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Radiation Induced Semi-Sterility for Genetic Control Purposes in the Onion Fly *Hylemya antiqua* (Meigen)

1. Isolation of Semi-Sterile Stocks and their Cytogenetical Properties

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Summary. In the preliminary stages of a study into the use of translocations for genetic control of the onion fly *Hylemya antiqua* (Meigen), irradiations were carried out in order to obtain chromosomal rearrangements. Several irradiation experiments, with X-rays or fast neutrons, were carried out on pupae and adults of both sexes at sub-sterilizing doses below 3.0 krad, to establish a favourable way of induction.

Because no visible markers are available for the genetic screening of induced rearrangements, and the reciprocal translocations or inversions in demand express themselves in the heterozygous condition by reduced fertility, a total of 234 F_1 individuals of both sexes were checked for reduced fertility. 50 F_1 individuals were suspected of carrying a translocation or inversion when they produced an egg hatch of between 30 and 60% (semi-sterility).

This category was passed for cytogenetic analysis. In the progeny of 25 suspect F_1 individuals, 9 different rearrangements were established, of which 7 were translocations. This means a yield of 4% for all the tested F_1.

After a discussion of the normal karyotype, some of the observed rearrangements are described.

Irradiation of males with 1.0 krad of X-rays is advised for the production of semi-sterile stocks carrying translocations. Fast neutrons were not found to be better than X-rays. At doses higher than 1.0 krad complex rearrangements and/or fragments were observed.

A translocation homozygote could be isolated in the case of an X-autosomal translocation, and this stock will be used for further genetic control purposes.

Introduction

The onion fly *Hylemya antiqua* (Meigen) was chosen by the Dutch Government in 1965 as a model for the development of genetic control methods (Ticheler and Noordink, 1968). It is an important pest in the Netherlands, and also in many other onion-growing countries in the Northern temperate zone. The feeding larvae cause losses in (export) quality and quantity of the crop. In most places the fly is resistant to chlorinated hydrocarbons, and in some areas it is also resistant to organophosphates. It lives in monocultures as the sole insect threat to the crop. The species belongs to the family Anthomyidae, which also includes other agricultural pests such as *Hylemya brassicae* (Bouché), *Hylemya cilicrura* (Rond) and *Psila rosae* (F).

The sterile release method was given primary attention. A method for continuous rearing of the onion fly has already been developed (Ticheler, 1971). A dose-effect curve for sterilization with X-rays has been determined (Noordink, 1971). The sterilizing dose is 3 krad for males and 2 krad for females. Untreated and irradiated gonads have been studied histologically (Theunissen, 1971). Population dynamics in onion fields is being studied by M. Loosjes (unpublished). Allied to this research team the authors are investigating the possibility of obtaining chromosomal rearrangements which could be useful in control programmes because of the genetic load they can introduce into the population in the field (Serebrovski, 1940). For example, a translocation induced in a field population at a suitable ratio puts a lasting genetic load on the insect population, which may slow down the rate of increase (Curtis and Hill, 1971) or even prevent the number of insects from increasing. Double translocations may enhance the genetic load on the population in the field (Curtis and Robinson, 1971). Still more complex genetic engineering has been suggested, such as the combination of multiple translocations with conditional lethals (Whitten, 1971). The authors were directly stimulated by Laven's work (Laven, 1969) and lectures.

Laven (1967) suggests the use of natural incompatibility as a means of genetic control, but no indication of natural incompatibility between geographic strains was found in the onion fly. The Dutch × Canadian onion fly cross and the reciprocal were fully fertile and produced fertile offspring. Chromosomal rearrangements, such as reciprocal translocations and inversions, also cause a fertility barrier. Irradiation facilities to induce chromosomal rearrangements were available. In the absence of visible genetic markers for genetic screening of induced rearrangements, it was necessary to design a

selection procedure on the basis of reduced fertility of the heterozygotes (Laven *et al.*, 1971).

Cytogenetic methods could be applied for definite proof of a rearrangement. The onion fly has 5 pairs of large distinguishable chromosomes (Boyes, 1954) and 2 or 3 small sex chromosomes. Somatic pairing enables cytogenetic screening to be carried out.

Pupae and adults of both sexes were irradiated with X-rays at sub-sterilizing doses to investigate the conditions for efficient production of translocations. Eventually, fast neutrons were used to confirm their expected high RBE (relative biological effectiveness) compared with X-rays and, in preliminary experiments, to investigate whether fast neutrons are advisable for the induction of translocations.

