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Translocations Induced by Fast Neutrons and X-rays in *Delia antiqua*

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Summary. A comparison was made using X-rays and fast neutrons for the induction of translocations in *Delia antiqua*. Using the same radiation dose, no difference in efficiency between the two radiation types could be observed. However, with fast neutrons many multiple translocations were induced, including a quadruple translocation involving 4 out of 5 autosomes. One male linked translocation was also induced.

The reciprocal translocations were assigned into two classes: symmetrical and asymmetrical, and ten of the latter were chosen for inbreeding to produce homozygotes. Asymmetrical exchanges were chosen so that translocation homozygotes could be differentiated cytologically from the normal karyotype. In seven different translocations, homozygous larvae were observed, but often at a low frequency. In four of these lines, viable adult homozygotes were observed. Subsequent random sib-crossing failed to produce a homozygous line.

Key words: Translocations – X-radiation – Asymmetrical exchanges – Sib-crossing – *Delia antiqua*

Introduction

In a variety of pest species of both agricultural (Wagoner et al. 1969; Curtis et al. 1972; Delrio and Cavalloro 1976; Foster and Whitten 1974) and public health importance (Baker et al. 1977; Baker et al. 1978; Laven et al. 1971; McDonald et al. 1978; Rabbani and Kitzmiller 1972; Rai et al. 1974) chromosomal translocations have been developed for use in genetic control. The theory behind the use of translocations has indicated the potential of this technique (Serebrovski 1940; Curtis 1968; van Ende 1978; Whitten 1971).

This approach to control has proceeded far further in insects of public health importance principally because the species which are important in this field, namely the mosquitoes, are well studied genetically and chromosomally they are very simple, possessing in the main only three pairs of chromosomes. In agricultural pests a different situation is apparent with a striking dearth of genetic information and in general a higher chromosome number.

The onion fly *Delia* (= *Hylemya*) *antiqua*, a pest of onions in the temperate regions of the world, has been studied as a candidate species for genetic control using the sterile male technique and chromosomal translocations. It possesses the following advantages for such approaches: the males can be sterilized with a relatively low dose of radiation; it is the main insect pest of the onion crop; much ecological data are available; it has only six pairs of chromosomes, all of which are recognisable.

In *Drosophila* (Savhagen 1960) irradiated spermatids are the most sensitive stage for the induction of translocations but in *D. antiqua* it is impossible to synchronize spermatogenesis in such a way. A compromise was therefore made in which homogeneity of the irradiated cells was guaranteed together with an expectation of the induction of a reasonable proportion of translocations. Fast neutrons induce a significantly higher frequency of translocations than X-rays (Gonzales 1972) when equivalent doses are applied. In addition, the RBE for translocations is higher than for radiation induced recessive lethals so that fast neutrons induce relatively more translocations and relatively fewer recessive lethals. Such lethals could be responsible for the difficulty in obtaining translocations as homozygotes. As there is no information on recessive lethal induction in the onion fly it was not possible to choose equivalent doses of fast neutrons and X-rays. Therefore the radiation dose was held constant in order to have a certain degree of comparison.

This paper reports on the induction, isolation and homozygous viability of translocation strains induced in male and female germ cells by X-rays and by fast neutrons, together with a comparison between the two types of radiation.

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Materials and Methods

a. *Rearing*

All pupae and adults were reared at 23°C and 70-80% R.H. with a 8D : 16L photoperiod. Larvae were initially reared on onion at 23°C but later an alternating temperature of 10°C for 8 h and 20°C for 16 h was used as this temperature regime was shown to improve the larval survival and pupal weight (Robinson and Zurlini 1979). All matings were performed in mass and the mated females were subsequently separated into individual cages. F_1 individuals, both males and females, descended from irradiated parents were used and progeny from separated females exhibiting a reduction in fertility were retained for cytological examination.

b. *Fertility Measurements*

Eggs were collected 2 or 3 times/week, counted and placed on moist filter paper on top of a piece of onion in an enclosed box. They were incubated at 29°C for three days. The number of hatched eggs was then determined and the unhatched eggs were classified into white eggs (probably unfertilized) and brown eggs (fertilized eggs dying late in embryonic development due to chromosome unbalance). Percent fertility is defined as

$$\frac{\text{no. hatched eggs}}{\text{no. brown eggs} + \text{no. hatched eggs}} \times \frac{100}{1}$$

c. *Cytology*

See Van Heemert and Robinson (in press).

