species C can be differentiated by the octanol dehydrogenase (*odh*) analysis[38] and ITS2-based PCR–RFLP[39] or ASPCR[40].

An. minimus s.s. and species C are malaria vectors and they occur in China, Laos, Thailand and Vietnam, whereas only *An. minimus s.s.* has been recorded from Taiwan and Cambodia[42]. Recently, species C has been reported from Myanmar[30]. Species E has a limited distribution in Japan[43], a malaria-free region. In India, so far only *An. minimus s.s.* has been reported from northeastern states[41] (Singh, O. P. *et al.*, unpublished data), Singhbhum hills[36] and Jalpaiguri, West Bengal (Singh, O. P. *et al.*, unpublished data). In India *An. minimus s.s.* is highly anthropophagic and is an efficient malaria vector mainly in northeastern states[44]. Among *An. minimus* complex, species C has been reported to be more exophagic and zoophilic as compared to species A[45].

An. fluviatilis and *An. minimus* are closely related species under Minimus subgroup of Myzomia series[3]. These two species are morphologically closely related and *An. minimus* has been misidentified as *An. fluviatilis*[5,27] probably due to variation in palpal ornamentation which has been described as 'hypermelanic form' of *An. minimus*[5]. Some authors considered *An. fluviatilis* S, an important malaria vector in India, as a synonym of *An. minimus* C[3,29,46] on the basis of homology of small region of rDNA sequence data and similarity in biological characteristics[29]. Later sequencing of d2–d3 domain of 28S rDNA, ITS2 and cytochrome oxidase II revealed that *An. fluviatilis* S and *An. minimus* C are in fact independent species[30].

Anopheles stephensi

Anopheles stephensi is a sub-tropical species and distributed throughout the Middle East and South Asia region (Afghanistan, Bahrain, Bangladesh, China, Egypt, India, Iran, Iraq, Oman, Pakistan, Saudi Arabia and Thailand) and is considered an important vector in India, Pakistan and Iran. So far there is no description of sibling species in *An. stephensi*; however two races, 'type form' and '*var. mysoriensis*' have been described based on egg-dimension and number of ridges present on the floats[47,48]. The 'type form' is an inhabitant of urban area and is a malaria vector whereas *var. mysorensis*, found in rural areas, is not a vector. Subbarao *et al.*[49] reported yet another form, i.e. 'intermediate'. All these three forms are found in India[49] and Iran[50]. The recent finding that 'type form' and *var. mysorensis* have different spiracular index[51] indicates that these two are ecological forms adapted to different ecological niche. The *var mysorensis* has lower spiracular index as compared to 'type form' and thus more adapted to dry climate.

An. stephensi is primarily a zoophilic species but considerable variability in human blood index (HBI) has been reported. In Kolkata, an urban area, as high as 100% HBI has been reported[52]. The specific breeding sites in urban areas are: building-construction sites, wells, garden ponds, cisterns, overhead tanks, ground level cement tanks, water coolers, etc. In rural areas it breeds in a variety of breeding sites such as streams and channels, tanks and ponds, seepages and wells. This is mainly an urban vector. Since a majority of breeding sites for *An. stephensi* in urban areas are man-made and limited, it is possible to control their breeding by community involvement, biological control and enforcement of legislative measures.

Dirus Complex

The Dirus Complex is mainly prevalent in the forest and forest-fringe area and its members are vectors in India, Bangladesh, Myanmar and Thailand. It is a complex of at least seven sibling species designated as *Anopheles dirus s.s.* (species A), *Anopheles cracens* (species B), *Anopheles scanloni* (species C), *Anopheles baimaii* (species D), *Anopheles elegans* (species E), *Anopheles nemophilous* (species F) and *An. takasagoensi*[53]. In India, only two species, *An. baimaii* and *An. elegans* are found. The former a highly anthropophagic[54] and an efficient malaria vector, is found in north-eastern states and the latter in Shimoga hills of Karnataka, the vectorial status of which is unknown[7,15]. The presence of *An. baimaii* in different north-eastern states of India has been confirmed by ITS2-rDNA sequencing and ASPCR[41] (Raghavendra, unpublished data). An ITS2-based ASPCR is available for distinguishing five members of the complex, *An. dirus*, *An. cracens*, *An. scanloni*, *An. Baimaii* and *An. nemophilous*[55].

