

PERICENTRIC INVERSIONS ON CHROMOSOME 2 IN *CULEX TRITAENIORHYNCHUS*

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ABSTRACT. Three pericentric inversions on chromosome 2 were isolated following radiation treatment which reduced crossing over between the 2 morphological mutants *e* and *Rs* (27–37 map units apart). One inversion, *In(2)1*, had only 2.2% crossovers between these markers while *In(2)2* and *In(2)3* reduced recombina-

tion to 5.9 and 6.7% respectively. The latter 2 inversions (possibly identical) were made homozygous, and both males and females were fertile. *In(2)1* appears to be lethal when homozygous. A scheme employing these inversions to detect additional mutations (visible and lethal) on chromosome 2 is presented.

The reduction in the recovery of recombinants is a necessary precursor in genetic systems for the detection of mutations. Most mutation detecting systems require the use of a balanced lethal stock carrying a dominant mutant marker with a lethal effect when homozygous or a codominant and a recessive mutant and crossover suppressors (inversions) which restrict recombination. For some species lack of crossing over in 1 sex is a natural phenomenon. In 1 species of mosquitoes, *Culex tritaeniorhynchus*, complete linkage is generally found in females, while crossing over normally occurs in males. Thus to restrict crossing over in males, the presence of inversions are needed to develop mutation detecting systems.

This paper reports data on 3 inversion lines produced in this mosquito for possible use in the detection of mutations on chromosome 2. Inversions have been produced on chromosome 1 in this species (Baker et al. 1971), and a mutation detection system has already been tested (Sakai and Baker 1972, 1974). The current linkage map for chromosome 2 is:

0	34	36	48
<i>Rs</i>	<i>Aph</i>	<i>e</i>	<i>Ldh</i>

Two of the loci, *Aph* (alkaline phosphatase, Sakai et al. 1973) and *Ldh* (lactate dehydrogenase, Sakai et al. 1975) are detected by gel electrophoresis while 2 are morphological, *Rs*, (red spotted eye, Rabbani

and Baker 1970) and *e* (ebony, Sakai et al. 1972).

MATERIALS AND METHODS

The stocks used in these experiments were:

A) *e Rs/e Rs*: This strain carries 2 linkage group II mutants, ebony, *e*, a recessive (Sakai et al. 1972) and red-spotted eye, *Rs*, a codominant mutant (Rabbani and Baker 1970, Sakai et al. 1972) which can be detected only in a homozygous white eyed background, *w/w* (linkage group I). The heterozygotes (*RS/+*) are clearly distinguishable from the homozygotes (*RS/RS*) by the amount of spotting present: the homozygotes have dense patches of spots, while in the heterozygotes the spotting is sparse. This stock is also homozygous for white eye.

B) *+/+/+*: This stock which is wild type for *e* and *Rs* is homozygous for white eye.

C) *e/e*: This stock is homozygous for ebony and white eye.

A diagram of the scheme used to detect, isolate and maintain inversions is given in Figure 1. The experiment was designed to detect only large inversions that suppressed crossing over between the *e* and *Rs* loci in males.

Sixty 3-day old *eRs/e Rs* males (stock A) were treated with approximately 3000 r gamma radiation from a ⁶⁰Co source. After treatment, the males were mass