d. *Irradiation*

X-rays – Two types of individuals were irradiated: for the comparison with fast neutrons, adult males (7 days old) were irradiated with 2 Gy and then mated to unirradiated females; in a supplementary experiment, fertilized females (12 days old) were irradiated with 1, 3 and 5 Gy and translocations were subsequently isolated in the progeny.
Fast neutrons – In the main experiment 7 day old males were irradiated with 2 Gy, but in supplementary experiments translocations were isolated from doses of 1, 1.5 and 2 Gy.

Results

a) Comparison of 2 Gy of X-rays and Fast Neutrons

The fertilities of the F_1 individuals derived from the irradiated males are shown in Figure 1 (fast neutrons) and Figure 2 (X-rays). For the fast neutrons a total of 396 F_1 individuals were testcrossed, of which 35 produced unfertilized eggs. Of the remainder, the majority (70.0%) had a normal fertility between 90 and 100%: in the semi-sterile F_1 females a total of 10 (6.1%) translocations were recovered and in the semi-sterile F_1 males 17 (8.6%). It was not possible to check all the progenies descended from females showing reduced fertility because of the early death of the female before sufficient eggs had been laid. Further, extremely sterile translocations would be lost during the rearing procedure and extremely fertile translocations would not be picked out during the initial fertility screening. Therefore, the percentages given above represents a significant underestimate of the frequency of translocations induced in the sperm. This argumentation applies equally well to the X-ray data.

As a consequence of the mass mating of the F_1 males to the control females, one male can mate with more than

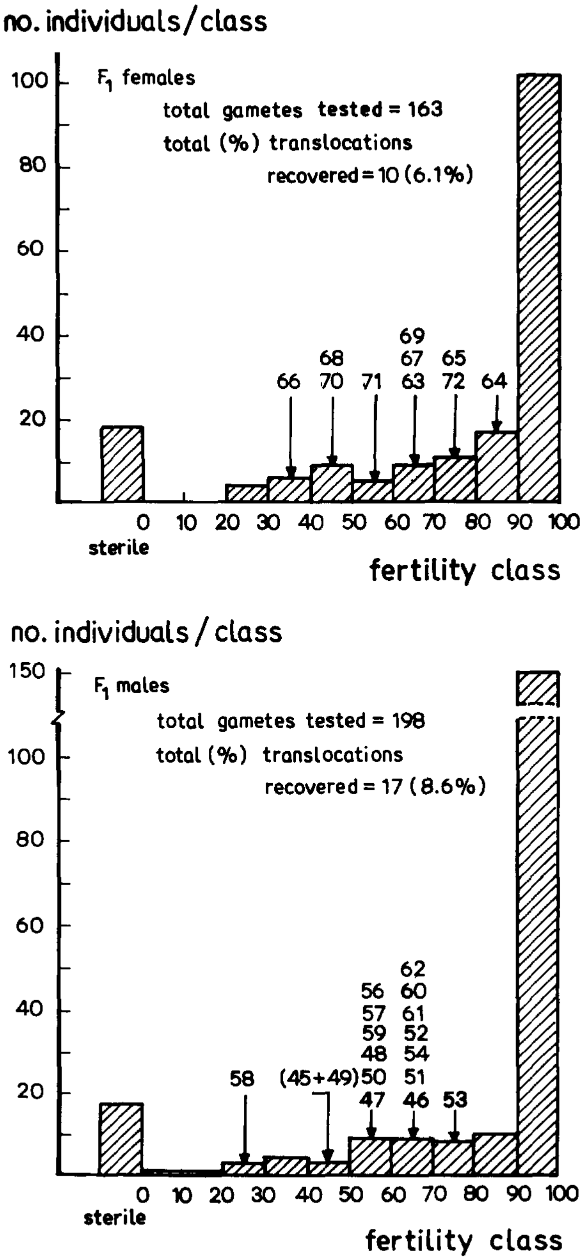

Fig. 1. Fertility of F_1 male and female *Delia antiqua* following irradiation of parental males with 2.0 Gy fast neutrons

