Available online at www.sciencedirect.com
ScienceDirect
Insect Biochemistry and Molecular Biology 37 (2007) 1168–1176
Insect Biochemistry and Molecular Biology
www.elsevier.com/locate/ibmb

Genetic sexing through the use of Y-linked transgenes

Kirsty C. Condon[a,b], George C. Condon[a], Tarig H. Dafa'alla[b], Guoliang Fu[b], Caroline E. Phillips[b], Li Jin[b], Peng Gong[a], Luke Alphey[a,b,*]

[a]*Department of Zoology, University of Oxford, South Parks Road, Oxford OX1 3PS, UK*
[b]*Oxitec Limited, 71 Milton Park, Oxford OX14 4RX, UK*

Received 25 May 2007; received in revised form 3 July 2007; accepted 6 July 2007

Abstract

Sterile insect technique (SIT)-based pest control programs rely on the mass release of sterile insects to reduce the wild target population. In many cases, it is desirable to release only males. Sterile females may cause damage, e.g., disease transmission by mosquitoes or crop damage via oviposition by the Mediterranean fruit fly (Medfly). Also, sterile females may decrease the effectiveness of released males by distracting them from seeking out wild females. To eliminate females from the release population, a suitable sexual dimorphism is required. For several pest species, genetic sexing strains have been constructed in which such a dimorphism has been induced by genetics. Classical strains were based on the translocation to the Y chromosome of a selectable marker, which is therefore expressed only in males. Recently, several prototype strains have been constructed using sex-specific expression of markers or conditional lethal genes from autosomal insertions of transgenes. Here, we describe a novel genetic sexing strategy based on the use of Y-linked transgenes expressing fluorescent proteins. We demonstrate the feasibility of this strategy in a major pest species, *Ceratitis capitata* (Wiedemann), and discuss the advantages and disadvantages relative to other genetic sexing methods and potential applicability to other species.

Keywords: SIT; Y chromosome insertion; Genetic sexing; Germ-line transformation; *piggyBac*; Marker; Mediterranean fruit fly; *Ceratitis capitata*

1. Introduction

The sterile insect technique (SIT) is a species-specific and environmentally non-polluting method of insect control. It relies on the mass rearing, sterilization and release of large numbers of insects. Mating with sterile insects leads to a decrease in the reproductive potential of the native insects and ultimately, if sterile insects are released in large enough numbers over a sufficient time period, to suppression or even local elimination of the pest population (Knipling, 1955; Msangi et al., 2000).

Highly successful, area-wide SIT programs have been conducted against the New World screwworm *Cochliomyia hominivorax* (Coquerel) in the USA, Mexico and Central America (Krafsur et al., 1987); also in Libya SIT was used in the successful control of a serious outbreak in 1989 (Lindquist et al., 1992). Other targets of area-wide SIT programs include Tephritid fruit flies, especially the Mediterranean fruit fly (Medfly, *Ceratitis capitata* Wiedemann), which is a major pest of citrus, coffee and other produce around the world. Worldwide production of sterile male Medfly for SIT is over 3 billion per week (Franz, 2005).

In most cases, it is considered highly preferable that the release population is free of females. There are several reasons why this is desirable. In mixed-sex releases, the males may court and mate the co-released sterile females rather than seeking out wild females, thereby reducing the efficiency of the program. For Medfly, co-release of females was shown to reduce the per-male effectiveness by a factor of three- to five-fold in large-scale field trials (Rendón et al., 2004). In addition, for some species the adult females are themselves damaging. This is particularly

*Corresponding author. Department of Zoology, University of Oxford, South Parks Road, Oxford OX1 3PS, UK. Tel.: +44 1865271157; fax: +44 1865310447.
E-mail address: luke.alphey@zoo.ox.ac.uk (L. Alphey).

0965-1748/$ - see front matter © 2007 Elsevier Ltd. All rights reserved.
doi:10.1016/j.ibmb.2007.07.006

clear in the case of mosquitoes, where females take a blood meal and males do not. Therefore, even sterile females may transmit pathogens and cause disease, in addition to providing a biting nuisance. Similarly, the adult females of some agricultural pests are directly damaging, for example Medfly, where oviposition damages fruit, even if the embryo itself fails to hatch. Even in the absence of these issues, if sterile females make no positive contribution to the control program, eliminating them from the final generation would reduce production and distribution costs without reducing the effectiveness of the program.