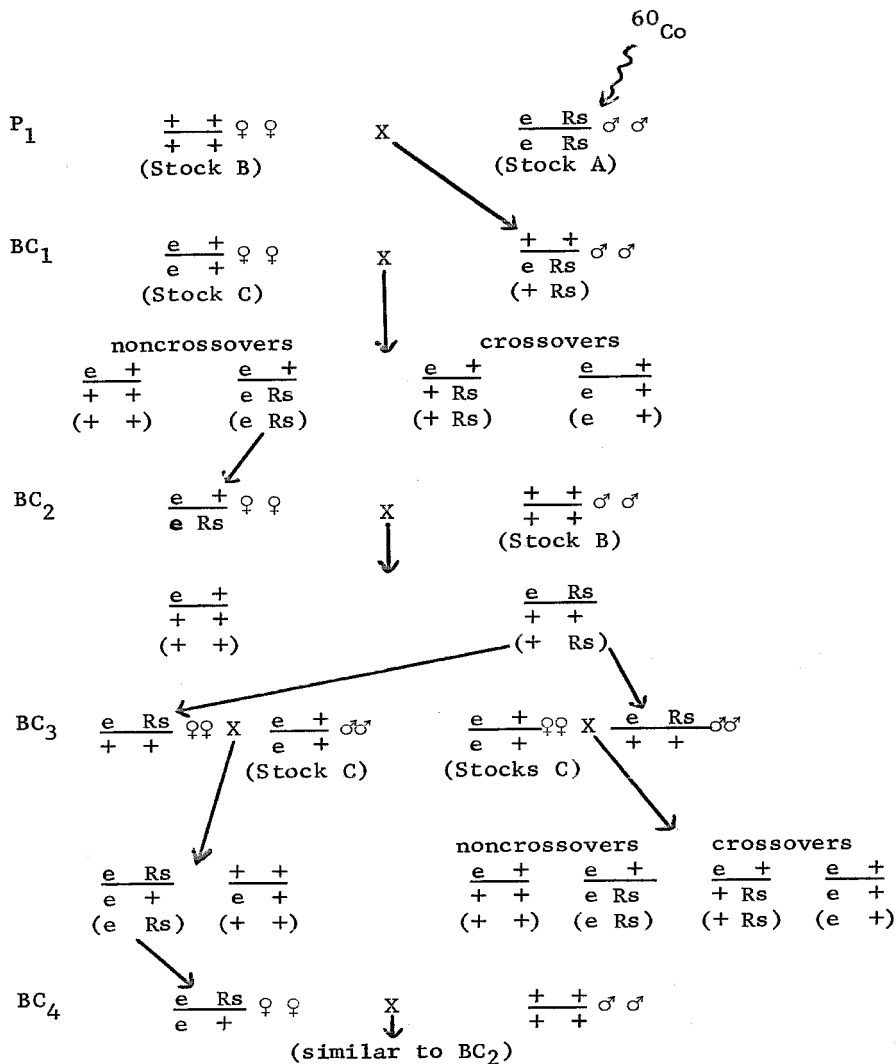


Fig. 1. Mating scheme for isolation of heterozygous inversions.

mated to $+/++$ females (stock B). The resulting F_1 heterozygous males were backcrossed (BC_1) to the ebony females (stock C) in 15 separate cages. One hundred and forty-two egg rafts were collected and those showing at least 20% sterility were reared and scored for recombinations between e and R_s among the resulting adults. Ebony-sparsely spotted females from families showing less than 11% crossing over were backcrossed (BC_2) to males of stock B and the resulting sparsely spotted females were backcrossed (BC_3) to the ebony males (stock C) to maintain the line unaltered by recombination while the sparsely spotted males were crossed to stock C females to assess recombination. With this mating scheme the frequency of crossing over can only be detected in males every other generation (BC_1 , BC_3 , BC_5 etc.). Methods used in handling larvae were similar to those reported earlier (Baker and Sakai 1972). The chromosomes from ovaries and testes were observed using previously reported techniques (Baker et al. 1971, 1977). Controls for these experiments consisted of crosses identical to those described above except the $e R_s/e R_s$ males not treated with ^{60}Co .

RESULTS AND DISCUSSION

A total of 142 rafts of the treated BC_1 were collected and counted. Fifty-five rafts with greater than 20% sterility were reared as individual families to the adult

stage. Of these only 9 families showed less than 11% crossovers. In the BC_3 , only 2 lines tested had less than 11% crossovers, (one other promising line was not tested until the BC_5 generation). The pooled data for all 3 lines, now designated $In(2)1$, $In(2)2$ and $In(2)3$, are given in Table 1 along with the control BC_1 crosses. $In(2)1$ had the lowest frequency of recombinants (2.2%) and the highest sterility (53.3%). $In(2)2$ and $In(2)3$ had similar crossover and sterility values ($P < .05$), and possibly originated from the same BC_1 heterozygous father. Both lines were isolated from the same BC_1 cage (15 cages were used in this generation), while $In(2)1$ was from a different cage. As expected no sterility was found in females heterozygous for the inversions since little crossing over is found in females of this species. The lack of sterility in females would suggest the absence of translocations (Baker et al. 1971).