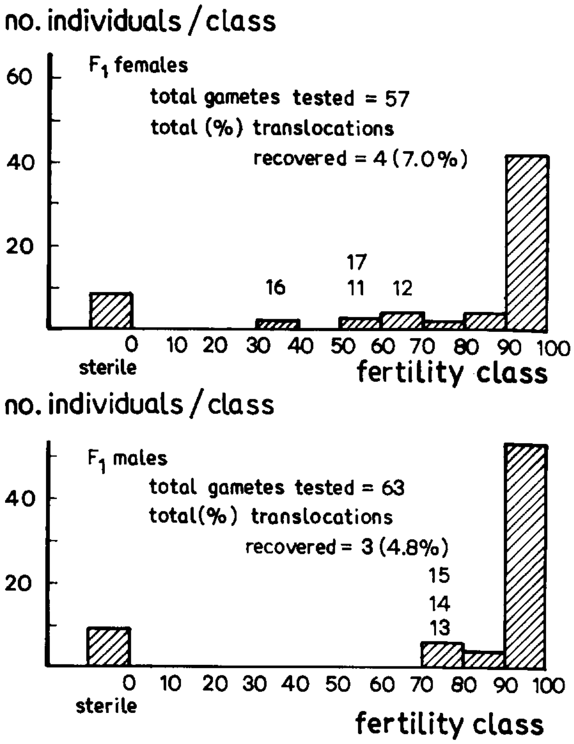

Fig. 2. Fertility of F_1 male and female *Delia antiqua* following irradiation of parental males with 2.0 Gy X-rays

one female. Indeed, T51 was identified in the progeny of three of the separated females, this of course can lead to an overestimation of the translocation induction frequency.

This multiple mating by males was also considered by Asman (1976). Of the F_1 individuals which had reduced fertility and produced sufficient progeny for cytological analysis, the majority had a chromosomal rearrangement. The observation that all 8 triple translocations and the single quadruple translocation were all induced by fast neutrons supports the *Drosophila* data in the efficiency of fast neutrons in producing chromosome breaks. Figure 1 also shows that in the F_1 generation, translocations were recovered from all fertility levels: there appeared to be no clustering around the 50% level. This spread in the fertilities of translocations in the F_1 generation was also observed in *Culex pipiens* (Laven et al. 1971).

Figure 2 gives the equivalent data for X-rays. Here fewer F_1 individuals were tested, a total of 137 of which 17 produced unfertilized eggs. The same fertility criteria used for fast neutrons were applied to initially screen for translocations and a total of 7 were recovered, 4 from the F_1 females and 3 from the F_1 males. The majority (79.2%) of the F_1 individuals had normal fertility.

For both X-rays and fast neutrons the absolute numbers of translocations initially induced could not be obtained, nevertheless, as the fertility and cytological criteria used were identical for both types of irradiation, a comparison of the recovery of translocations can be made. A X^2 test of the total numbers of translocations produced revealed no differences in translocation recovery frequency (X_1^2 = 0.37 n.s.).

b) Translocations Isolated from Other Irradiation Experiments

For completeness translocations produced and isolated in other experiments are given in Table 1. These involved irradiation of males with different doses of fast neutrons and irradiation of fertilized females with X-rays. The results of this latter experiment will be published elsewhere (Robinson in press). From fast neutrons a total of 12 translocations were isolated and from the X-irradiation, 15. Together with the translocations from the comparison experiment a total of 61 translocations have been recovered.

Table 1. Production of translocations by fast neutrons and X-rays in *Delia antiqua*

Fast neutrons (7 day old males)			X-rays (fertilized females)		
Dose (Gy)	Trans. nos.	Total	Dose (Gy)	Trans. nos.	Total
1.0	26-28	3	1.0	31, 33, 39-40	4
1.5	18	1	3.0	30, 34-37, 41-43	8
2.0	19-25, 29	8	5.0	32, 38, 44	3
		12			15
+ translocations from		27	+ translocations from		7
Figure 1		39	Figure 2		22
Total translocations recovered 61					

c) *Type of Translocation Isolated*

In Table 2 the translocations have been classified according to complexity, i.e. the number of chromosomes involved. Further, the reciprocal translocations (2 chromosome) have been classified as symmetrical or asymmetrical. This is an important classification for the subsequent isolation of translocation homozygotes where cytological analysis has to be used. Symmetrical translocations cannot be used as the homozygote cannot be differentiated from the normal karyotype. Of the 33 asymmetrical translocations recovered, 21 were induced by fast neutrons, however, this does not represent a significant effect when compared with the X-ray data. The quadruple translocation T18 involved 4 out of the 5 autosomes and had a fertility of around 20%. This low fertility led to the loss of this line. Since the Y chromosome constitutes only 4% of the total chromosomal length, male linked translocations will be rare events. Nevertheless T61 represents such a translocation. After backcrossing for several generations, this translocation became fully fertile (van Heemert and Vosselman 1980).

