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Chromosomal inversions and genetic control revisited: the use of inversions in sexing systems for higher Diptera

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Summary. Genetic sexing systems based on sex-linked translocations and deleterious mutations are subject to breakdown from genetic recombination in males. Including inversions in these strains may provide a solution to this problem, by ensuring selective elimination of recombinant products. Inversions could be used either in coupling to or in repulsion to the translocation. The latter system, requiring homozygous-viable inversions, would be more difficult to construct, but would offer several advantages not available with coupled translocation/inversion systems. A system proposed for the blowfly *Lucilia cuprina* is outlined, which combines homozygous-viable pericentric inversions in repulsion to existing sex-linked translocations. This system should both stabilize the genetic sexing system and increase the suppressive potential of such strains.

Key words: Genetic sexing – Translocation – Inversion – *Lucilia cuprina* – *Ceratitis capitata*

Introduction

Genetic sexing systems have been constructed in a number of dipteran species, either for use with the sterile insect technique (SIT) or with other genetic methods of population control (Whitten 1969, 1979; Whitten et al. 1977; Curtis et al. 1976; Foster et al. 1978, 1985; Kaiser et al. 1978; Baker et al. 1979, 1980; Rossler 1979; Suguna et al. 1981; Robinson and Van Heemert 1982; Saul 1984). These systems are all based on artificial linkage of deleterious autosomal mutations to sex, using Y-autosome (male-linked) translocations.

In species with frequent meiotic recombination in males (e.g., most lower dipterans), crossover-suppressing inversions are routinely included in genetic sexing systems, to ensure tight linkage of the deleterious genes to sex (Curtis et al. 1976; Kaiser et al. 1978; Baker et al. 1979, 1980). In species where male recombination is rare (most higher dipterans), inversions have not been considered necessary, since it was believed that male recombination in this group was sufficiently low to prevent serious breakdown of sexing systems (Whitten 1969; Robinson and Van Heemert 1982).

Unfortunately, the sexing systems developed so far for higher dipterans have been unstable under mass-rearing conditions. Sex linkage of the critical mutations has broken down from genetic recombination in males, followed by selection in rearing colonies favoring the recombinant genotypes. During several field trials of genetic control in the sheep blowfly *Lucilia cuprina,* frequent selection and replacement of the rearing colony was required to counter this type of strain deterioration (Foster et al. 1980, 1985; Vogt et al. 1985). Similar problems have been encountered in attempts to use genetic sexing systems in the Mediterranean fruit fly *Ceratitis capitata* (Rossler 1982a, b; Hooper et al. 1987; Busch-Petersen 1989).

As noted above, the use of inversions to suppress crossing-over in lower dipteran (mosquito) genetic sexing systems is not new. Robinson (1975) reviewed the historical development of this concept, and discussed the benefits and disadvantages of using inversions to generate genetic death in pest populations. The instability of genetic sexing systems in higher dipterans suggests that it is now appropriate to reconsider the use of inversions in these systems.

Suppression of recombination by inversions

In females of both higher and lower dipterans, the products of crossing-over within heterozygous inversions are

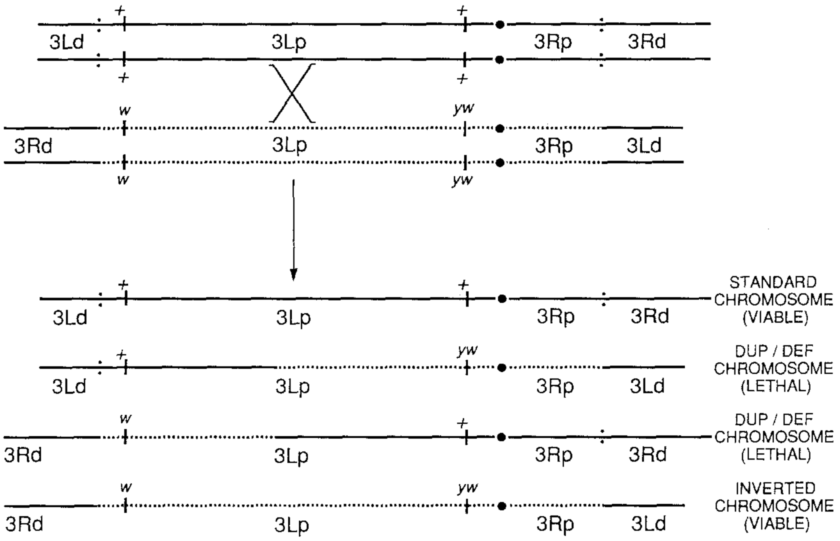

Fig. 1. Duplication-deficiency products of crossing-over within a hypothetical *L. cuprina* pericentric inversion on chromosome 3. *Solid line* = standard chromosome sequence; *dotted line* = inverted sequence; 3L, 3R = left and right arms of chromosome 3; p, d = proximal and distal portions of chromosome arms; w = white eyes; yw = yellowish eyes; + = respective wild-type alleles of eye mutations

