ANOPHELES CULICIFACIES COMPLEX: CYTOGENETIC CHARACTERIZATION OF RAMESHWARAM ISLAND POPULATIONS

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ABSTRACT. *Anopheles culicifacies sensu lato* collected from Rameshwaram island, Tamil Nadu state, India was identified as species B based on the diagnostic inversion karyotype Xab $2g^{1+h^1}$ as observed in polytene chromosomes. Among male mitotic karyotypes made from larval neurogonial cells, two types were observed: one with an acrocentric Y-chromosome and the other with a sub-metacentric Y-chromosome, both had sub-metacentric X and metacentric autosomes. The Rameshwaram population is identical to species B in its genetic relationship with species A and C as determined by experimental hybridizations (sterile and fertile male hybrids, respectively).

INTRODUCTION

Cytotaxonomic studies have so far identified 4 reproductively isolated populations in the taxon *Anopheles culicifacies* Giles. These populations have been given the species status and are provisionally designated as species A and B (Green and Miles 1980), species C (Subbarao et al. 1983) and species D (Subbarao et al. 1988a, Vasantha et al. 1991). The 4 sibling species were identified on the basis of positive assortative mating observed among populations differing in paracentric inversions. Sibling species are also distinguishable by structural variation in the Y-chromosomes (Vasantha et al. 1982, 1983; Suguna et al. 1989).

Extensive surveys were carried out in the country to establish the distribution of sibling species (Subbarao et al. 1988a). Longitudinal and spot surveys were also conducted to establish biological differences among sibling species (Subbarao et al. 1987, Joshi et al. 1988). Incrimination studies using a two-site immuno-radiometric assay established species A, C and D as vectors of malaria (Subbarao et al. 1988c, 1992). Further, it was shown that species B may play a negligible role, if at all, in malaria transmission.

On Rameshwaram island only species B was found (Subbarao 1984), but *An. culicifacies s.l.* had been incriminated and is considered as the major vector of malaria on the island (Sabesan et al. 1984). A similar situation exists in Sri Lanka (Subbarao 1988, Wickramasinghe and Samarasinghe 1991). To resolve this paradox, a cytogenetical study of *An. culicifacies s.l.* populations from Rameshwaram island and a few areas in Tamil Nadu state was undertaken: 1) to establish sibling species composition by polytene chromosome and mitotic chromosome examination, and 2) to analyze genetic relationships of the island population with other *An. culicifacies* sibling species identified by hybrid sterility/fertility in genetic crosses. Results are reported in this paper.

MATERIALS AND METHODS

Anopheles culicifacies adults were collected from: 1) Manali, about 40 km from Madras city, 2) Kolamanjanur, Rayandapuram and Vepur chekadi villages near the Sathanur dam area in North Arcot district, and 3) Karaiyur, Rajakoil, Natarajapuram and Tharvaithoppu villages on Rameshwaram island in the district Ramanathapuram in Tamil Nadu state (Fig. 1). Three collections were made in December 1990 and in May and June 1991 from Tharvaithoppu village on Rameshwaram island.

For sibling species identification, ovaries were removed from half-gravid females collected in the field and were fixed in 1:3 glacial acetic acid and methanol. Polytene chromosomes were prepared according to the method of Green and Hunt (1980) and sibling species were identified following the diagnostic inversion karyotypes given in Subbarao et al. (1988a).

Adult *An. culicifacies* collected from Rameshwaram island were brought to Delhi for establishing a laboratory colony. Mosquitoes were reared in the insectary maintained at 28 ± 1°C and 70–80% RH with simulated dawn and dusk conditions. Mitotic chromosome preparations were made from neurogonial cells of late III or early IV instar larvae by the squash technique of Breland (1961) with slight modifications. The brain was dissected from male and female larvae (sexed by examining the gonads) and placed in 0.1% colchicine for 1–1.5 h, stained in 2% lacto-aceto orcein for 10 min and squashed. Isofemale lines having different Y-chromosome karyotypes were isolated by examining 5–10 progeny of single females.

Stocks were used in crosses with *An. culicifacies* from Rameshwaram island as in Table 1. Reciprocal crosses among stocks were carried out in 30 × 30 × cm cloth cages. Only F_1 adults of the Rameshwaram population were used in all the crosses. Mosquitoes were offered fresh

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Fig. 1. Map of India showing areas surveyed.

Table 1. Stocks used in crosses with *Anopheles culicifacies* from Rameshwaram island.

