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 realized 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 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 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 electophoretic markers in some important malaria vector species—*An. culicifacies* (species A and B), *An. stephensi, An. sundaicus* and *An. minimus* and their inheritance pattern was also studied. A synoptic list of these markers and nature of inheritance is appended in Table 4.

Genetics of Egg-float Ridge Number in An. 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).

Table 4. List of genetic markers in malaria vectors

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

Genetics of Bacillus sphaericus Resistance in Mosquitoes

Culex quinquefasciatus

The *Bacillus sphaericus* was used in the breeding sites of *Cx. quin-quefasciatus* in Ghaziabad at fortnightly intervals. Within a year of spraying of *Bacillus 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 (LT_{50}) 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 (LC_{50} > 1600 mg/l) was crossed reciprocally to a susceptible strain *golden yellow larva* (LC_{50} > 0.08 mg/l). The F_1 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 An. culicifacies

Microsatellite markers are simple tandem repetitive sequences, mostly of 2 to 6 nucleotides that are

Table 5. 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
1	*AC43C30*	–	$(CA)_{38}$	CCTTGGAGAGGGCTGTAGAA ATCACAACACGCGGTACAGA	200
2	AN36	–	$(CA)_{8}$	GGGCAAACTGAAAAAGGTTG CACTGATGACGTTTCGTTGC	206
3	*AN59*	–	$(CA)_{6}$	TCCCACATACCGATACACCA GCGTAGGTCAACCGTAATGC	210
4	*AN61*	–	$(CA)_{3+4+3+4}$	TTCCTACTCACCAGCCGAAC CGAATGCATTTCGCTTGATA	204
5	*AN75*	–	$(GT)_{10}$	TCTGGAGATTGAGCACGAGT AACGCAGTCACAAGGCAGTA	91
6	ACIIB5	AJ417869	$(CA)_{3+1+2}$	CGGAAAACGTGCAACAAAATC ATCCAACCGTAGCCATAACAAGC	110
7	*ACIIB71*	AJ417870	$(GT)_{5}$	GCAGGCAGACCACTCACAATCTG GACTCTGCTGCTGCCACACTTG	149
8	*ACIVB129*	AJ417871	$(TG)_{7}$	TCTCCTTTTTGCATATCTTTCGTG TAGATTCGGTTGTAGTTTTCCTGC	107
9	*ACVB93*	AJ420078	$(GTG)_{3+4+1}$	GTCCTTTGCAAATCACATCGG TTAATGACTTCAATCCACAAACCC	140
10	*ACVB93A*	AJ420079	$(CA)_{2+3}$	GTGGCCGTTGTTCGTCCTTTTG 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 CAGCAGTTTGCCGGAAGGAGAG	168
14	*ACVIB213*	AJ420076	$(GA)_{7+1+5}$	ATAAAACGCCCCGCATCATACATG CACGGCACATTCCCTCCCATA	116
15	*ACVIIB46*	AJ420075	$(CAA)_{1+4}$	AACCGGAAGCAGTATCGCACAC GAGGCTCCTTCGTTATCCG	140
16	*ACVIIIB40*	AJ420077	$(CA)_{5}$	TCAAGCTGGACAATGTAACTCAAC GTTCAATCAAACCCAGCCAAAC	118
17	*ACVIIIB182A*	AJ420080	$(GA)_{1+6}$	GTTTAGCTTCGGGCCTTTCCATAC GAGATACAACCGGTGCGTCAGC	170

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, etc. 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, MRC for the first time isolated microsatellite markers from *An. culicifacies* species A. Partial genomic library was prepared and clones were screened for 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 5). 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 in species B population were found working in species B and were found polymorphic too.

Population Genetic Analysis of Malaria Vectors

Using Microsatellite Markers in An. culicifacies

Three populations of *An. culicifacies* species A collected from Gujarat, Haryana and Karnataka were genotyped using 12 microsatellite markers isolated by MRC. 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. 41. Study is in progress to test too 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 to11 alleles.

Using Paracentric Inversion Markers in An. annularis

Populations of *An. annularis* from six localities of India (Districts Shahjahanpur, Ghaziabad, Alwar,

