

Genetics of Malaria Vectors

The fact that resistance developed to insecticides in mosquitoes after their extensive use in the 1950s in disease control/eradication programme and that resistance is genetically controlled, have stimulated studies in mosquito genetics and created awareness among entomologists on the importance of genetics in control strategies. The need for mutant markers and their genetic analyses, and well-developed genetic maps was realised, particularly in the 1960s when genetic control of mosquito population was considered as an alternative strategy in disease control programmes. Genetic manipulation of mosquito population using molecular approaches now being considered for reducing or eliminating

vector-borne diseases, once again are underscoring the importance and need for well-developed genetic map and markers for studying the population structure and gene-flow.

Genetic Markers

Markers are useful as tools for genetic studies to map genes for insecticide resistance, resistance to malaria parasites, feeding preference, *etc.* in malaria vectors. Markers are also used in genetic studies to estimate the genetic distance and gene-flow between populations. Various types of markers are used for genetic study of mosquitoes such as morphological mutants, biochemical—isozyme

Table 5. List of genetic markers in malaria vectors

Linkage group	Markers	No. of alleles	Inheritance pattern	Reference
<i>An. culicifacies</i> Species A				
II	Vermilion eye (v)		Recessive	Adak <i>et al</i> 1983
<i>An. culicifacies</i> Species B				
I	White eye (w)		Recessive	Subbarao <i>et al</i> 1982
I	Malic enzyme (Me)	2	Co-dominant	Adak <i>et al</i> 1988
II	Creamish larva (cr)		Recessive	Subbarao <i>et al</i> 1982
III	Red thorax (rt)		Dominant	Subbarao <i>et al</i> 1982
<i>An. stephensi</i>				
I	Red eye (r)		Recessive	Sharma <i>et al</i> 1979
I	Malic enzyme (Me)	2	Co-dominant	Adak <i>et al</i> 1993
I	Creamish white eye (cr)		Recessive	Adak <i>et al</i> 1999
II	Colourless-eye (c)		Recessive	Sharma <i>et al</i> 1977
II	EST-4	3	Co-dominant	Adak <i>et al</i> 1984
II	IDH-2	2	Co-dominant	Adak <i>et al</i> 1991
III	Green larva (g)		Recessive	Subbarao <i>et al</i> 1978
III	Golden yellow larva (gy)		Recessive	Adak <i>et al</i> 1990
III	Black larva (Bl)		Semi-dominant	Adak <i>et al</i> 1990
III	6-PGD	2	Co-dominant	Adak <i>et al</i> 1992
III	MDH	2	Co-dominant	Adak <i>et al</i> 1992
III	AAT	2	Co-dominant	Adak <i>et al</i> 1996
<i>An. sudaicus</i>				
	Yellow larva (yl)		Recessive	Das <i>et al</i> 1997
<i>An. minimus</i>				
	ODH	1	Co-dominant	
	MDH-1	2	Co-dominant	
	MDH-2	2	Co-dominant	
	LDH	2	Co-dominant	
	MPI	1	Co-dominant	
	HAD	2	Co-dominant	

electrophoretic variations, microsatellites, RFLPs, etc. Some of the molecular markers have additional advantage that they can be physically mapped on chromosome, which would establish linkage relationship and indicate exact location in the genome.

Genetics of Phenotypic and Isozyme Electrophoretic Markers

We have isolated various phenotypic and isozyme electrophoretic markers in some important malaria vector species—*An. culicifacies* (species A and B), *An. stephensi*, *An. sundanicus* and *An. minimus* and their inheritance pattern was also studied. A synoptic list of these markers and nature of inheritance is appended in Table 5.

Genetics of Egg-float Ridge Number in *Anopheles stephensi*

In *An. stephensi*, two races—type form and variety *mysorensis* were described on the basis of differences of the egg length, width and the number of ridges on the egg-float. The type form was reported to inhabit urban areas and an efficient vector of malaria, whereas var. *mysorensis* inhabits rural areas and considered as a poor vector. In order to resolve the taxonomic status of rural and urban *An. stephensi* populations and the genetic basis of egg ridge number, crosses were made between laboratory colonies of type form (egg ridge number >16) and var. *mysorensis* (egg ridge number < 13) established from rural and urban localities of India. Reciprocal genetic crosses between these two forms indicated no post-copulatory barriers between populations. Likelihood analysis of the results of crosses and backcrosses indicated that variation in egg ridge number is controlled by more than one genetic factor (Subbarao *et al* 1987).