Species	Inversion genotype observable in polytene chromosomes	Metaphase Y-chromosome	Collection areas for establishing the stocks
A	$X+^{a}+^{b}\ 2+^{g^1}+^{h^1}$	Sub-metacentric	District Ghaziabad, Uttar Pradesh (UP) in July 1990.
B	$Xab\ 2g^1+^{h^1}$	Acrocentric	District Shahjahanpur, UP in August 1990.
C	$Xab\ 2+^{g1}\ h^1$	Sub-metacentric	District Allahabad, UP in March 1988.
R	$Xab\ 2g^1+^{h^1}$	Acrocentric + sub-metacentric	Rameshwaram island
RAC	$Xab\ 2g^1+^{h^1}$	Acrocentric	Rameshwaram island
RSM	$Xab\ 2g^1+^{h^1}$	Sub-metacentric	Rameshwaram island

Table 2. Y-chromosome types observed in the Rameshwaram colony of *Anopheles culicifacies* established from material collected in May and June 1991.

Period examined	No. examined	Y-chromosome		
		Acrocentric	Sub-metacentric	Unidentified
June, 1st week	47	10	9	28
June, 3rd week	8	3	3	2
July 1991	21	10	5	6
Sept. 1991	10	0	10	0
Jan. 1992	14	1	9	4

water-soaked raisins and 1% glucose water on cotton pads. Females were offered rabbits as a bloodmeal source. In all crosses, eggs were collected in a pool and counted after 2 days to determine the hatch rate. Larvae were reared and sexed at the pupal stage. Reproductive organs of hybrid males and females were dissected out and examined under the light microscope.

RESULTS

Anopheles culicifacies collected from Manali (n = 17), Sathanur dam area (n = 7) and Rameshwaram island 1 (n = 115) were of the Xab $2g^{1+h^1}$ karyotype. Hence, the populations were identified as species B. A colony was established with adults collected from Rameshwaram island.

Mitotic karyotype: Both male and female F_1 progeny of field collected adult females had 3 pairs of chromosomes ($2n$ = 6), females had homomorphic and males heteromorphic sex-chromosomes. Among 39 F_1 male larval neurogonial preparations made, two different mitotic karyotypes at the metaphase stage were observed: 23 with the Y-chromosomes having an arm-ratio ranging between 3.6 to 4.8 and 16 with Y-chromosome having an arm-ratio of 1.5 to 2.1. In both karyotypes, the X-chromosome had an arm-ratio ranging between 1.4 and 2.3. Y-chromosomes having a higher arm-ratio would be referred to as acrocentric and that with the lower as sub-metacentric and also the X-chromosome as sub-metacentric (Fig. 2). In the F_2 generation, of the 26 preparations examined, only 10 could be identified, of which 3 had acrocentric and 7 sub-metacentric Y-chromosomes. The frequencies of the 2 types of Y-chromosomes observed in the colony established subsequently from the mosquitoes collected in May and June 1991 are given in Table 2.

Isofemale lines having acrocentric and those having sub-metacentric Y-chromosomes had the Xab $2g^{1+h^1}$ (species B) inversion genotype in polytene chromosomes.

Genetic crosses: Results from reciprocal crosses between species A and B, and between strains from Rameshwaram and sibling species A, B and C are given in Table 3.

Results from crosses between species A and B (crosses 1 and 2) were as reported earlier (Subbarao et al. 1988d), i.e., the hatch rate was normal in A female and B male cross and 0% hatch in the reciprocal cross. Hybrid females were fertile and hybrid males had extremely underdeveloped or rudimentary testes lobes and vasa deferens and fully developed ejaculatory duct and accessory glands, i.e., sterile.

Results from reciprocal crosses between species A and *An. culicifacies s.l.* from Rameshwaram island (R), and between species A and the R line with acrocentric Y-chromosome (RAC) were similar to those from species A and B. In the cross between female species A and the male sub-metacentric line (RSM) there was no egg laying, and in the reciprocal cross there was egg laying but the hatch rate was 0.7%. Males from the Rameshwaram colony (at the time when males were of predominantly sub-metacentric type) when crossed with A females produced sterile hybrid progeny (Table 3).

In the reciprocal crosses between species B and the R strain and in those between species C and the R strain, fertility was >90% and hybrid females and males had fully developed reproductive organs. Backcrosses of F_1 males and females to both parental strains were fertile (data not shown in the table).

DISCUSSION

Anopheles culicifacies species B from districts Faridabad (Haryana), Ghaziabad and Nainital (UP) and Aurangabad (Maharashtra) had acrocentric Y-chromosomes (Vasantha et al. 1982). Lines established recently in 1990 from Shahjahanpur (UP) and Alwar (Rajasthan) also had acrocentric Y-chromosomes. Suguna et al. (1983, 1989) reported the same from North Arcot district, Tamil Nadu state. In Rameshwaram, two populations that are homosequential for the polytene chromosome arrangements but different male mitotic karyotype were observed: population 1—sub-metacentric Y-chromosome and population 2—acrocentric Y-chromosome.