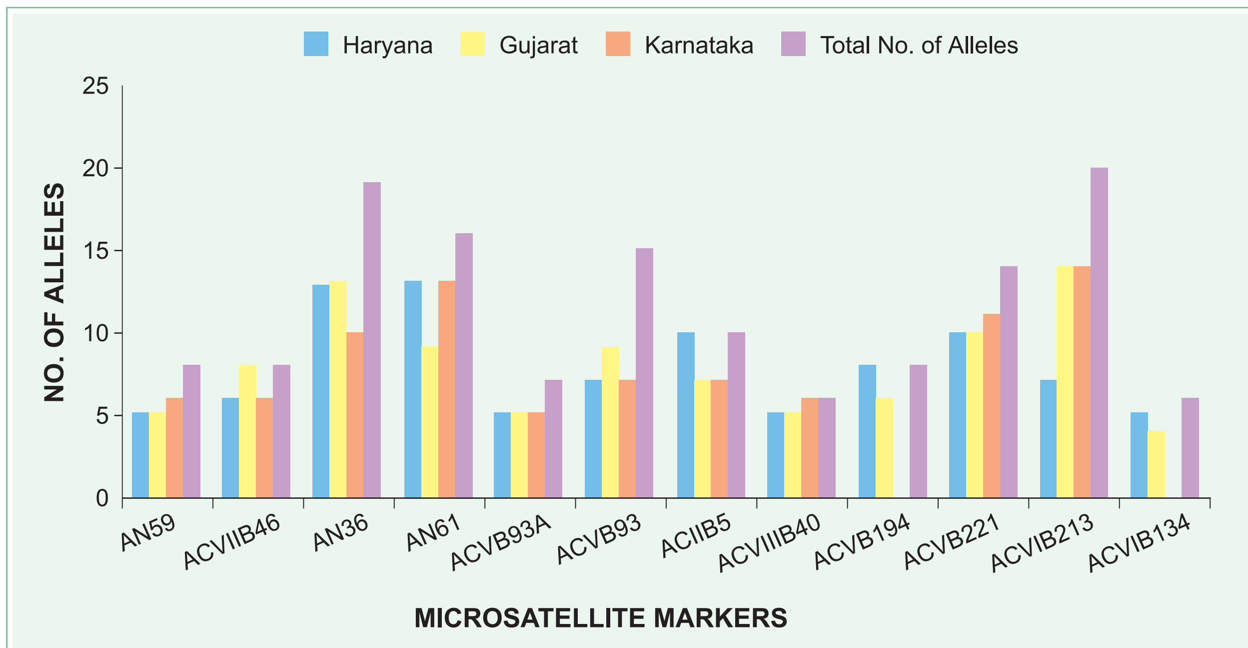

Fig. 41: **Allele distribution of 12 microsatellite loci in three populations of *An. culicifacies* species A**

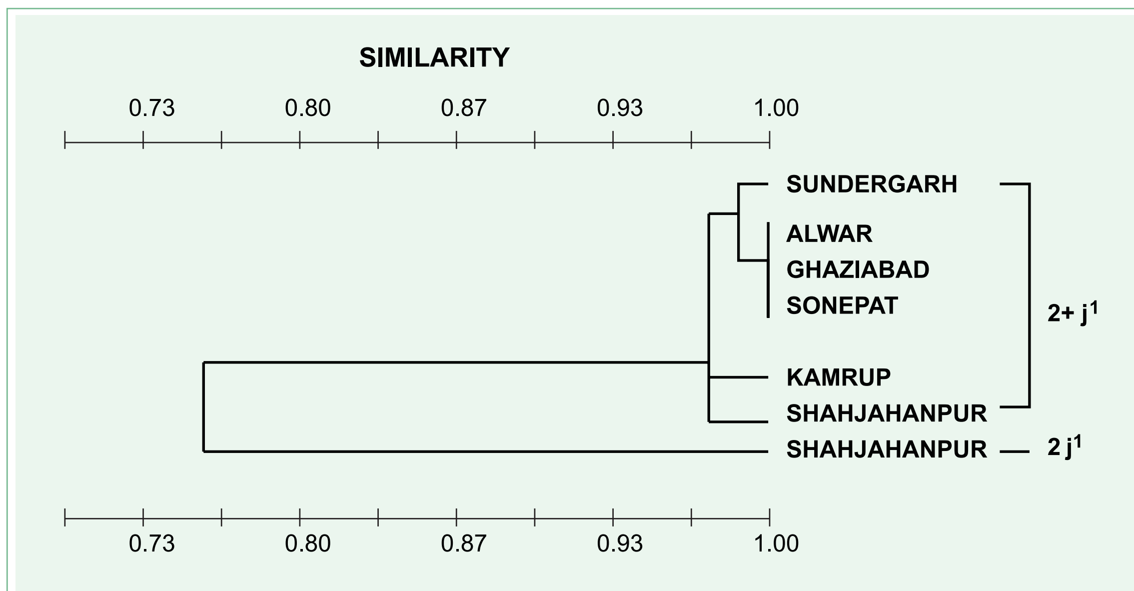

Fig. 42: **A phenogram of *An. annularis* population of India produced by the unweighted pair group method of Nei with arithmetic average (UPGMA) cluster analysis of the average similarity matrix (Cophenetic correlation = 0.989)**

Sundergarh, Sonepat and Kamrup) were genotyped for inversion polymorphism by examining ovarian polytene chromosomes (Atrie *et al.,* 1999). A total of 9 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^1on chromosome arm 2, which was taken as 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 $2 j^1$. The BIOSYS-1 computer programme of Swofford and Selander revealed two clusters of the phenogram (Fig. 42), one of 6 populations with $2+j^1$ (species A) and another of $2j^1$ population (species B). The analysis further showed a good correlation between genetic distances and geographical distances among populations of species A (loc. cit.).

Using Paracentric Inversions in Urban and Rural Populations of An. 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 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, 1996). In addition to urban population, population from 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). ■