eliminated either pre- or post-zygotically, depending on whether the inversions are paracentric or pericentric (reviews by Robinson 1975; Roberts 1976). With paracentric inversions, most crossover products are excluded from the functional egg nucleus and fertility is thus only minimally affected. With pericentric inversions, crossing-over generates duplication/deficiency (Dp/Df) products (Fig. 1), which have a 50% probability of inclusion in the functional egg nucleus. These crossover products are generally inviable and fertility of heterozygotes is consequently reduced.

The expected semisterility arising from exchange in a pericentric inversion heterozygote is half the frequency of tetrads containing at least one exchange event within the inverted segment. The maximum sterility arising from exchange within a pericentric inversion is thus 50% (Robinson 1975; Suguna and Seawright 1980).

In males, crossing-over within either type of inversion apparently leads to lethally defective sperm, from breakage of anaphase bridges in paracentric inversions (Bhalla 1970a, b) and from Dp/Df chromosomes (Fig. 1) in pericentric inversions. In species with frequent male recombination, the presence of either type of inversion leads to a reduction in male fertility (Bhalla 1970a; Rabbani et al. 1977). However, where male recombination is rare, the small amount of defective sperm generated by crossing-over within inversions would have an insignificant effect on fertility.

Using inversions in sexing systems

The choice of type and configuration of inversions in genetic sexing systems will depend partly on the intended use of the sexing system and partly on availability of particular inversion types. The latter in turn will depend on the ease of genetic manipulation of the species concerned and the number and linkage relationships of available mutations. Among the choices to be considered are: (1) inversions either coupled or in repulsion to the translocation, (2) paracentric or pericentric inversions, and (3) homozygous-lethal or -viable inversions.

Assembly of strains with inversions contained on the same autosome as the sex-linked translocation (coupled) would be a relatively easy task. In species with little male recombination, this would involve a serial process, with construction of the inversion first (by selection for crossover suppressors in females), then construction of the translocation (by selection for sex linkage of the inversion). Homozygous viability would not be required, and either a paracentric or a pericentric inversion would suffice, provided it gave the required suppression of recombination between the male-determining gene and the sex-separation mutation.

This type of inversion-translocation strain would be appropriate for a genetic sexing system whose use was solely to eliminate females prior to release, e.g., in a SIT program. If, however, circumstances required changing the inversion or the translocation (e.g., to accommodate a new sex-separation mutation or to increase strain fertility for mass rearing), the entire complex rearrangement would need to be replaced.

Inclusion of inversions in repulsion with the translocation would be more versatile and, in some cases, would provide additional benefits. Strains would be synthesized using separately constructed inversions and translocations. As new rearrangements became available, either the translocation or the inversion, but not necessarily both, could be replaced. In theory, this could be done using inviable inversions with similar break-points (pseudohomozygous inversions) (Suguna et al. 1981). However, the fecundity of such strains may be too low for efficient mass rearing, because half the female zygotes would be inviable. Using homozygous-viable inversions would be preferable even though their isolation is more laborious.

In systems with inversions and translocations in repulsion, either paracentric or pericentric inversions could be used in SIT programs, where the released genetic material is not inherited in viable offspring. However, if the offspring of released males contribute to further genetic death, pericentric inversions offer a distinct advantage. In addition to suppressing male recombination, they would increase the rate of genetic death imposed on field populations through the inviable products of crossing-over in heterozygous females (Robinson 1975; Suguna and Seawright 1980).

Improving field-female killing systems in *L. cuprina*

The existing field-female killing (FK) system in *L. cuprina* combines recessive eye-color mutations with a sex-linked translocation. Females are homozygous for the mutations and have white eyes, and they are functionally blind. In cages they are not severely debilitated, but if released they are unlikely to survive in the field.

Males carry the wild-type alleles on the translocation and have normally pigmented eyes. They are competitive in the field and transmit the translocation to their sons and the mutations to their daughters. In field populations, homozygosis for the mutations and semisterility of the translocations combine to give genetic death rates approaching 94% (Whitten et al. 1977; Whitten 1979; Foster et al. 1985, 1988).

This type of system is effective in suppressing field populations of *L. cuprina* (R. J. Mahon and G. G. Foster, unpublished results), and for long-term suppression campaigns it is likely to be more cost-effective than sterile-male release (Foster et al. 1988). However, strain instability due to male recombination is still an impediment to commercial application of this system.

