COMPLEX Y-LINKED TRANSLOCATIONS IN *DELIA ANTIQUA* PRODUCED BY IRRADIATION OF A FERTILE Y-LINKED TRANSLOCATION

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SUMMARY

In the onion fly, *Delia antiqua*, a fertile, Y-linked translocation involving chromosomes Y and 2 was irradiated with fast neutrons to induce new complexes involving the Y-chromosome. This chromosome is male determining in the onion fly. Such complexes can be used for the development of genetic sexing systems and also for the introduction of sterility into field populations following release.

Irradiation reduced egg fertility by 54 per cent and significantly reduced larval survival but it had no effect on the F_1 sex ratio. By measuring the fertility of 807 F_1 males following outcrossing, 112 semi-sterile progenies were isolated of which 11 were lost, 29 showed no inheritance of the semi-sterility, 59 were new autosomal translocations and 13 were new complex Y-linked translocations. This classification was accomplished by checking the fertilities of outcrossed F_2 males and females. Following cytological observation it was revealed that one of these new complexes involved four chromosome pairs, the remainder involved three. There appeared to be no correlation between the fertility of the translocation and the complexity of the rearrangement. The utilization of these rearrangements in the development of the genetic sexing technique for the onion fly is discussed, together with an assessment of their use for fertility reduction in natural populations.

1. INTRODUCTION

MALE (Y)-linked translocations can be used in insect control in two ways, firstly to introduce sterility into populations (Wagoner *et al.*, 1969; Rabbani and Kitzmiller, 1975a; Baker *et al.*, 1978) and secondly to enable genetic sexing techniques to be developed (Seawright *et al.*, 1978; Baker *et al.*, 1978; Whitten *et al.*, 1976; Curtis *et al.*, 1976; Curtis, 1978). Y-linked translocations with their associated semi-sterility are amongst the simplest types of rearrangement which can be used in genetic control as the translocation is passed to all the sons and none of the daughters, therefore they can be easily maintained in stock. Theoretical studies (Curtis, 1975; Seawright *et al.*, 1979) indicate the usefulness of this type of rearrangement and laboratory (Laven, 1969; Rabani and Kitzmiller, 1975b) and field (Laven *et al.*, 1972) studies have supported this promise.

In the onion fly, *Delia antiqua*, Y-linked translocations are being isolated both for population sterility induction and genetic sexing. One basic difficulty faced is that the Y-chromosome comprises only 4 per cent of the mitotic chromosome length and as translocations are generally recovered in a frequency relative to their length (Muller and Altenberg, 1930) Y-linked translocations will be disappointingly rare. Out of 71 translocations isolated

only one was Y-linked (T 61). When first isolated the fertility of translocation males was reduced by 30 per cent but in subsequent generations it regained normal fertility (Van Heemert and Vosselman, 1979). The effective length of the male determining chromosome was therefore increased to 21 per cent of the total chromosome length; this fact together with the full fertility of the translocation made irradiation of this line an attractive proposition for inducing complex Y-linked translocations.

For genetic sexing in the onion fly, dieldrin resistance (*DD*) and susceptibility (*dd*) are being used (Towgood and Brown, 1962). T 61 males were backcrossed to the resistant line *DD* and made homozygous for the resistant allele and irradiated with fast neutrons. In the F_1 progeny new complex Y-linked translocations were isolated and their fertility and cytology were studied.

2. MATERIALS AND METHODS

(i) *General procedures*

Adults, larvae and pupae were reared at 23°C and 70–80 per cent R.H. Adults were fed on dried food and larvae reared on onion slices. Egg sterility is used as an initial screen for translocations and eggs were incubated at 29°C and 90 per cent R.H. for two days. In the onion fly duplication/deficiency gametes from translocations lead to death in a late embryonic stage causing brown eggs. The presence of such eggs provides a very good marker for the identification of translocations as it is possible to distinguish between fertility reduction caused by translocations and that caused by other factors for example, non-fertilization of the egg. Sterility of translocations is defined as the percentage of brown eggs in the total of brown and hatched eggs.