Table 2. Classification of translocations produced by fast neutrons and X-rays in *Delia antiqua*

Translocation type	Translocation nos.	Total
Reciprocal		
a) Asymmetrical	11, 14-16, 21, 23, 25-29, 31-33, 35, 40-45, 47, 50-53, 58-63, 65, 67-70, 72	37
b) Symmetrical	12, 13, 17, 19, 22, 34, 36-39, 55-57, 71,	14
Triple	20, 24, 46, 48, 49, 54, 64, 66	8
Quadruple	18	1
Uncertain	30	1
Total		61

d) *Fertility and Homozygous Viability of Selected Translocations*

It is not proposed to give a full list of the fertilities of all the translocations, they fell within the range 40-70% fertility, but only to report on those which were selected for intensive sib-crossing. More extensive cytological data on the result of sib-crossing translocations are given in a subsequent paper (van Heemert and Robinson in press). The fertility of translocation heterozygotes has always to be interpreted with care. Normally in the literature, fertility is equated with egg hatchability however it is often observed that duplication/deficiency zygotes survive to the larval stage and even to the adult stage (Ved Brat and Rai 1974). This observation is further discussed by van Heemert and Robinson (in press).

The data in Table 3 show that with the exception of T's 31 and 52 all the translocations and a fertility averaging around the 50% level. There was no difference between males and females notwithstanding the fact that males are achiasmate. The use of T 54 for inbreeding, which was initially isolated as a non-cyclic triple translocation (Table 2), was made possible due to recombination in the differential segment in a female translocation carrier, liberating two reciprocal translocations. One of these was asymmetrical and was used for inbreeding.

The absence of sufficient genetic markers necessitates the use of random mating during inbreeding studies. Following mass mating of translocation heterozygotes and wild types of both sexes, the females were separated and those exhibiting the highest sterility were chosen for further study. However, a clear discrimination between TN X TN mating and TN X NN matings could not always be made. The expectation is that 25% of the matings will be of the type TN X TN. Of the 10 translocations inbred, TT larvae were not found in T's 35, 40 and 68 and in five

Table 3. Fertility and homozygous viability of ten translocations subjected to inbreeding in *Delia antiqua*
(Chrom. = Chromosome)
(Rad. = Radiation)

Trans. no.	Rad. type	Chrom. Involved	Fertility mean ± S.E.		No. pairs inbred	No. pairs selected for cytology	No. pairs identified as containing T/T larvae and their fertility	T/T viability as adult
			♂	♀				
16	X	5-6	51.0 ± 2.3	50.1 ± 3.7	113	23	2 (49.6 ± 2.3)	+ both sexes
31	X	3-6	71.7 ± 3.7	67.4 ± 2.6	177	21	1 (45.4)	–
33	X	5-6	54.6 ± 1.9	55.7 ± 2.1	230	36	4 (40.8 ± 3.7)	+ inferred from mating
35	X	2-6	59.1 ± 2.1	63.1 ± 6.4	34	9	0	
40	X	4-6	53.7 ± 6.6	63.4 ± 1.9	50	6	0	
42	X	2-3	50.5 ± 4.1	55.4 ± 3.9	150	27	10 (41.8 ± 3.6)	+
47	F.N.	2-6	54.3 ± 3.2	52.1 ± 4.1	47	11	2 (43.4 ± 0.9)	–
52	F.N.	2-3	63.8 ± 2.7	75.3 ± 2.9	130	21	12 (52.6 ± 3.7)	+ both sexes
54	F.N.	2-6	46.0 ± 4.1	53.5 ± 3.3	50	5	1 (47.0)	– (some half-eclosed)
68	F.N.	4-5	59.9 ± 1.4	57.6 ± 2.2	50	5	0	

other T's 16, 31, 33, 47 and 54 very few of the matings contained TT individuals. It was noticeable that in the matings involving TN individuals as both parents, the frequency of the TT individuals in the progeny was often very low. In T's 42 and 52 a sufficient number of pairs producing TT individuals were identified, however in T 42 homozygous adults could not be identified. In T 52 viable adults of both sexes were identified cytologically. Viable adult homozygotes were also observed in T's 16 and 33. Further inbreeding of the translocation lines containing homozygotes failed to produce a homozygous line.