It is proposed to stabilize the FK strains in *L. cuprina* by including a homozygous-viable pericentric inversion on each of the nontranslocated chromosomes carrying the eye color mutations. Females would be homozygous for the inversions and mutations, with males heterozygous.

In the rearing colony, most products of crossing-over in males would carry inviable duplications and deficiencies and would thus be eliminated (Fig. 1). Released males would transmit the mutations and inversions to their daughters, which would be heterozygous for both the inversion and mutations. They would be up to 75% semisterile, regardless of the type of male they mated with, because of the inviable crossover products from two inversions. Thus, both the stability and suppressive power of the FK system would be improved.

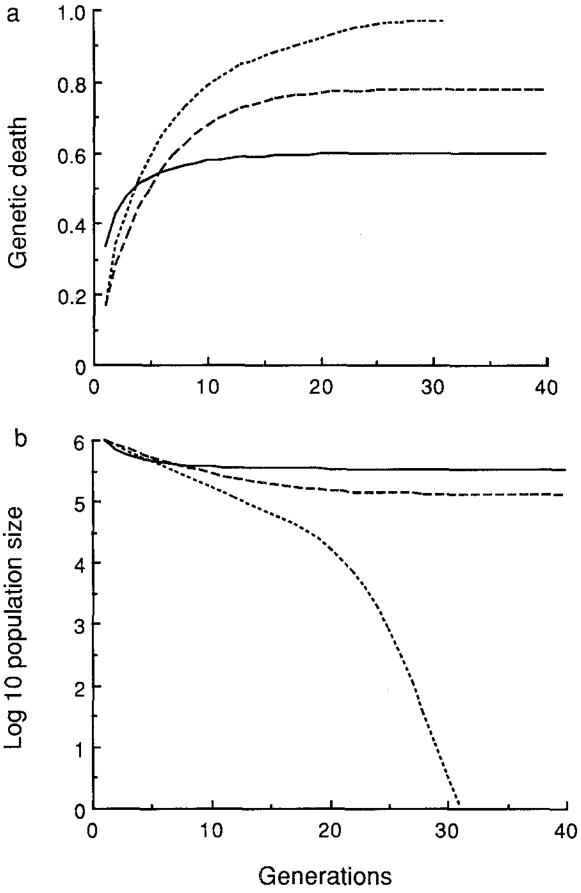

Fig. 2 a and b. Simulations of genetic control using sterile males or *L. cuprina* T(Y;3;5)/w yw;to translocations with sterility = 50%. The inversion modelled is a chromosome 3 pericentric inversion with exchange-tetrad frequencies within the inversion of 1.0 between w and yw, and 1.0 between yw and the right break-point (sterility 50%). Populations are entirely density-dependent with $R_{\max} = 10$, initial release ratio = 0.5:1 (hard releases). *Solid line* = sterile males; *dashed line* = FK strain without inversion; *dotted line* = FK strain with inversion. **a** Genetic death, **b** population size

Computer simulations

The genetic control simulation program GENCON (Foster et al. 1988) has recently been modified to include recombination within inversions in FK systems (G. G. Foster, unpublished results). Simulations indicate that FK systems that contain inversions should give higher genetic death rates and population suppression than present strains (Fig. 2).

In the simulation shown in Fig. 2 (comparable to Fig. 5 d of Foster et al. 1988), release of an inversion-containing FK strain leads to eradication under conditions where SIT and a noninversion FK strain do not.

Discussion

The modified FK strain proposed for *L. cuprina* is similar in karyotype to strains constructed more than a decade ago in two *Anopheles* species (Baker et al. 1980; Suguna et al. 1981). Several homozygous-viable pericentric inversions have recently been isolated in *L. cuprina* and are being evaluated for inclusion in field-release strains (G. G. Foster, G. L. Weller and D. G. Bedo unpublished results).

The simulations suggest that population suppression using this type of genetic control system may be more effective than a SIT program, particularly under low-budget conditions which necessitate a low ratio of released to wild insects. The potential suppressive power of FK systems compared to the conventional SIT should lead to consideration of this method for other major pests such as *C. capitata* and the screwworms *Cochliomyia hominivorax* and *Chrysomya bezziana.*

Large areas of South America remain infested with *C. hominivorax* (Krafsur et al. 1987) and, if the recent introduction of this species to North Africa is not contained, it may become a long-term pest there (Gabaj et al. 1989). The SIT as practiced in North America may be simply too expensive to use in these regions.

With the recent advances in genetics and cytogenetics of *C. capitata* (Bedo 1987; Malacrida et al. 1990; Rossler and Rosenthal 1990; Zacharopoulou 1990), isolation of inversions should be a routine procedure in this species. Since several mutations are now available (both in the eye pigment pathway and elsewhere) which may be inviable under field conditions, construction of a stable FK system in *C. capitata* may simply be a matter of time.

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