(ii) *Induction and isolation procedure*

Adult males T 61 (Y-2) *DD* were irradiated with 200 rads fast neutrons (fig. 1) and mated to dieldrin susceptible virgin females (15 cages each with 15 males and 15 females). Two other cages with unirradiated males were used as controls. The fertility of a sample of eggs from each cage was assessed twice. Larval survival was measured by allowing known numbers of first instar larvae access to ample onion, and counting the number of pupae; these were allowed to emerge and pupal survival and adult sex ratio were measured.

The vast majority of the eggs were placed directly on onion and the F_1 pupae were reared and stored at 2°C. Periodically, samples were placed at 23°C and F_1 males were mass-mated to *dd* females (fig. 1). (As only Y-linked translocations were needed, the F_1 females were discarded.) Following mating, the females were separated and the sterility assessed. Those females showing in excess of 20 per cent sterility were retained and the F_2 generation reared. As both autosomal and Y-linked translocations will be included in this selected group, F_2 adults of both sexes were again out-crossed to *dd* females (fig. 1). Where a new Y-linked translocation had been induced all the F_2 males showed sterility but none of the females. In the case of a new autosomal translocation, half the F_2 males and half the F_2

FIG. 1.—Mating scheme for the isolation of complex Y-linked translocations D/d = Dieldrin locus; A = an additional autosome.

females showed sterility. The presence of putative Y-linked translocations was confirmed by cytology (for methods see Van Heemert and Vosselman, 1979).

3. RESULTS

(i) Radiation-induced dominant lethality

Table 1 shows the egg fertility of the unirradiated and irradiated Y-linked translocation males. The original Y-linked translocation males exhibit full fertility (92 per cent hatch) but following irradiation the fertility was reduced to 38 per cent. Irradiation of normal males with the same dose lead to a fertility in the treated males of 34 per cent (Robinson, 1976). The sterile eggs could be classified into the two types (see Materials and Methods) and both showed a significant increase in frequency following irradiation.

TABLE 1

Effect of radiation on the fertility of male *Delia antiqua* carrying a fertile Y-linked translocation (n = number of cages)

Treatment	Classification of Eggs		
	Sterile	Fertile	
	Percentage unhatched white $\pm$ S.E.	Percentage unhatched brown $\pm$ S.E.	Percentage hatched $\pm$ S.E.
0 rad (n = 2)	5.60 $\pm$ 3.40	2.35 $\pm$ 1.65	92.05 $\pm$ 5.05
200 rad F.N. (n = 15)	50.20 $\pm$ 1.22	11.33 $\pm$ 0.82	38.47 $\pm$ 1.09

Radiation-induced lesions leading to early egg lethality and white eggs are probably caused by early inhibition of mitosis due to the formation of chromosome bridges (Smith and Von Borstel, 1972) and in *Drosophila* (Von Borstel and Rekemeyer, 1959) they constitute the major portion of radiation induced dominant lethality. However, late embryonic lethals (brown

eggs) are probably caused by chromosome imbalance as this type of lethality can be mimicked by aneuploid gametes from different types of chromosomal rearrangements (Robinson, 1977).

The vast majority of radiation induced dominant lethals are egg lethals but nevertheless a small proportion can be expressed in later stages (table 2).

TABLE 2

Effect of radiation on larval and pupal survival following radiation of male Delia antiqua carrying a fertile Y-linked translocation

	Larval survival (% $\pm$ S.E.)	Pupal survival (% $\pm$ S.E.)	Percentage females $\pm$ S.E.	Percentage males $\pm$ S.E.
0 rad	73.60 $\pm$ 4.06	90.05 $\pm$ 3.41	50.95 $\pm$ 3.10	49.05 $\pm$ 3.10
X^2_{HET} d.f. = 9	45.29***	25.96**		8.95 n.s.
200 rad F.N.	62.81 $\pm$ 5.80	89.32 $\pm$ 1.60	54.16 $\pm$ 4.31	45.84 $\pm$ 4.31
X^2_{HET} d.f. = 9	72.16***	7.41 n.s.		17.38*
X^2 (0 rad v.s. 200 rad) d.f. = 1	13.44***	1.64 n.s.	X^2_1 (sex ratio 1:1) = 0.42 n.s.	