Genetics of *Bacillus sphaericus* Resistance in Mosquitoes

Culex quinquefasciatus

The *Bacillus sphaericus* was used in the breeding sites of *Cx. quinquefasciatus* in Ghaziabad at fortnightly intervals. Within a year of spraying of *B. sphaericus*, the *Cx. quinquefasciatus* developed 7-fold resistance. This strain was further selected in laboratory for resistance against *B. sphaericus*. As a result, 52000-fold (LT₅₀) resistance was developed in this strain. To study the inheritance pattern of resistance the reciprocal and backcrosses were carried out between homozygous resistant and susceptible strain of *Cx. quinquefasciatus*, which revealed that the resistance was recessive, autosomal and controlled by more than one gene (Adak *et al* 1995). No maternal effect was observed in the expression of resistance.

Anopheles stephensi

A resistant strain of *An. stephensi* was selected

in laboratory against *B. sphaericus*. The resistance strain (LC₅₀ > 1600 mg/l) was crossed reciprocally to a susceptible strain *golden yellow larva* (LC₅₀ > 0.08 mg/l). The F₁ progenies of both the reciprocal crosses were inbred and backcrossed. Results of these crosses revealed that the resistance is recessive and autosomally inherited. No linkage between *B. sphaericus* and *golden yellow larva* was established. As the resistance gene is autosomal and *golden yellow larva* is on chromosome 3, this gene has been assigned to linkage group II.

Development of Microsatellite Markers in *Anopheles culicifacies*

Microsatellite markers are simple tandem repetitive sequences, mostly of 2 to 6 nucleotides that are randomly distributed throughout the genome. The fact that are highly polymorphic in a population, good number of markers can be isolated in comparatively short duration, can be physically mapped on chromosomes and can be used for positional cloning of specific genes, makes them attractive markers for genetic studies.

Not much work has been done on microsatellite markers except in *An. gambiae*, *An. funestus*, *An. maculatus* and *An. dirus*. In India, NIMR for the first time isolated microsatellite markers from *An. culicifacies* species A. Partial genomic library was prepared and clones were screened for the presence of microsatellite loci using poly GA/GT probes. The clones positive for poly GT/GA probes were sequenced and primers were designed for amplification of 17 such microsatellite loci (Table 6). These primers have been tested in laboratory populations, out of which 12 microsatellite loci were found to be polymorphic which have been selected for further genetic analysis of *An. culicifacies* field populations. A few of these markers tested on species B population were found working and polymorphic too.