Discussion

In this study a total of 61 translocations were isolated in the onion fly using fast neutrons and X-rays. In an experiment involving a comparison of 2 Gy of both fast neutrons and X-rays, an average of 7.5% and 8.5% of translocations respectively were recovered in the F_1 progeny. For the reasons indicated previously this represents a significant underestimate. Although a higher frequency of translocations was recorded using fast neutrons the difference was not significant. This may seem rather surprising considering that for dominant lethal induction in the onion fly fast neutrons are three times more effective than X-rays (Robinson 1977). It remains unclear why the difference in the translocation frequency between the two types of irradiation was not greater.

Comparison of this induction frequency with data from other species presents some problems as firstly different absolute doses of radiation have been used, secondly doses have been used inducing different amounts of genetic damage i.e. insects vary widely in their sensitivity, and thirdly different techniques have been used to isolate translocations (pseudo-linkage or fertility screening). These factors result in a wide divergence of translocation recovery, from 0.7% (McDonald et al. 1978) in one mosquito, up to 50% (Laven et al. 1971) in another mosquito. This last figure does appear to be a clear exception as in the majority of studies the frequency of translocations induced is usually below 20%. This includes intensive investigations in *Drosophila* (Sankaranarayanan and Sobels 1976). The use of a low radiation dose would appear to be critical when the data are extrapolated from *Drosophila* (Sobels 1972; Ytterborn 1970); this is especially so when translocations have to be made homozygous. In order that meaningful comparisons of translocation frequency and homozygous viability can be made between different species and between different laboratories it could be suggested that a radiation dose inducing equivalent genetic damage could be chosen. The dose could be related to dominant lethal induction as this is simple to measure; for example a dose inducing 20% dominant lethality in irradiated sperm could be suggested.

Of the 61 translocations induced in this study, 10 were chosen as candidates for inbreeding to produce homozygotes and intensively studied. The choice was determined by the geometry of the exchange. Firstly, symmetrical exchanges were excluded and secondly, of the asymmetrical exchanges, those producing as homozygotes very easily recognisable karyotypes were retained. Reciprocal translocations involving each of the autosomes were used. In the three translocations in which viable homozygous adults were identified, no homozygous line was established. This highlights a difficulty which is apparent when directed matings cannot be made because of lack of genetic markers and reliance is placed instead on random mating followed by cytological examination to determine parental karyotypes. Notwithstanding this difficulty, two translocations have been made homozygous in the onion fly in other studies (Vosselman 1980; van Heemert 1977). If translocations are to be developed in pest species the initial induction and characterization of useful mutants should be seriously considered. It is also essential that suspected translocations can be cytologically confirmed. In a recent paper (Reid and McEwen 1977) it has been suggested that translocation homozygous lines in the onion fly can be obtained without cytological confirmation, however, the certainty that cytological analyses provide is well worth the expenditure of the cytological preparations.

To maximise the chances of producing translocation homozygous lines, certain protocols can be routinely adopted. Firstly, a low dose of radiation can be used instead of a high dose; secondly translocation lines can be backcrossed for at least 4 generations before inbreeding is begun (preferable through the female if males are achiasmate); thirdly a translocation should be maintained in separate lines during backcrossing; fourthly the use of cytology to assess homozygous viability should be restricted to the adult stage.

For the reasons indicated earlier fast neutrons have been used to induce translocation in this study. However, from the small numbers of translocations so far inbred, a comparison between the two as regards homozygous viability cannot be made. Experiments using fast neutrons and X-rays in *Drosophila* have also lead to inconclusive results (Robinson unpublished results). Therefore, at present there does not appear to be evidence of any difference in the efficiency of fast neutrons or X-rays in producing viable homozygous translocations.

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