* $P < 0.05$ ** $P < 0.01$ *** $P < 0.005$.

n.s. = not significant.

They significantly reduce larval survival in the irradiated series when the overall totals were compared. However, in both series there was significant heterogeneity between replicates. This often occurs during larval rearing of the onion fly where onion quality plays an important role in larval survival. Pupal survival and adult sex ratio were unaffected by the radiation (table 2).

(ii) Sterility of the F_1 generation

Over a period of a year samples of F_1 pupae were removed from the 2°C storage and emerged. The F_1 males (Dd) were mass-mated with dd females and the sterilities of these females is illustrated in fig. 2; it is very similar to that recorded when normal males were irradiated with 200 rads of fast neutrons (Robinson and Van Heemert, 1980a). Out of 807 separated females 695 (86 per cent) showed no clear increase in sterility and were discarded. The remaining 112 females exhibited sterilities ranging from 20 per cent to over 80 per cent, however there did appear to be a peak around the 45-50 per cent level. Eleven females in the right hand section of the distribution did not leave sufficient progeny in order to produce an F_2 generation leaving 101 semi-sterile progenies.

(iii) Classification of the F_2 progenies

F_2 progenies, both male and females were mass-mated to dd flies (fig. 1). Subsequently the mated females were separated and their fertility assessed. Using this information conclusions could be drawn concerning the genetic constitution of the F_1 male.

(a) If both male and female F_2 showed normal fertility then a factor or factors other than an inheritable rearrangement caused the reduction in

FIG. 2.—Fertility distribution of F_1 males descended from irradiated T 61 males and the classification of the F_2 progenies.

fertility in the F_1 male. In fig. 2 these cases are represented by N and as expected more were found at slightly reduced fertility levels.

(b) If half the F_2 males and half the females show a decreased fertility this gives a strong indication that an autosomal translocation was present in the F_1 male. In fig. 2 these cases are represented by A and 59 were observed. They were found at all fertility levels.

(c) If all the F_2 males showed a reduced fertility but all the F_2 females had normal fertility then the presence of a Y-linked translocation was suggested. In fig. 2 these are represented by Y together with the F_1 number; a total of 13 was isolated.

The overall frequency of translocation induction was 8.92 per cent which agrees well with equivalent data when normal males are irradiated (Robinson and Van Heemert, 1980a). However, what is different is the proportion of the translocation which was Y-linked, 18 per cent, a frequency which agrees very well with the expectation based on chromosome length (21 per cent) of the Y-2 translocation complex.

(iv) *Fertility and cytology of the complex Y-linked translocations*

In all cases Y-linked translocations were confirmed by cytological analysis and it became clear that in two cases a single F₁ male had mated with two females. Translocations T 447 and T 473 were identical and translocations T 903 and T 919 were also identical (table 3). However, this did

TABLE 3

Cytology and sterility of complex Y-linked translocations in Delia antiqua

Translocation Number	Chromosomes Involved	Percentage Sterility $\pm$ S.E.	No. males tested for sterility
T 108	Y-2-5 ¹	—	—
T 165	Y-2-6 ¹	30.72 $\pm$ 1.80	12
T 347	Y-2-5 ¹	38.52 $\pm$ 1.55	18
T 447 } T 473 }	Y-2-3 ¹ -6 ¹	50.57 $\pm$ 1.85	13
T 474	Y-2-3 ¹	34.32 $\pm$ 2.14	14
T 619	Y-2-6 ^s	63.15 $\pm$ 4.86	10
T 698	Y-2-6	17.56 $\pm$ 1.51	9
T 710	Y-2-6?		
T 887	Y-2-3 ¹	31.90 $\pm$ 1.36	6
T 903 } T 919 }	Y-2-3 ¹	35.93 $\pm$ 1.84	19
T 936	Y-2-3 ^s	26.27 $\pm$ 1.62	13