Population Genetic Analysis of Malaria Vectors

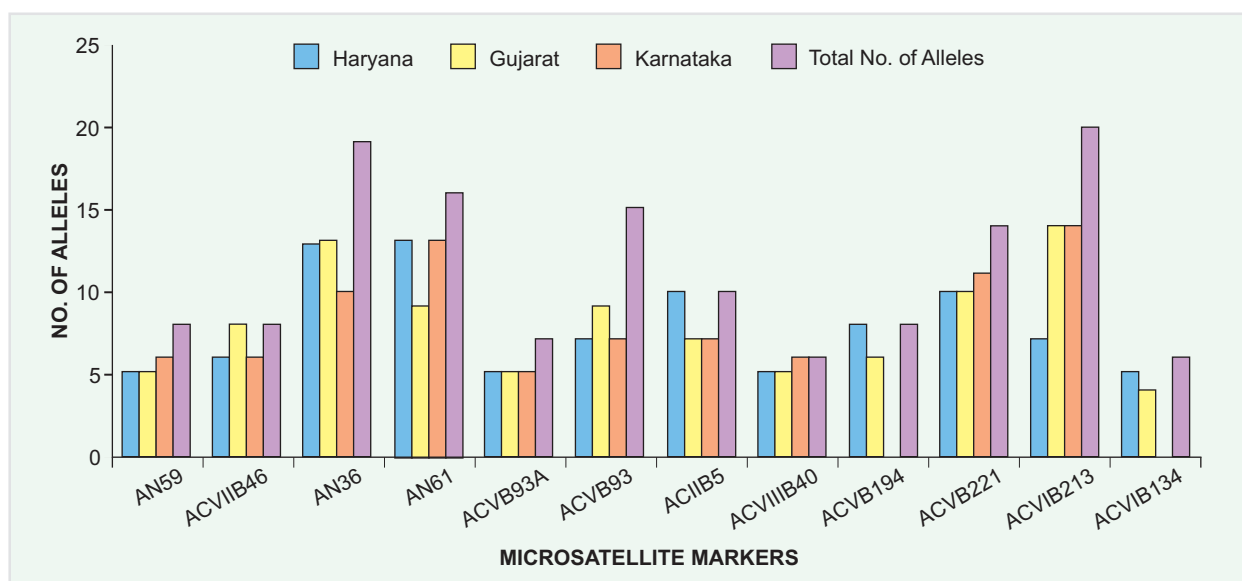
Using Microsatellite Markers in *Anopheles culicifacies*

Three populations of *An. culicifacies* species A collected from Gujarat, Haryana and Karnataka states were genotyped using 12 microsatellite markers isolated by NIMR. All the markers were found to be highly polymorphic in all the populations. The number of alleles found with each of the markers in the three populations are shown in Fig. 43. The study is in progress to test two more populations and genetic analysis of the results.

These microsatellite markers were tested against *An. culicifacies* species B from Gujarat and Haryana. Nine sets of primers developed for amplification of microsatellite loci in species A were successfully used for species B also, and all the nine markers were found to be multi-allelic having 3 to 11 alleles.

Table 6. Microsatellite markers and amplifying primer pairs for *An. culicifacies* species A

S. No.	Locus	Gene bank accession No.	Repeat motif	Primer sequence (5'-3')	Sequenced allele size units
1.	AC43C30	–	(CA) ₃₈	CCTTGGAGAGGGCTGTAGAA ATCACAACACGCGGTACAGA	200
2.	AN36	–	(CA) ₈	GGGCAAACGAAAAAGGTTG CACTGATGACGTTTCGTTGC	206
3.	AN59	–	(CA) ₆	TCCCACATACCGATACACCA GCGTAGGTCAACCGTAATGC	210
4.	AN61	–	(CA) ₃₊₄₊₃₊₄	TTCCTACTCACCAGCCGAAC CGAATGCATTTTCGTTGATA	204
5.	AN75	–	(GT) ₁₀	TCTGGAGATTGAGCAGGAGT AACGCAGTCACAAGGCAGTA	91
6.	ACIIB5	AJ417869	(CA) ₃₊₁₊₂	CGGAAAACGTGCAACAAAATC ATCCAACCGTAGCCATAACAAGC	110
7.	ACIIB71	AJ417870	(GT) ₅	GCAGGCAGACCACTCACAATCTG GACTCTGCTGCTGCCACACTTG	149
8.	ACIVB129	AJ417871	(TG) ₇	TCTCCTTTTGCATATCTTTCGTG TAGATTCGGTTGTAGTTTTCTCGC	107
9.	ACVB93	AJ420078	(GTG) ₃₊₄₊₁	GTCCTTTGCAAATCACATCGG TTAATGACTTCAATCCACAAACCC	140
10.	ACVB93A	AJ420079	(CA) ₂₊₃	GTGGCCGTGTTTCGTCCTTTTG CAGTGCTCGTGGCGTTCGCG	117
11.	ACVB194	AJ420081	(GT) ₆₊₁	TGTCGTGAAGGCATGTTTGAG ATTATTGCATTCTAGCGGGTGA	184
12.	ACVB221	AJ420073	(CT) ₃₊₇	ACTCACGGGAAGCCAAAATACC AAGGAGAAGGATACATCGATGGAG	115
13.	ACVIB134	AJ420074	(CA/AC) ₃₊₁₊₂₊₆	CTGGCGATGATGATGATGGCG CAGCAGTTTCCCGGAAGGAGAG	168
14.	ACVIB213	AJ420076	(GA) ₇₊₁₊₅	ATAAAACGCCCCGCATCATAATG CACGGCACATTCCCTCCATA	116
15.	ACVIIIB46	AJ420075	(CAA) ₁₊₄	AACCGGAAGCAGTATCGCACAC GAGGCTCCTTCGTTATCCG	140
16.	ACVIIIB40	AJ420077	(CA) ₅	TCAAGCTGGACAATGTAACCTAAC GTTCAATCAAACCCAGCCAAAC	118
17.	ACVIIIB182A	AJ420080	(GA) ₁₊₆	GTTTAGCTTCGGGCCTTTCATAC GAGATACAACCGGTGCGTCAGC	170