The normal metaphase chromosomes for this species ($2N = 6$) are shown in Figure 2a; the correlation between the three pairs of chromosomes and their respective linkage groups have been reported (Baker, et al. 1971, 1977; Sakai et al. 1971). The smallest metacentric chromosome (1) has been correlated to linkage group I. From translocation experiments, the large submetacentric chromosome (2) has been correlated to linkage group II. Moreover, it has been suggested that the R_s locus is associated with the short arm, 2L, and the e locus with the long arm, 2R (Sakai et al. 1971, Baker

Table 1. Pooled results for backcrosses involving $c +/e + \varphi \varphi \times ++/e R_s \delta \delta$ from lines carrying an inversion on the $e R_s$ chromosome and the control.

Line	F ¹	# Eggs	% S ²	% R ³	% e-Rs Crossovers	Progeny Genotype			
						$++$ e +	$e +$ c +	$+ R_s$ e +	$e R_s$ e +
$In(2)1$	54	6468	53.3	72.1	2.2	1086	29	20	1043
$In(2)2$	50	4940	44.6	79.6	5.9	1065	51	77	986
$In(2)3$	37	3784	44.3	69.8	6.7	757	43	55	617
Control	20	2299	4.6	76.8	27.5	720	221	242	503

¹ = Number of families.

² = Percent sterility = total unhatched eggs/total eggs laid \times 100.

³ = Percent successful rearing = total adults/total first instar larvae \times 100.

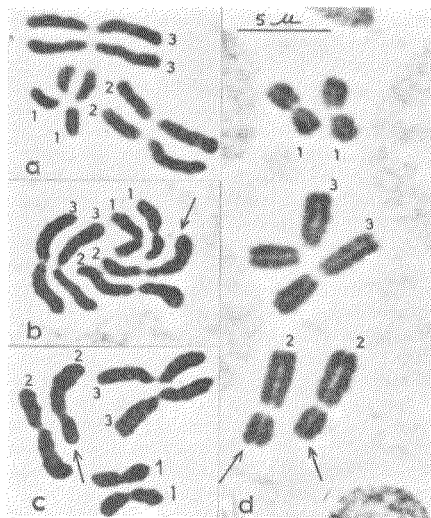


Fig. 2. Metaphase chromosomes from adult ovaries. Arrows point to pericentric inversion chromosomes. a- Normal "wild type" complement. b- *In(2)1* heterozygote. c- *In(2)2* heterozygote. d- *In(2)2* homozygote.

et al. 1977). The chromosomal complements from those adults heterozygous for *In(2)1* and *In(2)2* are given in Figures 2b and c respectively. Chromosomes 1 and 3 appear unaltered but the centromere in chromosome 2 is in a new position, resulting in a partial disruption of the close pair-

ing between homologues. This centromeric shift suggests the presence of a pericentric inversion. In addition, examination of anaphases in the testis gave no evidence of chromatid bridges or fragments.

ATTEMPTS TO ISOLATE HOMOZYGOUS INVERSIONS. Attempts to produce homozygous pericentric inversion stocks were carried out by crossing heterozygous inversion females and males to each other (*e Rs/++ ♀♀* × *e Rs/++ ♂♂*). The results of these crosses are given in Table 2. Since the inversions are on chromosomes marked with *e* & *Rs*, *e Rs/e Rs* individuals would be expected to be homozygous for the inversions. *In(2)1* had no homozygous *e Rs/e Rs* adults suggesting that this inversion carries at least one recessive lethal. Both *e Rs/e Rs* females and males were found in the *In(2)2* and *In(2)3* crosses, and cytological examinations of ovaries and testes confirmed that these individuals were indeed inversion homozygotes (Figure 2d). The homozygous females and males for both *In(2)2* and *In(2)3* are fertile.

Thus with the presence of an inversion on chromosome 2 marked with a codominant mutant and recessive mutant (*Rs* and *e*), it is now possible to screen for additional mutants on chromosome 2 in laboratory populations marked with white eyes following chemical and radiation treatments. Figure 3 outlines one possible method.

Table 2. Pooled results for crosses involving females and males heterozygous for the inversions. (*e Rs/++ ♀* × *e Rs/++ ♂*) with the *e Rs* chromosomes carrying the inversion.

Line	F ¹	# Eggs	% S ^a	% R ^a	% Crossovers	Progeny Genotype				
						Noncrossovers			Crossovers	
						++	++ e Rs	e Rs	e Rs e +	e Rs + Rs
<i>In(2)1</i>	10	1319	53.3	60.9	3.2	133	230	0	5	7
<i>In(2)2</i>	8	1415	54.9	64.5	3.6	105	240	51	10	5
<i>In(2)3</i>	10	1544	38.2	67.0	2.3	148	387	89	12	3

¹ = Number of families.

