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Short communication

A simple method for the isolation of allelic series using male-linked translocations

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Summary. Using *Adh* null alleles a genetic sexing technique is being developed in *Ceratitis capitata.* In order to facilitate the isolation of a whole series of null alleles at this locus a technique utilizing male-linked translocations is described. It provides a simple efficient and general method for the isolation of any allelic series in species where little genetic information is available.

Key words: Translocations – *Adh* – Genetic sexing

Introduction

There is considerable interest for developing a genetic sexing system which would produce only males of the Mediterranean fruit fly, *Ceratitis capitata* (LaChance 1979; IAEA 1980) and significant progress has already been made (Rössler 1979; Robinson and Riva 1983; Wood and Busch-Petersen 1982). When using the sterile insect technique for the control of this species, there are many advantages if only males are released (Robinson 1983). One technique being considered exploits the alcohol dehydrogenase (*ADH*) locus. In *Drosophila,* individuals homozygous for a null allele at this locus are sensitive to ethanol (Vigue and Sofer 1976). Using translocations which linked the positive *ADH* allele to the male-determining chromosome in combination with a homozygous *ADH* null strain a genetic sexing system producing only males was developed in this species (Robinson and Van Heemert 1980). A similar system is now being developed in *C. capitata.*

The problem

Translocations linking a positive *ADH* allele with the male determining chromosome have been isolated (Riva and Robinson 1983). The major difficulty remaining is the isolation of a whole series of *ADH* null alleles which can be assessed for their feasibility for use in a genetic sexing system, i.e. they must show high viability and fitness and be "non-leaky". The absence of relevant genetic strains for isolating such alleles means that the isolation of a single null allele can only be achieved through the post-mating electrophoretic screening of thousands of F_1 individuals following mutagenesis (Dickinson and Sullivan 1975). Data from *Drosophila* indicate that the induction rate for null alleles at this locus is in the order of one in several thousands (O'Donnell et al. 1977; Aaron 1979), representing, therefore, an enormous task in *C. capitata.* This is compounded by the fact that not all *ADH* null alleles will show good viability or fitness (Ashburner et al. 1982). It is clearly impractical to isolate a series of such alleles using the above technique and, thus, the following approach is suggested.

The solution

The method is outlined in Fig. 1. Using irradiation, the original null allele is translocated to the male determining chromosome. Males (Adh^{n1}/Adh^{n1}) are irradiated and mated to Adh^s/Adh^s females. The F_1 males are individually backcrossed to Adh^f/Adh^f females and a sample of the F_2 progeny are examined electrophoretically. A family is identified in which all the males show one band T (Y-Adh^{n1}/Adh^f) and all the females three bands (Adh^f/Adh^s). Males from this line are then mated in mass to mutagenized Adh^s/Adh^s females. The progeny from this cross are reared in a medium containing allyl alcohol which positively selects for homozygous null individuals (Woodruff and Ashburner 1979). All survivors will be males and *Adh* homozygous null, with the original null allele linked to the male determining chromosome. These males can then be individually mated to Adh^s/Adh^s females,

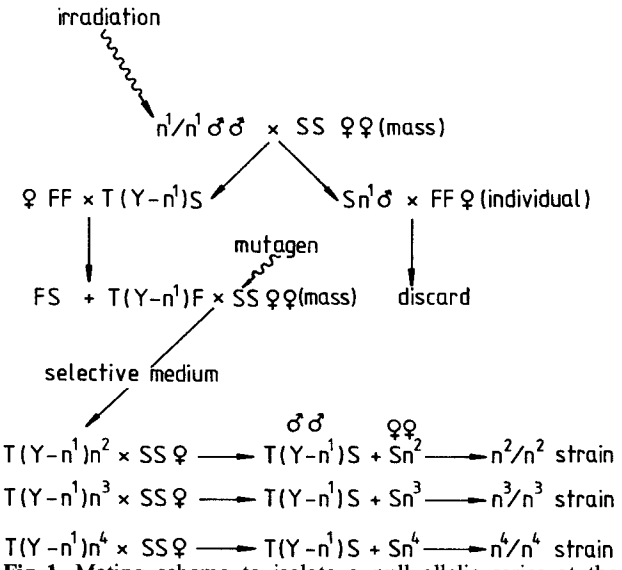

Fig. 1. Mating scheme to isolate a null allelic series at the *ADH* locus in *Ceratitis capitata*

producing the original translocation males and females heterozygous for the new null allele (Adh^s/Adh^{n2}). The viability and fitness of this new null allele can then be tested by outcrossing and inbreeding. One complicating factor in this procedure could be the presence of the maternal effect. In *D. melanogaster* when *Adh* heterozygous females are crossed to homozygous *Adh* negative males, all the larvae die when exposed to pentynol, even though half are genetically *ADH*-negative (O'Donnell et al. 1975). It might therefore be necessary to postpone the selective procedure until the adult stage. This scheme can also be used when the original null allele is homozygous lethal by irradiating heterozygous males. However, as half of the male-linked translocations will be with the positive allele, more F_1 males have to be screened.

Three *Adh* null alleles have now been isolated in the species (Riva and Robinson, in preparation) and if their fitness or viability proves to be inadequate, this method will be used to generate new mutants.

Conclusion

The use of this technique enables a whole series of *Adh* null alleles to be isolated in *C. capitata,* which can be subsequently assessed as to their usefulness for incorporation into a genetic sexing system. The technique, of course, is not locus specific and it can be used for the isolation of any allelic series in species where the genetic hardware is at a minimum if two conditions can be fulfilled: firstly that crossing-over is absent in the male and, secondly, that a male determining chromosome is present.

The principle can also be used to maintain autosomal recessive alleles by translocating them to the male determining chromosome. It is then simple to assess the specific locus mutation rate by checking the sex ratio following irradiation. The heterozygosity inherent in the XX, XY sex determination system can provide a genetic shelter for recessive lethals.

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