Materials and Methods

Experimental work with the onion fly was started in October 1969. The insect was reared for 6 or more generations under laboratory conditions at the Institute for Phytopathological Research, Wageningen. Hundreds of pupae of this stock were used. The offspring of irradiated parents and of the control groups were reared in small 8 cm ⌀ perspex cages, 16 cm high, in a climate room with 21 °C–23 °C, 80% R. A. H. and 18hr light per day of 1300 lux. Fresh flies had been collected from onion fields on the island of Goeree, June 1971. Their offspring were reared in small colonies of 15–20 flies in larger cages.

When the pupae are 4–7 days old and their cuticle has hardened, they can be stored at 2 °C, 90% R. A. H. They may be kept for a year but eclosion percentages will decrease in time. The flies were irradiated at different stages: pupae just before eclosion; 13 days old at 23 °C; and newly emerged males or females. Late pupal stage is the most suitable for manipulation in mass irradiation. Irradiation is usually carried out at this stage for the sterile release method as the pupae can withstand high doses without immediate effects on fitness, and after release the males are able to compete for females and inseminate them. Spermatozoa are already present, but all preceding stages of spermatogenesis are also present (Theunissen, 1971).

Due to the unstable way of storing the pupae, their developmental stage is not precisely defined. At the moment of eclosion all flies have reached the same stage of development. Flies in the first 6 hours after eclosion are therefore better suited for the comparison of irradiation effects.

When females are irradiated, either as old pupae or as young adults, their ovaries are still developing (Theunissen, 1971).

The following apparatus was used for irradiation:

X-rays were applied with a Philips 250/25 deep therapy apparatus, operating at 250 kVp and 15 mA, without an additional filter. The dose rate applied was 200 rad/min. X-ray doses were determined with a Philips Universal Dosimeter connected to a hose-shaped intracavity ionization chamber.

Van de Graaff electron generator, producing X-rays at an energy of 1.5 MeV, was used as a substitute for the X-ray machine.

Fast neutron irradiation was carried out in the BARN (Biological Agricultural Reactor Netherlands) reactor. Fast neutron doses were determined using acetylene equivalent and muscle tissue equivalent ionization chambers. The fast neutron spectrum has an average energy of 1.7 MeV. The γ-contamination amounts to 80 rad/h.

The material was irradiated in flat boxes so that the dose was distributed equally, and in ordinary air. The doses applied were all below the sterilizing dose of 3 krad as established by Noordink (1971). Dose rate was as high as possible in order to exclude dose-rate effects.

After irradiation the adults were crossed with non-irradiated mates, either individually 1 ♂ × 3 ♀♀ or in small groups of 5 irradiated to 10 non-irradiated mates. When the irradiated pupae had emerged, the sexes were separated and the tested sex was outcrossed to untreated mates. Eggs were collected after a pre-oviposition period of 7–10 days, 3 times a week, and incubated at 23 °C, 80% R. A. H., for 2–3 days, during which time embryonic development is usually complete.

The percentage empty eggs of all collected eggs (% egg hatch) has been used as a measure of fertility of the treated flies and their offspring. The remaining full eggs may consist of:

1. defective eggs, often very small and glassy;
2. non-fertilized eggs, which preserve their white colour;
3. fertilized eggs

a. without any observable embryonic development, these eggs are also white;

b. with short embryonic life, the eggs being somewhat coloured;

c. with a clear embryonic development. Segmentation and/or jaws are visible, but the larvae die before or during hatching. These eggs are brown in colour.

The percentage of unfertilized eggs fluctuates; it is relatively high in the first egg batch, then decreases, and increases as the female grows older. In the first selection series, unfertilized eggs were included while calculating the % egg hatch. The number of defective eggs also increases as the females grow older. Defective eggs were excluded from calculation. The percentage egg hatch used is the mean of egg hatch during the 2–4 weeks of egg collection, not corrected to the control value.

The symbol P is used for irradiated flies and their untreated mates; their offspring are called F_1, and were backcrossed to untreated mates (B_1 cross), to yield the B_1 generation. The following backcross is called B_2 etc.

The first score gives the immediate effects of irradiation on the reproductive capacity of the P generation. The fertilized full eggs are thought to represent dominant lethal mutations in which embryonic development usually ceases at an early stage. Attempts were made to backcross 25–30 individuals of the F_1 with control mates for each treatment, in order to investigate their individual fertility by scoring the egg hatch.