Sundaicus Complex

Anopheles sundaicus is an important malaria vector in coastal areas in Southeast Asian region. Its distribution extends from northeastern India to southern Vietnam, south to the Nicobar, Andaman, and Indonesian islands[56]. In India it was reported from West Bengal, Orissa, the coastal areas of Andhra Pradesh and Andamans[57], but its presence is now restricted to Andaman and Nicobar Islands[58] and Kuch of Gujarat state[59]. It prefers to breed in saline/brackish water, though it has been reported to breed in freshwater also. Sukowati *et al.*[60,61] reported three sibling species in *An. sundaicus* in Thailand and Indonesia on the basis of chromosomal and biochemical (isozyme) evidences and designated as species A, B and C. Species *An. sundaicus s.s.* has been formally designated from Malaysia[62]. Species A has been formally designated as *An. epiroticus*[63]. Recently a new cytotype D from Car Nicobar island has been reported[64]. Being allopatric, specific status of cytotype D as a new sibling species could not be assigned. Molecular characterization of this cytotype using ITS2 region revealed no difference in population from brackish and freshwater habitats, but is different from *An. sundaicus* A of Vietnam and *An. sundaicus s.s.* of Malaysia[65]. *An. sundaicus* has adaptability to breed in wide salinity conditions from freshwater to brackish water[56].

Vector control

Vector control, an essential component of malaria control, has become less effective in recent years, partly due to poor use of alternative control tools, inappropriate use of insecticides, lack of an epidemiological basis for interventions, inadequate resources and infrastructure, and weak management[66]. Changing environmental conditions, the behavioural characteristics of certain vectors and resistance to insecticides have added to the difficulties[66]. The options available for vector control are mainly, indoor residual spraying (IRS) of insecticides, personal protection measures, larval control, biological control and environmental managements. The World Health Organization's Global Malaria Programme recommended the use of IRS as a major means of malaria vector control to reduce and eliminate malaria transmission, and distribution of insecticide-treated nets (ITNs) to achieve full coverage of populations at risk of malaria, as primary interventions that must be scaled up in countries to effectively respond to malaria, towards achieving the Millennium Development Goals for malaria[67] by 2015.

In India, vector control mainly relies on indoor residual spraying of DDT, malathion or synthetic pyrethroids (SPs) in rural areas and source reduction and anti-larval measure in urban areas. Approximately 60–70% of total malaria control budget goes to the IRS. In the past, the IRS has shown excellent result in controlling malaria in many parts of the world. In India, use of DDT as IRS resulted in bringing down malaria from 75 million cases to an all time low of 0.1 million cases[68] in the year 1966. The spectacular success in malaria control by DDT IRS paved the way for possibility of malaria eradication. In 1958 the National Malaria Control Programme was converted to the National Malaria Eradication Programme (NMEP). However there were serious setbacks to the NMEP from 1968 onwards due to various factors including insecticide resistance in vectors. Another insecticide, benzene hexachloride (BHC, gamma isomer), was banned for public health use since 1997 owing to health concerns. The other insecticides being used in public health are malathion and pyrethroids. In spite of the fact that spraying of insecticides in malaria control programme over the last five decades has resulted in resistance to insecticides and behavioural changes in vector population, IRS still remains the main vector-control strategy in India and DDT remains the main choice of insecticide in most situations. The World

Health Organization has recently recommended effective implementation of IRS with DDT or other recommended insecticides as a central part of national malaria control strategies where this intervention is appropriate[66]. During the last two decades, SPs such as deltamethrin, cyfluthrin and lambdacyhalothrin have been introduced into public health programme as residual insecticide and for impregnation on mosquito nets. However, reports on resistance against SPs have already surfaced in areas where SPs are in use[69,70].

