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A Method for Chromosome Location of New Autosomal Recessive Mutations of *Ceratitis capitata* (Wiedemann) (Diptera: Tephritidae) Based on Crosses With Three Strains Bearing Autosomal-Y Translocations

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Un Método para la Localización Cromosómica de Nuevas Mutaciones Autosómicas y Recesivas en *Ceratitis capitata* (Wiedmann) (Diptera: Tephritidae) Basado en Cruzamientos con Tres Líneas Portadoras de Translocaciones Y-Autosoma

RESUMEN – Se propone un método para la localización de cualquier nueva mutación autosómica recesiva en la mosca del Mediterráneo *Ceratitis capitata* (Wied). La localización cromosómica se logra mediante una serie de cruzamientos que detectan pseudo-ligamiento entre sexos y la mutación, empleando tres líneas con translocaciones ligadas al cromosoma Y.

PALABRAS CLAVE: Insecta, mosca del Mediterráneo, cromosoma Y, pseudo-ligamiento.

ABSTRACT – A method is proposed to locate any new autosomal and recessive mutation in the Mediterranean fruit fly, *Ceratitis capitata* (Wied.). A series of crosses searches for pseudo-linkage between sex and the mutation by employing three strains with Y-linked translocations, thereby indicating its chromosome location.

KEY WORDS: Insecta, Mediterranean fruit fly, Y chromosome, pseudo-linkage.

The first mutations in the Mediterranean fruit fly, *Ceratitis capitata* (Wiedmann), were described in the middle 1970s (Sharp & Chambers 1973, Rossler & Koltin 1976). Since then, the number of laboratories working on the isolation of new mutants has increased rapidly. Many of those efforts were sponsored by the International Atomic Energy Agency (IAEA) through a Co-ordinated Research Programme begun in 1980. The ADH locus was the first to be assigned to a medfly chromosome in 1986 by cytological analysis of a translocation (Zapater & Robinson 1986).

The chromosome location of any new mutation normally requires a series of crosses and flies scoring along two successive generations using markers already assigned to their respective chromosomes. A technique using multiple marker strains has been proposed (Saul & Rossler 1984) to map any new mutations to their chromosomes. Possible genetic interaction between mutations may also require investigation to determine the mutant's location within the genome.

A method employing three Y-linked translocations is here proposed to locate any new autosomal recessive mutation in the medfly. A series of crosses searches for pseudo-linkage between sex and the mutation, indicates its chromosome location.

The basis of this method lies on the association established by the translocation between the new mutation and the Y

chromosome.

This is due to the inviability of aneuploid zygotes that inherit part of the translocation. These aneuploid segregation products for autosomes and Y chromosomes will not be recovered.

Material and Methods

Isolation of Translocation Strains. Translocations were isolated by the pseudo-linkage technique in the laboratory. Wild-type Castelar males were irradiated with 3 krads. of X-rays and mass-crossed with a triple marker strain named G, homozygous for dark pupae, dp, (Rossler & Koltin 1976), apricot eyes, ap, and white pupae, wp, (Rossler 1979), mutations. These markers are located on chromosome 3, 4, and 5 respectively. F₁ males were individually backcrossed to 8 G females and their progeny scored for translocations.

Description of the Translocations. The following three translocations, one of them single and the other two double, were employed in this protocol: T: (Y, 3, 5) 11 wp+ dp+, T: (Y,4) 104 ap+ and T:(Y; 2, 3) 97 dp+. The stocks isolated 30 generation ago (ca. 3 yrs.) were passed to each succeeding generation with 150 pupae per cycle. No recombinant or contaminant flies were observed during routine screening.

The translocation genotypes were confirmed by additional crosses with known markers (Zapater 1990). Additionally, the translocations T: (Y, 3, 5) 11 were confirmed cytologically (A. Zacharopoulou, University of Patras, Greece, personal communication).

Strain Fitness. Single pair crosses were set up in each of the three translocation strains and in the control (Castelar); progeny were evaluated for egg hatch, larval survival, adult emergence, overall survival and sex ratio (Table 1). Egg hatch was recorded as the percentage of eggs laid which hatched. Larval survival was the percentage of hatched eggs that pupated. Adult emergence was the percentage of pupae which

(Table 2). From those combinations, the chromosome location is deduced. The mutation will be expressed only in female of strains that have the translocation involving the same autosome containing the new mutation. On the other hand, male and female mutants will appear in strains having translocations that do not involve the autosome where the mutation is located. Additional consideration should be given to locating less than fully penetrant mutations where both mutant and wild-type females would appear in a strain showing pseudo-linkage. In these cases, only males but not females from the F₂ should be screened.

Male recombination could alter the expected protocol results. This process could generate just a few mutant males

Table 1. Egg hatch, larval survival, adult emergence, overall survival and sex ratio for the T: (Y, A)s strains and control in percentages of *C. capitata*.