Stocks of B_1 crosses with 60–30% egg hatch (semi-sterile) were passed for cytogenetic investigation. Stocks with an egg hatch of between 75–60%

and a high percentage of brown eggs, or with a very low fertility (30—15%) but with enough larvae, were also analyzed cytogenetically. In suspected cases, or when too few offspring could be obtained, a B_2 backcross was made with 5 B_1 males and 5 B_1 females individually, so as to enlarge the stock and/or to see if the reduced fertility was stable (in some of the offspring), or sex-linked.

For cytogenetic screening, testes and ovaries were used just after eclosion of the adults, and brains from 7—9 day-old larvae were used for analyzing the karyotypes. After anaesthetizing the males with chloroform vapour they were put into a soap solution for a few minutes to promote wetting of the cuticle. The caudal 4—5 segments of the abdomen were torn away and the testes were dissected in a physiological saline solution under a dissecting microscope (12 × magn.) with a pair of fine needles (Theunissen, 1971). Distilled water was added for 5—10 minutes in order to spread the chromosomes, after which staining was carried out in 2% lacto-acetic-orceine, overnight, at room temperature. Squash preparations were then made in 45% acetic acid. Larval brains, ovaries and young eggs (11 hours old at 24 °C after oviposition) could be prepared in the same way, but the tissue had to be crushed with fine needles before squashing. If larval brain tissue was used, the larvae were supplied with additional onion two days before, to ensure that they were in good condition. Most photographs were taken with a Zeiss Photo-microscope on Agfa Copex Ortho high-contrast negative film.

Results

A survey of the treatments, fertility scores and cytogenetic data of the experiments up to date is given in table 1. This scheme illustrates the irradiation procedure, selection and cytogenetic analysis. It can be seen that there were few individuals per treatment, and sometimes interesting stocks could not be maintained for further analysis. Treatments aimed for comparison were often carried out on different material with different antecedents.

Fertility of the F_1 Generation

The fertility of B_1 crosses ranged from nearly 100% egg hatch to complete sterility. The variation in egg hatch of the B_1 crosses is illustrated in fig. 1, on the left for irradiated males, on the right for irradiated females. A small experiment with fast neutrons has been omitted from this figure. The figure at the top right hand corner shows the range of egg hatch in control crosses.

Although semi-sterility is generally considered to be a property of individuals carrying a reciprocal translocation or pericentric inversion, chromosomal rearrangements may be carried by individuals with an almost normal egg hatch. However, the group with an apparently reduced fertility is more interesting with regard to any application of the rearrangements. Too high a degree of sterility impedes the rearing of the stock. If a mean egg hatch of between 60 and 30% was found, the tested parent was suspected of carrying a chromosomal rearrangement.

