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Genetic sexing in the mediterranean fruit fly, *Ceratitis capitata*, using the alcohol dehydrogenase locus

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Summary. Using the alcohol dehydrogenase (ADH) locus a genetic sexing system is being developed in the Mediterranean fruit fly *Ceratitis capitata* based on the sensitivity of ADH null mutations to environmental ethanol. A series of null mutants have been induced at this locus, however, none proved viable as homozygotes. One of these null mutants was translocated to the male determining chromosome and this line can be used for genetic sexing. When larvae from this line were reared on larval medium containing various concentrations of allyl alcohol, 97% of the emerging adults were males; in the absence of the allyl alcohol the sex ratio in the line is distorted in favour of the females. It is proposed that the higher ADH activity of the females (homozygous positive) in comparison with the males (heterozygous null) is responsible for their lower survival in larval medium containing allyl alcohol. ADH converts the allyl alcohol to the lethal ketone. The possible use of this line to sex large populations of medflies for use in sterile insect release programmes is discussed.

Key words: Genetic sexing – ADH – Allyl alcohol – *Ceratitis capitata* – Medfly

Introduction

A genetic sexing system is being developed in the Medfly, *Ceratitis capitata*, based on the model demonstrated in *Drosophila* using the alcohol dehydrogenase locus (Robinson and van Heemert 1982). Genetic sexing in *C. capitata* brings with it appreciable economic and biological advantages for the development of the sterile insect technique (Robinson 1983). Polymorphism has been demonstrated in *C. capitata* at this locus and fast (Adh^F/Adh^F) and slow (Adh^S/Adh^S) lines have been isolated (Riva and Robinson 1983). These lines have been successfully used in an extremely time-consuming scheme to isolate a series of radiation induced null mutations at this locus (Riva and Robinson, in press), however, none of the mutations were viable as homozygotes. To improve the selection efficiency for new *Adh* null mutants a novel scheme was proposed (Robinson and Riva 1984) involving the translocation of one of the original null mutants to the male determining chromosome. This paper reports the induction and isolation of such a line together with its genetic sexing characteristics.

Materials and methods

The three strains used were: (a) Adh^F/Adh^F and Adh^S/Adh^S – two variants at the alcohol dehydrogenase locus (in the text abbreviated to FF and SS); (b) EK 15, a line containing a radiation induced null mutation at the ADH locus, inviable as a homozygote; (c) T(Y-2) 128, a male-linked translocation linking the EK 15 ADH null mutation to the Y chromosome.

Larval rearing medium and general rearing conditions were as Robinson and van Heemert (1982).

Selective larval rearing medium

The constitution of the medium was as follows: 25.6 g carrot powder, 9.6 g yeast, 144 cc H_2O, 8.8 cc 1N HCl, 1.0 cc 4% formaldehyde, 1.2 cc proprionic acid and varying amounts of allyl alcohol. Populations to be treated were collected daily as eggs and placed under parafilm for 2 days at 28 °C. At this time all the eggs had hatched and the larvae were transferred in 15 cc of water to the selective medium in a Petri dish. The lid was replaced on the Petri dish, sealed with parafilm and the medium placed at 28 °C for 2 days. The parafilm was then removed but the lid remained for a further 3 days. The

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	♂♂ ♀♀		Genotype in progeny ♂♂		♀♀	
			SN	FS	SN	FS
P–2	EK15 FN×SS	→				
	EK15 (FS×SS)	discard				
P–1	EK15 SN×FF	→	FN	FS	FN	FS
	EK15 (FS×FF)	discard				
P	EK15 FN×SS	→	SN	FS	SN	FS
	EK15 (FS×SS)	discard				
F_1	EK15 SN×FF	→	FN	FS	FN	FS (discard)
	EK15 (FS×FF)	discard				
	T(Y–S)N×FF	→		T(Y–S)F	FN	(discard)
	T(Y–N)S×FF	→	T(Y–N)F128			FS (retain)

Fig. 1. Mating scheme used to maintain the ADH null mutation and to isolate a translocation linking an ADH null mutant to the male determining chromosome in *Ceratitis capitata*

medium dish was then transferred to a larval rearing box. Ten–twelve days later the pupae were collected and allowed to emerge and the sex ratio noted.