not invalidate the calculation of the translocation frequency if it is assumed that all F₁ males have an equal chance of mating twice. In one case, T 447 (T 473) a complex involving four chromosome pairs was identified, the rest all involved three chromosome pairs. Four involved chromosomes Y-2-3, two involved Y-2-5 and three or four involved Y-2-6. Chromosome 4 was conspicuous by its absence from these complexes (table 3).

Photomicrographs of two types (a triple and a quadruple) are presented in fig. 3 together with a normal karyotype (fig. 3a) and the original Y-linked translocation T(Y-2) (fig. 3b) between chromosome arm 2¹ and Y (acrocentric). T 165 (figs. 3c and e) has been derived from T(Y-2) by a break at about the original breakpoint in the short arm of chromosome 2, close to the centromere. The other breakpoint was proximal in the long arm of chromosome 6. Together with three bivalents a hexavalent can be observed in fig. 3c, in which three chromosome pairs are included. This translocation is schematically represented in fig. 3e. T 447 (or T 473) has chromosomes 3 and 6 in the complex and in fig. 3d an octavalent is present in meiotic prophase as well as the two bivalents from chromosomes 4 and 5. Following radiation three breaks occurred and a cyclic triple translocation was produced superimposed on T(Y-2). The three breaks occurred in the long arms of the chromosomes 2, 3 and 6. Thus in total five breakpoints occurred to form a complex of eight chromosomes (fig. 3f).

The translocations exhibited a wide range of partial sterility from 17 to 63 per cent when males were testcrossed in subsequent generations. Interestingly the two translocations exhibiting the lowest levels of sterility, T 936 and T 698 were isolated from F₁ males from the lowest sterility class (fig. 2); the same was true for the least fertile translocation T 619 (fig. 2). So far none

Plate I

FIG. 3.—Meiotic configurations of male *Delia antiqua* ($2n = 10 + XY$); a, normal karyotype; b, Y-linked translocation (T61); c, Y-linked complex translocation T165; d, Y-linked complex translocation T447; e and f, schematic representations of T165 and T447 respectively.

of the translocations has recovered fertility as occurred with the original Y-linked translocation and there appears to be no relation between the complexity of the translocation and the sterility.

4. DISCUSSION

Following irradiation of a Y-linked translocation (Y-2), eleven new complex Y-linked translocations were isolated, but two were lost. The sterilities showed a wide range of values and there was no obvious clustering round the 50 per cent level. The male linked translocations were isolated firstly to develop a genetic sexing system and secondly for use to exert a genetic load in a natural population. For these two uses different fertility criteria apply. For genetic sexing high fertility is necessary, so that mass rearing can be carried out efficiently but for the introduction of a genetic load a lower degree of fertility is desired, for example that exhibited by T 619.

What was unexpected was the lack of any relationship between the complexity of the translocation and its sterility. This is in contrast to most other species where an increase in complexity of rearrangements is always accompanied by an increase in sterility (Wagoner *et al.*, 1969; McDonald *et al.*, 1978).

In a previous report (Van Heemert and Robinson, 1981) concerning breakpoint distribution in 70 autosomal translocations it was shown that chromosome 4 was seldom recovered in rearrangements; the same appears now to be true for Y-linked complex translocations.

The recent successful development of genetic sexing in *Drosophila* by using the ADH locus (Robinson and Van Heemert, 1980b), the localization of the ADH locus in the onion fly on chromosome 6 (Van Heemert and Witteveen-Pillen, 1980) and the presence of a Y-linked translocation with chromosome 6 suggest also this approach for the development of a genetic sexing system in the onion fly.

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