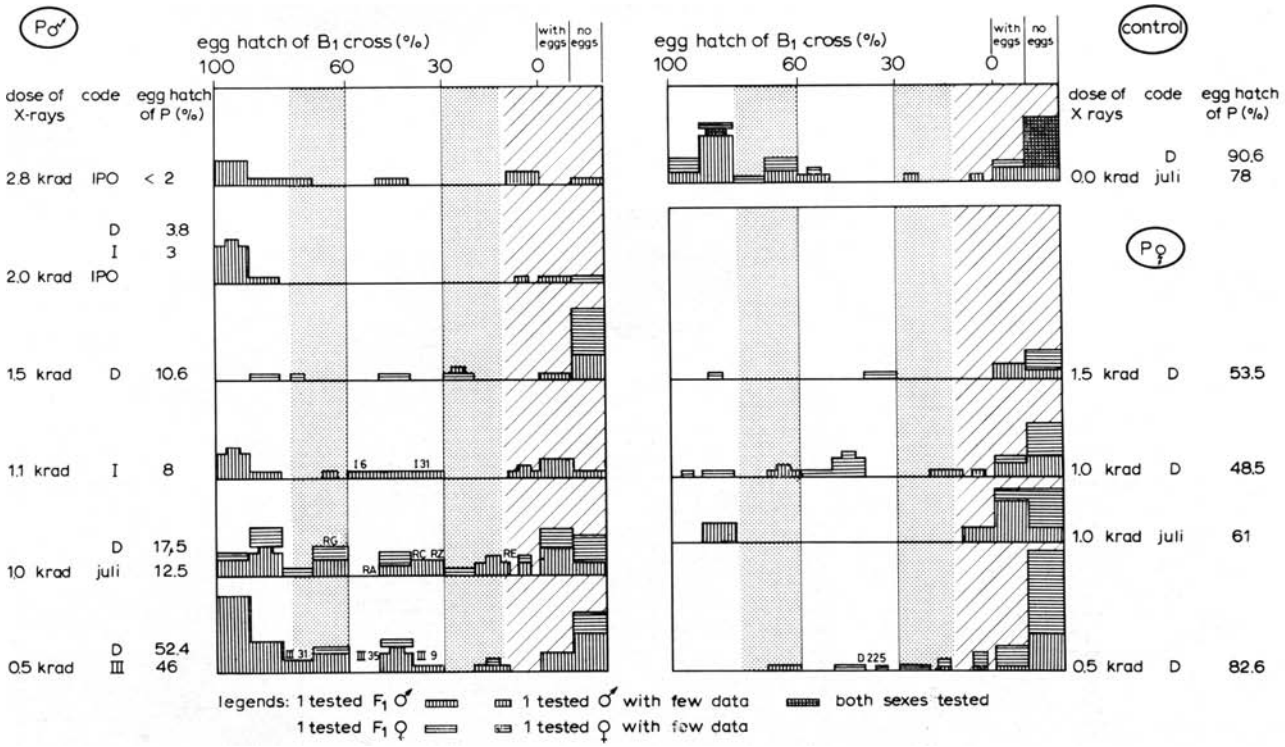

Fig. 1. Diagram of the range of egg hatch of B_1 crosses after different irradiation treatments of P ♂ or P ♀. Dotted areas and area between 60—30% E. H. contain the F_1 stocks suspected of carrying a chromosomal rearrangement. Shaded area contains the failures

Table 1. *Survey of experimental data*

Treatment of *P* Generation					Selection in B_1 crosses									Cytogenetics			
Code		Dose in krad	Sex	stage	% E.H.	n ♂	n ♀	Fertility of F_1 N	BN	T?	BS	S+	S−	Stocks analyzed	No structural mutation*	Structural mutations*	Comments
I	Sept. '69	2.0 X-rays	♂	13d. pupa	3	3		2				1		I 1 γ	0/21		
		1.1 X-rays	♂	13d. pupa	8	16		6	1	3	2	3	1	I 1 $\gamma(\delta)$	0/32		
														I 6 α		11/34	non-disjunction occurs
I		Control			78									I 6 γ		5/13	
														I 6 q	0/5		
														I 31 δ		7/15	cyclic translocation $3^l - 5^l - 6^l$ ∓
III	Oct. '69	0.5 X-rays	♂	1d. adult	46	30		17	5	3	1	3	1	III 7 η	0/24		
														III 9 α		5/11	$3^l - 5$ ∓
III		Control			96									III 22 v	0/5		
														III 22 w	0/6		
														III 23 δ	0/16		
IPO	Winter '70	2.8 X-rays	♂	13d. pupa		10		5 1→		1	2		1	III 26 α	0/11		
		2.6 X-rays		13d. pupa		1							1	III 26 γ	0/2		
		2.0 X-rays		13d. pupa		6		6						III 31 γ		4/9	recipr. transl.
														III 31 η	0/6		
														III 35 a		? 5/34	(recipr. transl.)
D	April '70	2.0 X-rays	♂	13d. pupa	3.8	1	1					1	1	IPO 8 δ	0/6		
		1.5 X-rays	♂	13d. pupa	10.6	6	11	2		1	2	1	11	IPO 14 q	0/18		
		1.0 X-rays	♂	13d. pupa	17.5	4	10	2		1→	1	6	4	D 26	0/9		
		0.5 X-rays	♂	13d. pupa	52.4	7	6		1	3	1		8	D 122	0/3		
		1.5 X-rays	♀	13d. pupa	53.5	3	4	(3)		1		3	3	D 129	0/30		
		1.0 X-rays	♀	13d. pupa	48.5	9	13	2	2	5	2	4	7	D 131	0/4		
		0.5 X-rays	♀	13d. pupa	82.6	10	22	1		2	←1 2	4	22	D 133	0/40		
														D 225 *B*		? 1/1	(recipr. transl.)
D		Control			90.6	10	(10)	2	1				7	RA 2		3/3	$5^s - 6^l$ ∓
														8		8/11	$5^s - 6^l$
														RE 11		2/4	$3^l - X$
July	'71	1.0 X-rays	♂	1d. adult	12.0	18	8	9	4	5	5	1	2	14	0/8		
		1.0 X-rays	♀	1d. adult	51.9 ⌀	13	8	3			2	9	7	20		15/20	$3^l - X$ ∓
		1.0 fast neutrons	♂	1d. adult	3.4	9	4	5		2	2	1	3	RG		4/9	invers. + struct. rearr.
		1.0 fast neutrons	♀	1d. adult	— ⊘									25	0/9		
														30		2/9	invers. + transl.?
July		Control			72.8	20	6	12	3	2	1	3	5	RW	0/7		
														RC		1/3	extra fragment
														42		4/10	transl. (+ invers.)
														46		5/9	recipr. transl.
														47		6/8	transl. (+ invers.)
														RZ 54		? 3/13	(recipr. transl.)
														NJ 61		5/15	recipr. transl.