Results

Induction of a translocation in the EK 15 line linking the null mutation with the male determining chromosome

As the null mutation in the line (EK 15), chosen for the induction of the translocation, was homozygous lethal it was maintained as a heterozygote, as shown in Fig. 1. Males from the mutant line were mated individually with SS females (P-2) and then checked electrophoretically; the mutant males (single electrophoretic band) were identified and the progeny of such males was retained. In the next generation, males (SN or FS) were mated individually to FF females and mutant males again identified electrophoretically and their progeny retained. At the P generation, males were irradiated with 5 krad of X-rays before being paired individually with SS females. FN males were electrophoretically identified after mating and the progeny retained. F_1 males from this cross were individually mated to FF females and F_2 progenies from males having single band (SN) were retained. To identify a male-linked translocation, F_2 females were checked electrophoretically and families retained in which all FS females were kept. A total of 254 F_1 males were mated of which 167 were FS and 87 SN. From the latter, 60 F_2 progenies were examined and 3 had male linked translocations: 2 linked with the S allele and 1 linked with the null mutation, T(Y-2) 128. The translocation induction frequency was 5%. Line T (Y-2) 128 was maintained by crossing these males to SS virgin females, and at the next generation by mating the translocation males to FF virgin females. In this way the strain is characterized by heterozygous females (FS) which show 3 electrophoretic bands and males (FN or SN) which show 1 electrophoretic band, and it can be regularly checked for contamination.

Genetic sexing in line T (Y-N) F 128 using allyl alcohol

This translocation is at present being used to isolate a series of null mutants as proposed by Robinson and Riva (1984). During this process larvae carrying mutagenized chromosomes are exposed to medium containing allyl alcohol. This alcohol should preferentially select individuals having low ADH activity as it is converted into a lethal ketone by ADH. During preliminary experiments to determine the concentration of allyl alcohol to be used in the experiments it became apparent that a high degree of discrimination was also possible between the translocation carrying males (heterozygous for the null mutation) and normal females, with the result that only males survived the treatment.

It can be seen from Table 1 that the survivors from egg collections originated from crosses in which the male parents were T (Y-2) 128 males. The progeny were reared in medium containing varying amounts of allyl alcohol. From 306 survivors, 299 were males and of the 7 females six originated from the lowest allyl alcohol concentration. It is clear that males exhibit a higher survival in the selective medium than females. Controls for this observation can be seen in Table 2. Larvae from the control population (Adh^F/Adh^F) are

Table 1. Number and sex of surviving *Ceratitis capitata* individuals from matings involving line T(Y-2)128 in larval medium contained allyl alcohol

Oviposition date	% allyl alcohol (v/v)	No. adults ♂♂	No. adults ♀♀	
13/12	0.09	33	6	
19/12	0.07	15	0	
20/12	0.11	62	0	(+2 intersexes)
22/12	0.11	5	0	
24/12	0.11	1	0	
27/12	0.11	55	0	
29/12	0.11	3	0	
31/12	0.11	54	1	
2/1	0.10	43	1	
3/1	0.10	28	0	(+1 intersex)
		299	7	

Table 2. Effect of strain and larval medium on adult sex ratio in *Ceratitis capitata*

Strain	Allyl alc. conc. (%)	No. eggs	No. larvae	No. pupae	No. adults ♂	No. adults ♀
T(Y-2)128	0.0	482	418	282	75	112
	0.08	500	407	67	52	0
	0.092	494	346	5	5	0
Adh^F/Adh^F	0.0	778	615	546	241	286
	0.08	1,060	791	4	2	0
	0.092	1,090	798	0	0	0

unable to survive in the larval medium containing 0.08% of the allyl alcohol whereas 16.5% of larvae of the T (Y-2) 128 strain survive the same concentration and only males are produced (Table 2). In the absence of allyl alcohol the T (Y-2) 128 population produces an excess of females.

Discussion

Allyl alcohol, like 3-penten-1-ol, is converted to a lethal ketone by the enzyme alcohol dehydrogenase (Sofer and Hatkoff 1972) and as such has been used in *Drosophila* and maize as a selective agent to aid in the isolation of mutants exhibiting no ADH activity (Sofer and Hatkoff 1972; O'Donnell et al. 1975; Freeling and Cheng 1976). It is this phenomenon which has been exploited in the present study to differentiate between individuals with reduced ADH activity (males) and individuals with normal ADH activity (females).

Extrapolating from the *Drosophila* data it was not expected that such a clear discrimination would be demonstrated between heterozygous null individuals and positive individuals. In general, although the activity of heterozygous null individuals is reduced by 50% as compared to wild-type individuals (Oakeshott 1976), strains showing even a 95% reduction in activity differ only marginally in survival on medium supplemented with pentynol (O'Donnell et al. 1975) as compared with wild-type positive strains. This emphasises the qualitative aspect of the present results and further studies are necessary to quantify the observed differences in survival between the sexes to determine when the larvae die.

As far as the suitability of the line for genetic sexing is concerned the following can be stated. Males from the line T (Y-2) 128 can be differentiated from females by supplementing the larval medium with 0.11% allyl alcohol. However, several developments are necessary before the system can be exploited for the mass rearing of males without females. Firstly, as already suggested, the observed differences in survival have to be quantified; secondly, exposure of larvae to the allyl alcohol has to be more precise and thirdly, the reduced fertility of the line (to about 20% of normal fertility) will have to be taken into account when considering the productivity of the colony.

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