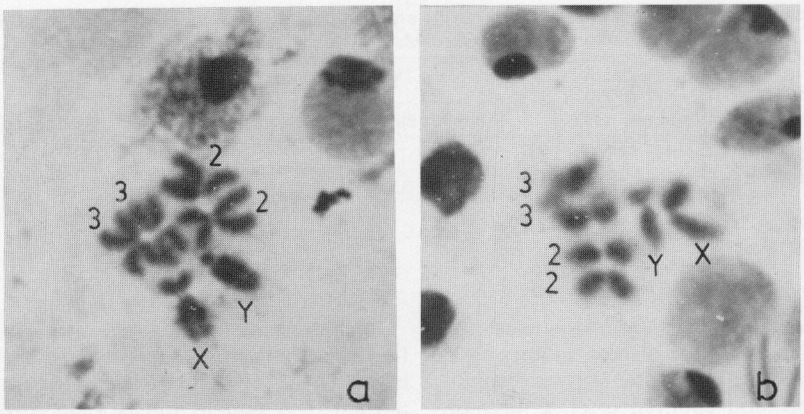

Fig. 2. Male mitotic karyotypes of *An. culicifacies* from Rameshwaram island. a) Karyotype with acrocentric Y-chromosome, b) karyotype with sub-metacentric Y-chromosome.

Table 3. Results from crosses between different strains of *Anopheles culicifacies*.

Cross no.	Cross		No. eggs laid	Percent hatch	Male reproductive organs	
	Female	Male			No. examined	Status
1	A	B	193	92.2	25	S
2	B	A	140	0	—	—
3	A	R*	262	97.3	42	S
4	R*	A	2488	0.7	—	—
5	A	RAC	159	94.3	20	S
6	RAC	A	285	0	—	—
7	A	RSM	0	—	—	—
8	RSM	A	207	0.75	—	—
9	A	R**	866	94.2	96	S
10	R**	A	690	0	—	—
11	B	R*	152	94.7	55	N
12	R*	B	221	98.2	50	N
13	C	R*	473	95.1	33	N
14	R*	C	477	92.5	50	N

N = Fully developed reproductive organs with sperm.
S = Partially developed or atrophied reproductive organs and no sperm.
* = F_1 progeny of females collected from field.
** = From the cyclic colony. At the time of setting up the cross, one acrocentric and 9 sub-metacentric Y-chromosomes were observed and all 18 male progeny examined from the cross had sub-metacentric Y-chromosomes.
R = *An. culicifacies s.l.* from Rameshwaram island.
RAC = *An. culicifacies* from Rameshwaram with acrocentric Y-chromosome.
RSM = *An. culicifacies* from Rameshwaram with sub-metacentric Y-chromosome.
A = *An. culicifacies* from Ghaziabad, UP.
B = *An. culicifacies* from Shahjahanpur, UP.
C = *An. culicifacies* from Allahabad, UP.

Anopheles culicifacies from Rameshwaram island produced bidirectionally fertile hybrid males with species B, and was identical to species B in its genetic relationship with species A and C. Within the Rameshwaram colony, a slow elimination of acrocentric Y-chromosomes leaving sub-metacentric Y-chromosomes in the population was observed, however, the sample size was small. Crosses between acrocentric and sub-metacentric Y-chromosome lines were not made as the 2 lines could not be maintained long enough to do so. Since polytene chromosomes are homosequential in the 2 lines, a prezygotic barrier could not be ascertained.

In laboratory colonies established from mixed populations of species A and B, the $X+^{a}+^{b}$ $2+^{g^1}+^{h^1}$ arrangement of species A disappeared and in those of species B and C, the Xab $2+^{g^1}h^1$

arrangement of species C disappeared (Subbarao et al. 1988d). In "choice-mating" experiments (loc. cit.) species A and B were found to mate with their own kind, although a few matings between species A and B were observed. Between species B and C, species C were found to prefer to mate with species B and a low frequency of matings was observed within species C. Thus, male hybrid sterility observed between species A and B was not the reason for the elimination of the species A arrangement. In spite of bidirectional hybrid male fertility between B and C, species C was eliminated. Therefore, the possibility of species varying in their biological fitness was suggested (Subbarao et al. 1988d).

Species B populations examined from other parts of India were of the acrocentric Y-chromosome type, and epidemiological (Subbarao et al. 1988c) and incrimination data (Subbarao et al. 1988b) from several areas suggested that species B is not a vector. Hence, the possibility of the new population on Rameshwaram island, i.e., species B having sub-metacentric Y-chromosome being a vector should be examined. In Sri Lanka where only species B was found and *An. culicifacies s.l.* was incriminated (Wickramasinghe and Samarasinghe 1991), a similar study is suggested.

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