**Fig. 43: Allele distribution of 12 microsatellite loci in three populations of *An. culicifacies* species A**

Using Paracentric Inversion Markers in *Anopheles annularis*

Populations of *An. annularis* from six localities of India (Districts Shahjahanpur, Ghaziabad, Alwar, Sundargarh, Sonapat and Kamrup) were genotyped for inversion polymorphism by examining ovarian polytene chromosomes (Atrie *et al* 1999). A total of nine autosomal paracentric inversions located on different arms of chromosomes were found to be polymorphic. In Districts Shahjahanpur and Ghaziabad there were no heterozygotes for inversion j^1 on chromosome arm 2, which was taken as the evidence for reproductive isolation between these two forms. The two forms were provisionally designated as species A and B with diagnostic arrangements $2+j^1$ and $2j^1$. The BIOSYS-1 computer programme of Swofford and Selander revealed two clusters of the phenogram (Fig. 44), one of six populations with $2+j^1$ (species A) and another of $2j^1$ population (species B). The analysis further showed a good correlation between genetic and geographical distances among populations of species A (loc. cit.).

Using Paracentric Inversions in Urban and Rural Populations of *Anopheles stephensi*

An. stephensi is one of the anopheline species in which inversion polymorphism is extensive. So far 26 floating paracentric inversions have been identified in this species. In a population studied from Delhi (urban areas), we have found 10 floating inversions. A photomap of polytene chromosome of this species with inversion break points of all the inversions marked is given elsewhere (Subbarao

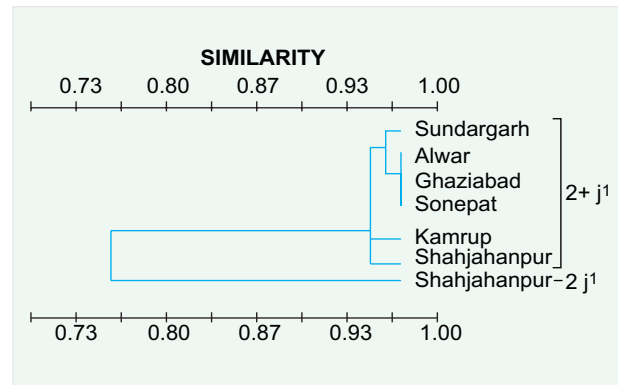


Fig. 44: A phenogram of *An. annularis* population of India produced by the unweighted pair group method of Nei with arithmetic average (UPGMA) cluster analysis of the average similarity matrix (Cophenetic correlation = 0.989)

1996). In addition to urban population, population from a rural area near Delhi was examined. While this species exhibits extensive inversion polymorphism in urban area, in rural population one very common inversion, $2b$ (found in urban area) and another inversion $2h^1$ in one specimen in heterozygous state were found. This study has shown that urban and rural *An. stephensi* populations are distinctly different. This confirmed earlier finding that rural and urban populations are distinct with reference to prevalence of two ecological races—in rural areas variety *mysorensis* (characterized by low egg-float ridge number) is prevalent while in urban areas type form (characterized by high ridge number) is prevalent (Subbarao *et al* 1987). □