N % egg hatch > 75
BN % egg hatch 75—60
T? % egg hatch 60—30
BS % egg hatch < 30
S+ no hatch of eggs laid

S− no eggs laid
{ sib strains
* ratios are numbers of individuals with rearrangements divided by total number of individuals from each stock, which were checked cytologically.

∓ see figures *a*—*h*
⌀ reduced fecundity
⊘ no eggs laid
→ borderline case

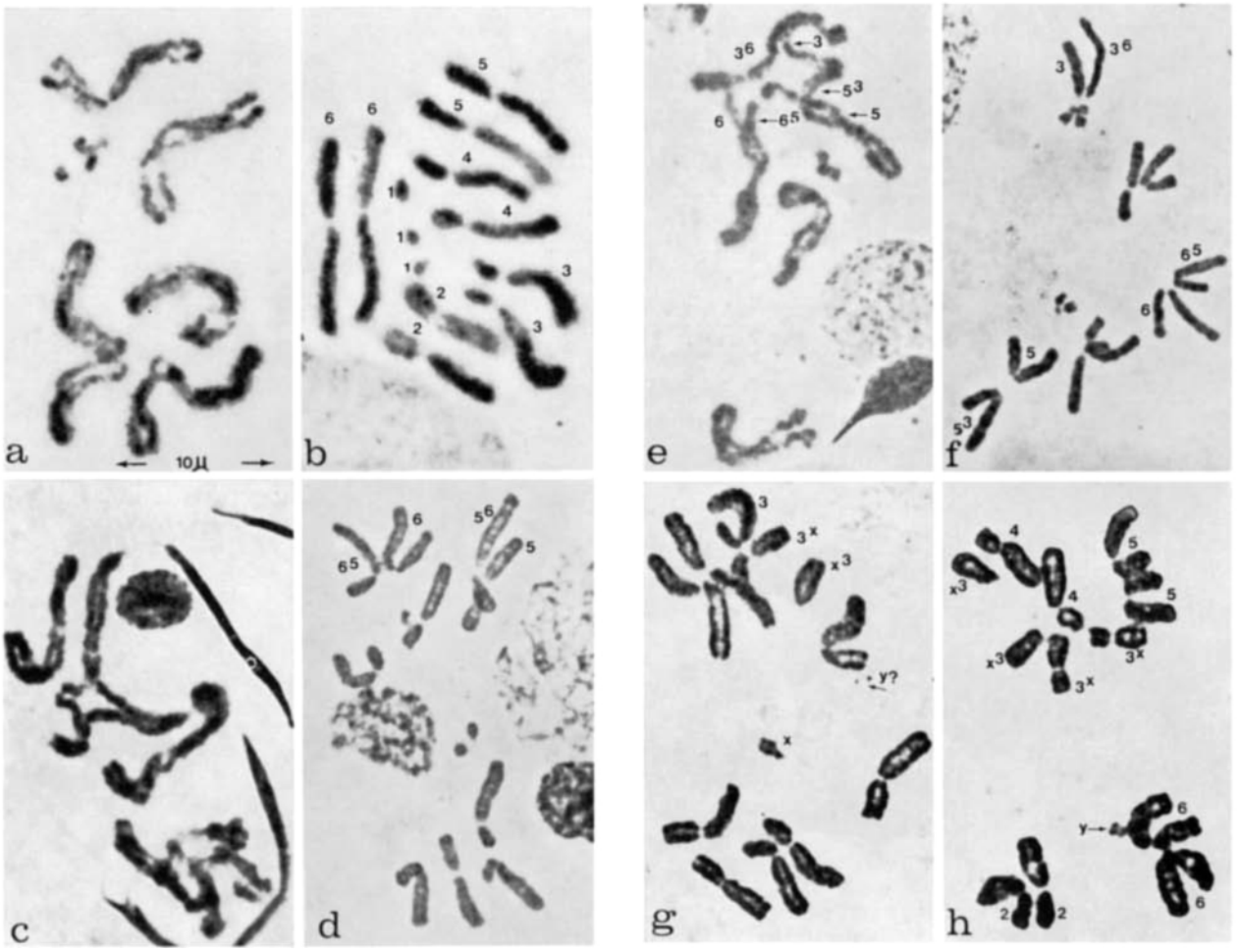

Fig. 2. Photographs of the normal and translocated karyotypes in the onion fly.