² = Percent sterility = total unhatched eggs/total eggs laid × 100.

³ = Percent successful rearing = total adults/total first instar larvae × 100.

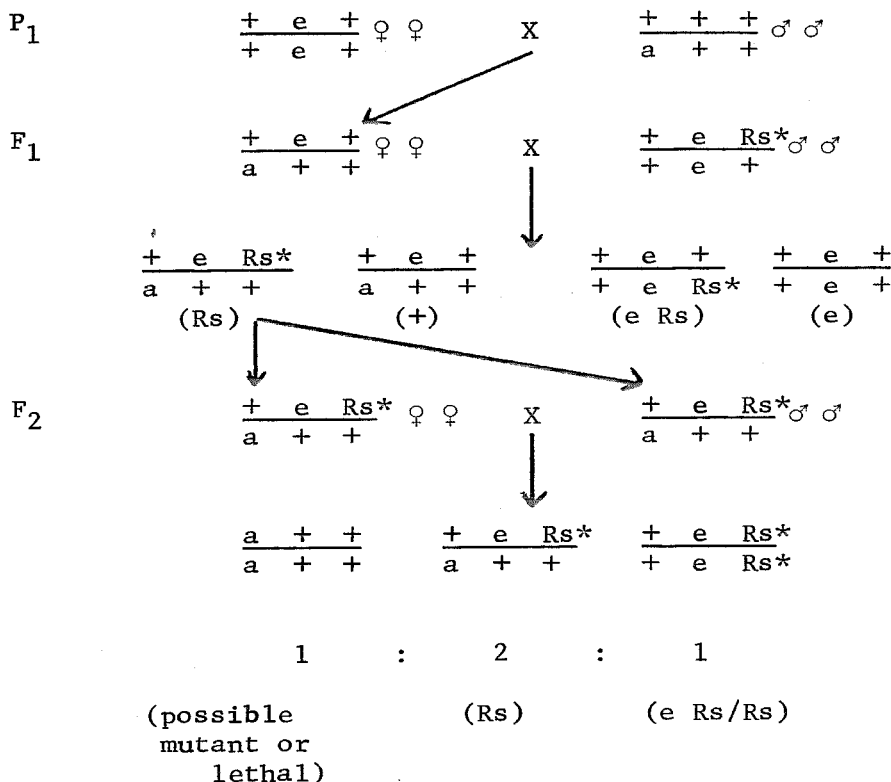


Fig. 3. Diagrammatic representation of the crosses employed to achieve homozygosity of chromosome 2(a) from white eyed populations. The + e Rs* chromosome contains a crossover suppressor.

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SHORT WING, A RECESSIVE MUTANT ON CHROMOSOME 1 IN *CULEX TRITAENIORHYNCHUS*

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ABSTRACT. A recessive, sex-linked mutant, short wing (*sw*), was isolated from a strain which contained an induced translocation. Genetic crosses revealed that the gene sequence is w^{re}

(rose eye) - $sw - M$ (sex) and the recombination frequencies are as follows: $w^{re} - sw = 7.3\%$, $sw - M = 5.9\%$ and $w^{re} - M = 13.2\%$.

During routine culling of a strain of *Culex tritaeniorhynchus* containing an autosomal translocation, a number of individuals with short wings were found. The wings of these individuals were greatly shortened and broadened with very prominent venation; in addition, the segments of the legs were usually shorter and thicker than the wild type. The larvae and pupae of this mutant appear to develop normally, but there is considerable mortality during and shortly after emergence of the imago. Some of the adults are unable to extricate their legs from the pupal case while others seem unable to fly from the surface of the water and consequently drown. A pure line of short wing was

selected without the presence of the translocation. The morphological characteristics as well as the viability problems associated with this mutant are similar to those of the short wing mutant in *Aedes aegypti* (Uppal et al. 1976). The results of the genetic analysis of short wing (*sw*) in *Cx. tritaeniorhynchus* are reported.

MATERIALS AND METHODS

The following strains of *Cx. tritaeniorhynchus* were used in the crosses:

1) Balloki—a wild type strain colonized from Balloki Headworks with the M^1 sex allele (Baker and Sakai 1976).

2) rose (w^{re})—this strain contains the