Strain	N° eggs	Egg hatch Mean±S.D	Larval survival mean±S.D.	Adult emergence mean±S.D.	Overall survival mean±S.D.	Adult sex ratio ♂ : ♀
Control	831	90.5 ± 1.9a	87.0 ± 2.9a	97.8 ± 2.9a	76.8 ± 2.8a	1 : 0.99
T(Y,3,5)11	745	44.0 ± 4.9b	68.2 ± 4.1b	93.1 ± 3.6b	27.9 ± 4.3b	1 : 0.80
T(Y,4)104	911	63.6 ± 3.1c	79.3 ± 4.3c	86.5 ± 5.3c	41.9 ± 3.9c	1 : 1.19
T(Y,2,3)97	870	63.5 ± 7.0c	84.5 ± 3.6a	70.0 ± 4.3d	37.4 ± 5.1d	1 : 1.07

Means followed by the same letter within columns do not differ significantly at 5% level.

eclosed. Overall survival was the percentage of eggs which eclosed as adults.

Single crosses were set up between translocation males and females from the three strains. Egg hatch, larval survival, adult emergence and sex ratio are presented in Table 1.

Crosses and Protocol Proposed. The protocol proposed to locate any new mutation involves the following steps: a) males from the three T: (Y, A) are separately mass-crossed to virgin homozygous females for the new mutant; b) F₁ male offspring are back-crossed to mutant females and c) 20-30 individuals are examined for the mutant phenotype in the F₂.

Statistical analysis was carried out by one way ANOVA; significant differences among treatments were determined by Duncan's multiple range test.

Results and Discussion

The chromosome location of any new autosomal recessive mutation can be detected by scoring the descendants from the three crosses described above.

The protocol proposed works because only the euploid males and females, resulting from alternate segregation products, survive (Fig. 1). Aneuploid individuals do not reach the adult stage in these strains, but die as eggs, larvae or pupae.

Depending in which chromosome the mutation is located, five different combinations could result from the three crosses

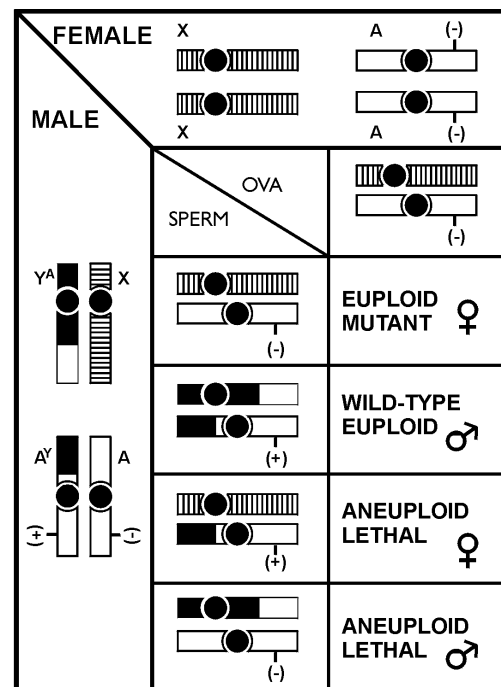


Figure 1. Expected results for a cross between F₁ translocation males and mutant females in *C. capitata*. Euploid individuals result from alternate segregation products, while aneuploid individuals result from adjacent segregation products.

Table 2. Detection scheme for the chromosome location of any new autosomal, recessive mutation in *C. capitata*. It is indicated pseudo-linkage between sex and this mutation for each autosome and translocation. (autosome = A)

T (Y,A)	A = 2	A = 3	A = 4	A = 5	A = 6
T(Y,3,5)11	no	yes	no	yes	no
T(Y,2,3)97	yes	yes	no	no	no
T(Y,4)104	no	no	yes	no	no

and/or wild-type female (opposite to current expectation) in the translocation strains. Low levels of male recombination have been confirmed in the medfly (Rossler 1982). Although this phenomenon has not been observed in the three original stocks, the use of other mutations could result in a variable level of recombination. If an apparent recombination is observed, the cross should be repeated with larger number of individuals.

Yellow body, *ye*, (Zapater & Battista 1993), was located on chromosome 2 using this protocol (Table 3). As a control, white pupae, *wp*, was also evaluated and assigned to chromosome 5, as expected (Rossler 1990). To confirm the location of *ye*, additional crosses were performed with the mutant black body, *bf*, located on chromosome 2 (Gerald Franz, personal communication); *ye*, males were mass-crossed with *bf* females and the reciprocal crosses were also done. Phenotypes in each of the reciprocal crosses were scored for 188 and 97 adults. The absence of double mutants in the F₂ from both crosses confirmed that *ye* and *bf* are located on the same chromosome.

Van Heemert & Witteveen-Pillen (1980) used a similar system of one Y-linked and three autosomal translocations to map the ADH locus in the onion fly, *Hylemya antiqua*. The protocol here proposed is an improvement over their methods because it locates markers on all chromosome, double translocations reduce the number of crosses required, and Y-linked translocations strains are easier to maintain in the laboratory.

The protocol, described here for morphological mutants,

will also be useful for locating molecular genetics markers. When a number of molecular markers have been assigned to a particular chromosome, exact mapping can then be conducted to determine gene order.

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Table 3. Segregation obtained from F₂ crosses of the three tester translocations with the mutants yellow body (*ye*) and white pupae (*wp*) of *C. capitata*.

Strain	Yellow body				White pupae			
	Wild-type		Mutant		Wild-type		Mutant	
	♂	♀	♂	♀	♂	♀	♂	♀
T(Y,4) 104	15	15	19	24	10	9	6	11
T(Y,2,3) 97	11	-	-	13	9	4	5	8
T(Y,3,5) 11	18	22	11	15	13	-	-	10

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