Some species, for example *Aedes aegypti* (L.) and tsetse (*Glossina* spp.), have sufficient sexual dimorphism to allow large-scale, automated sex separation with reasonable accuracy (Dowell et al., 2005; Klassen and Curtis, 2005). However, for many species this is not the case. Large-scale sex separation then requires the induction of such a dimorphism by genetics. The resulting strains are known as genetic sexing strains (GSSs). Several such strains have been made using classical genetics, by inducing a chromosome translocation such that a chromosomal fragment including a selectable marker becomes attached to the Y chromosome. Selectable markers could be visible, e.g., pupal colour, or a conditional lethal, e.g., insecticide resistance or temperature-sensitive lethality. For example, pupal colour markers have been identified and used in various Tephritid fruit flies (Robinson, 2002a, b) as has insecticide resistance in a range of Diptera including *Stomoxys calcitrans* (L.), *Hylemya antiqua* (Meigen), *Lucilia cuprina* (Wiedemann), *Culex tarsalis* (Coquillett), and *Anopheles albimanus* (Wiedemann) (Klassen and Curtis, 2005; Robinson, 2002b). Perhaps the most sophisticated of these translocation-based systems is the *temperature-sensitive lethal* (TSL) system developed in Medfly by the IAEA/FAO Entomology Unit (Franz, 2005; Robinson, 2002a). Females of the strain are homozygous for a recessive *tsl* allele on chromosome 5; males have one copy of this allele, a wild-type allele of this gene on a chromosome segment translocated to the Y chromosome, and another version of chromosome 5 with a deletion of the translocated segment. The net effect is that females can be killed by exposure to elevated temperatures; males survive such temperatures as their Y chromosome carries a wild-type allele of the *tsl* gene.

However, these classical methods have several shortcomings, particularly for development in new pest species. The chromosome translocations lead to reduced productivity due to unbalanced segregation of translocated chromosomes resulting in a significant fraction of the progeny having non-viable (aneuploid) genomes. The mutant genetic background and chromosome rearrangements will tend to reduce the performance of the flies. The strains are not entirely stable; two types of recombination can produce flies that do not carry the whole system. These two types of recombination are estimated to occur at around 10^{-3}–10^{-4} and 10^{-5}–10^{-6} per fly per generation, respectively, for the Medfly TSL system (Franz, 2002, 2005), which is significant in view of the very large numbers of insects used in SIT programs. Since the recombinants are fitter than the desired genotypes, they will tend to increase rapidly in frequency within the colony. Special rearing systems, so-called 'clean filter' systems have been developed to maintain the strains at adequate purity despite this instability (Franz, 2005; Robinson, 2002a).

Perhaps the major limitation of the classical genetic approach is that the special chromosomes—mutants and chromosome rearrangements—made for one species cannot normally be transferred to any other species. Suitable markers are lacking in the vast majority of pest species for which genetic sexing could be a benefit to SIT programs; inducing mutations in a new species is a very unpredictable process, with no guarantee that usable selective markers will be identified (Franz, 2005). In contrast, one of the key hopes and rationales for the various transgenic approaches to genetic sexing is that the strategies, molecular components, and perhaps even entire constructs may work equally well in other species. In this case, though developing a novel strategy in one species may require a significant investment of resources, this should be substantially less for the application of the same strategy in other species, at least where an efficient germ-line transformation system has been developed for that species.

Several recombinant DNA strategies have been proposed or developed to allow genetic sexing. One of these is to selectively kill females by conditional expression of a lethal gene or genetic system, i.e., sex-limited expression, probably from an autosomal insertion (Christophides et al., 2001; Fu et al., 2007; Heinrich and Scott, 2000; Thomas et al., 2000). Another is based on the differential expression of a non-lethal marker in males and females. There are potentially two ways to achieve this—sex-linked expression using an insertion on a sex chromosome and sex-limited expression using an expression system (e.g., promoter) that is differentially active in males and females. Sex-limited expression of a fluorescent protein by use of a male-specific promoter has been demonstrated for several species (Catteruccia et al., 2005; IAEA, 2006; Smith et al., 2007). Each of these studies used a β2-tubulin promoter to give expression in the male germ-line only. For *Anopheles stephensi* (Liston), automated sex separation based on this differential expression was shown to be possible (Catteruccia et al., 2005). However, use of such promoters has some disadvantages, notably that expression is detectable only rather late in development and only in one specific tissue. Since most sex-specific promoters are active relatively late in development, being associated with sexual differentiation and function in the adult, very early sex-specific expression of a marker may be difficult to achieve by this method. One potential alternative would be to use sex-specific alternative splicing to control gene expression (Fu et al., 2007). Here we demonstrate another method—the use of sex-linked transgenes. We show that such markers can be scored from the earliest larval stage onwards.