The first report of resistance to DDT has appeared[71] in *An. culicifacies* in 1958 and has now become widespread. Resistance against HCH, dieldrin and malathion was also reported quickly after their short use[72–74]. One of the major reasons for the resurgence of malaria in mid-1970s has been insecticide resistance in vector species[75]. Presently, *An. culicifacies*, the main malaria vector in India, has developed resistance to DDT in 286 districts and to DDT and malathion in 182 districts of India[76]. Resistance to SPs has also reported from some areas[69,70] despite early optimism that because of its rapid toxicologic action this newest large class of insecticides would not produce resistance[77].

Limited number of chemical groups of effective insecticides is available for vector control. Furthermore similarities in the mode of action across some of these chemical groups and the phenomenon of cross-resistance explain why, in some situations, vector populations can develop resistance very rapidly to newly introduced insecticides. It is therefore important to identify the mechanisms involved once resistance has appeared in a vector population for better management of insecticide resistance. The major mechanisms of resistance include glutathion-S-transferase-based degradation of DDT, carboxyl esterase-dependent hydrolysis of malathion, altered acetyl cholinesterase activity[78,79] to organophosphate and carbamate, cytochrome P-450 monooxygense[80,81] and *kdr* type of resistance against SPs[82]. Behavioural resistance is a genetic phenomenon that involves modification in the central signalling system or peripheral signal receptors[83] and develops independent of physiological resistance.

Due to rapid increase in insecticide resistance in vectors and due to non-availability of effective new molecules of insecticides in near future, management of insecticide resistance becomes important[84]. Resistance management can be attempted using insecticide-based approaches in conjunction with other non-insecticidal vector-control methods. The proposed strategies of insecticide management are: (i) rotational strategies based on two or preferably more insecticide classes with different modes of action over time, (ii) the use of mixtures of insecticides (classical example for such strategy is Onchocerchasis Programme in Africa for the management of temephos resistance in Similium[85]), (iii) mosaic approach, i.e. spatially separated applications of different compounds against the same target vector, and (iv) minimal use of insecticides based on the distribution of sibling species and their susceptibility to insecticides[86]. However for successful management of insecticide resistance, knowledge of the mode of action of the available insecticide products and resistance monitoring should be an integral part of vector control programmes.

The use of insecticide-treated nets (ITNs) is being promoted because the application of a residual insecticide greatly enhances the protective efficacy of bed-nets. Trials of ITNs in the last two deacdes showed that ITNs reduced deaths in young children significantly in Africa. Pyrethroids are the only insecticides that have been used for impregnation of bed-nets due to very low mammalian toxicity. The rapid knock-down effect, even at very low doses, and high residual effect provide added advantages. Deltamethrin was the first insecticide that was used for impregnation of bed-nets with remarkable success followed by cyfluthrin, lambda-cyhalothrin, bifenthrin and alphacypermethrin. Owing to the success of these ITNs, which require re-treatment at periodic intervals, long-lasting insecticide-treated nets (LLINs) became available, in which insecticide is incorporated into the net fibres. Trials of various brands of LLINs impregnated with permethrin, deltamethrin and alphacypermethrin are underway. These nets are said to have increased activity for longer periods of time (reportedly five years) unlike the earlier treated nets that need re-impregnation generally after six months. The emerging pyrethroid resistance in vectors is a serious threat to the success of pyrethroid-treated nets. Search for alternative insecticides with novel mode of action and use of mosaic or mixture of insecticides to prevent insecticide resistance is being advocated.

As part of alternate strategies, the bio-environmental control approach was implemented in India after successful multi-centric field trials conducted by the National Institute of Malaria Research (formerly Malaria Research Centre). Larvivorous fishes have also been found to be very effective in controlling malaria in certain situations in Karnataka[87]. The National Vector Borne Disease Control Programme is presently implementing this strategy as part of integrated disease control in many states.

Two biocides from the bacterium *Bacillus thuringiensis* var. *israelensis* (*Bti*) and *Bacillus sphaericus* (*Bs*) were extensively field tested in India. The *Bti* is now being used in public health programmes as anti-larval measure in urban areas; however *Bs* developed resistance soon after its application[88].

Yet another strategy being considered is the genetic manipulation of vectors by using modern biological techniques to render them ineffective as carriers of disease which, in principle, involves introduction of foreign genes into the vector using transposable elements.

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