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Genetic Sexing in *Drosophila melanogaster* Using the Alcohol Dehydrogenase Locus and a Y-linked Translocation

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Summary. By incorporating ethanol (4% v/v) into the larval rearing medium of a specially constructed *Drosophila melanogaster* strain it was possible to produce only male adults; the female larvae died.

In this strain, the male determining chromosome was linked with a positive Alcohol dehydrogenase (ADH) allele by a translocation. The females were homozygous for the null allele and hence sensitive to ethanol.

This genetic sexing method is discussed in relation to its use in the genetic control of insects.

Key words: Genetic sexing – Alcohol dehydrogenase – *Drosophila*

Introduction

The efficiency of the sterile male technique for the control of insects pests is greatly increased if systems for the removal of females during mass rearing, prior to release, can be developed. This premise holds true in many cases where in some species the females are disease vectors; in others they cause damage during oviposition and in others they are more radiation resistant than the males, therefore necessitating a higher radiation dose. In two mosquito species (Curtis 1978; Seawright et al. 1978) genetic sexing systems have been developed in which an insecticide is used to kill susceptible female zygotes selectively. The males are resistant and the resistance gene is linked to the male determining gene by a translocation. In a further mosquito species (Baker et al. 1978) a dominant temperature-sensitive lethal has been used. We present here a genetic sexing system in *Drosophila melanogaster* using ethanol as the killing agent. The use of ethanol as a toxicant has an advantage over the use of insecticides in that there is no fear of accidentally introducing insecticide resistance genes into natural populations.

Results and Discussion

Ethanol (4% v/v) incorporated into larval rearing medium poisons larvae which exhibit no alcohol dehydrogenase activity (homozygous 'null' mutants): ADH positive larvae, both heterozygotes and homozygotes, survive (Vigue and Sofer 1976). In *Drosophila melanogaster* ADH is coded for by a single locus mapped at position 50.1 in chromosome 2 (Grell et al. 1965). Males homozygous for the active allele and carrying a translocation between the male-determining chromosome and chromosome 2 were mated in mass to virgin females exhibiting no ADH activity, i.e. homozygous for the 'null' allele(n) (Fig. 1). The heterozygous (*Fn*) F_1 males were again crossed with 'null' homozygous females (*nn*) and a known number of 1st instar larvae from this cross were transferred to a medium containing 4% ethanol (Bijlsma-Meeles 1979). Larvae also were transferred to normal medium without ethanol. The number of emerging males and females were recorded and are shown in Table 1. It can be seen that the addition of 4% (v/v) ethanol resulted in lethality in the female larvae (all homozygous 'null' individuals) and resulted in a 100% effective sexing system producing only males (all heterozygous *Fn*). That the ethanol is toxic to female larvae is

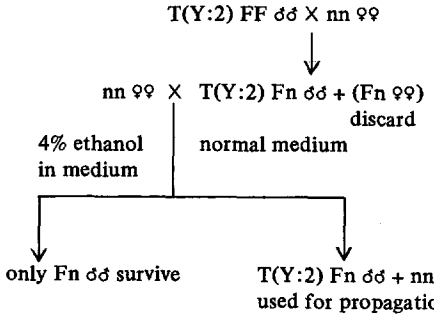

Fig. 1. Mating scheme used for the genetic sexing system producing only males

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Table 1. Effect of ethanol on the survival of male and female larvae of *Drosophila* differing in their ADH activity

Larval genotype ♀	Larval genotype ♂	Larval medium	No larvae transferred	Replicates	Mean no. (%) emerged adults ♀	Mean no. (%) emerged adults ♂	Mean survival % ± S.E.
nn	*Fn*	0% ethanol	200	3	82.6 (48.6%)	84.0 (51.4%)	83.3 ± 1.2
nn	*Fn*	4.0% ethanol	300	3	0 (0%)	115.1 (100%)	39.4 ± 3.2

also evident from larval survival which was reduced by about 50% when ethanol was incorporated into the medium (Table 1). The crucial characteristic of Y-linked translocations which is exploited here is that such translocations (in this case linking the Y chromosome with the ADH locus) are inherited by all the sons and none of the daughters. As crossing over is virtually absent in *Drosophila* males, this genetically contrived male sexing strain can be easily propagated every generation without selection on normal medium. When only males are required, the eggs have simply to be transferred to medium containing 4% ethanol as the ovipositing *nn* females exposed to 4% ethanol will quickly die.

Frequently, experimental design requires not the production of males but the production of virgin females. Such a system can be easily produced by inducing a Y : 2 translocation in an ADH 'null' homozygous line and mating males from this line to ADH positive females in a scheme similar to Figure 1. Production of females has the added technical advantage that as the females are resistant to ethanol they can lay their eggs directly on the 4% ethanol medium.

Work is now in progress at this laboratory to develop a genetic sexing system in the Mediterranean fruit fly, *Ceratitis capitata*, using the ADH locus to produce males for release for the control of this species by the sterile insect technique.

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Literature

Baker, R.H.; Sakai, R.K.; Saifuddin, U.T. (1978): Genetic sexing technique for a mosquito sterile male release. Nature 274, 253-255

Bijlsma-Meeles, E. (1979): Viability in *Drosophila melanogaster* in relation to age and ADH activity of eggs transferred to ethanol food. Heredity 42, 79-89

Curtis, C.F. (1978): Genetic sex separation in *Anopheles arabiensis* and the production of sterile hybrids. Bull. Wld. Hlth. Org. 56, 453-456

Grell, E.H.; Jacobson, K.B.; Murphy, J.B. (1965): Alcohol dehydrogenase in *Drosophila melanogaster*: Isozymes and genetic variants. Science 149, 80-82

Seawright. J.A.; Kaiser, P.E.; Dame, D.A.; Lofgren, C.S. (1978): Genetic method for the preferential elimination of females of *Anopheles albimanus*. Science 200, 1303-1304

Vigue, C; Sofer, W. (1976): Chemical selection of mutants that affect ADH activity in *Drosophila*. III Effects of ethanol. Biochem Genet. 14, 127-135

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