a — Normal karyotype, diplotene in male meiosis, 5 autosomal pairs and one complex of three sex chromosomes;

b — Normal karyotype, spermatogonial metaphase, $2n = 13$, clear somatic pairing;

c — Translocation heterozygote III 9 α, diplotene in male, see cross figure, cell incomplete;

d — Translocation heterozygote RA, mitotic metaphase in larval brain cell, exchange 5^l-6^l;

e — Translocation heterozygote I 31 δ, diplotene in male, exchange $3^l-5^l-6^l$;

f — Translocation heterozygote I 31 δ, mitotic metaphase in larval brain cell;

g — Translocation heterozygote RE, mitotic metaphase in larval brain cell, exchange 3^l-X. Chromosome X and X^3 acrocentric;

h — Translocation homozygote RE, mitotic metaphase in larval brain cell, chromosomes 3^X and X^3 in duplo. See the Y chromosome

The dotted areas with egg hatches of 75—60% and 30—15% also show structural rearrangements, as had been proved cytogenetically. Scores in the dotted areas are especially suspect when reduced fertility was accompanied by brown eggs. This is an expression of late embryonic death, probably due to unbalanced genotypes. In the case of a translocation, this feature is the result of adjacent meiotic segregations of the chromosomes in the tested parent.

The shaded area does not give much information on the tested parents, either because they were fully sterile (S—), had no egg hatch at all (S+), or had a very low egg hatch (< 10% E. H.), which is not reliable. This class of failure is rather large. Although it may be a delayed effect of irradiation, failure to mate must have played a role in the high frequency, because the controls also contained a high number of failures.

Normal Karyotype of Hylemya antiqua

In order to obtain a clear picture of the normal karyotype and somatic pairing, many larval brain cells were studied. Boyes (1954) has described the karyotype of *Hylemya antiqua* in detail. His classification of the different chromosomes was used. The chromosome number is 12—13, depending on the sex. Each pair of the five pairs of large sub-metacentric chromosomes, 9—12 μ long at mitotic metaphase,

could be distinguished by looking at the total chromosome length, arm ratio and secondary or tertiary constrictions (fig. 2b).

Two or three very small chromosomes, presumably the sex-chromosomes, $\pm 2\,\mu$ long, were also present (figs. 2a and b). Boyes asserts: Twelve chromosomes were regularly present in larvae studied, of which a small pair of chromosomes are considered to be the sex chromosomes. Contrary to Boyes findings three small chromosomes were usually found in approximately 50% of the larvae checked cytogenetically, and two small chromosomes in the other 50%. In larvae with 13 chromosomes, in spermatogonial metaphases as well as in male meiosis, it could be seen that there were nearly always two acrocentric chromosomes present, as well as a somewhat smaller metacentric one. Sometimes only the 2 acrocentric chromosomes were visible, but the metacentric one was not detectable probably due to its small size. Oogonial metaphases always showed only two small acrocentric chromosomes.

In male meiosis the three small chromosomes sometimes form a trivalent, so a multiple sex determination system is being considered, the male being the heterogametic sex (fig. 2a).

Presumably there is a XXY/XX system and not a Y_1Y_2X/XX system involved, as could be concluded from a translocation (RE) of the acrocentric chromosome and one of the large chromosomes (unpublished).

Cytogenetic Analysis of Chromosomal Rearrangements

When an asymmetrical exchange between two pairs was induced by irradiation, the translocation could be established fairly easily and sometimes a somatic quadrivalent could be seen. When the exchanged chromosome segments had approximately the same length and were rather short, or if a multiple or cyclic translocation or an inversion was involved, pachytene or diplotene were the only stages at which the presence of a structural mutation could be established cytogenetically.

As seen in table 1, some of the semi-sterile stocks originating from 25 suspected F_1 males or females, which were cytogenetically analyzed, carry a reciprocal translocation. Eight different translocations were found, six of them being reciprocal translocations, I 31 δ a cyclic one in which three pairs were involved (figures 2e and f), and the RE translocation appeared to be an X-autosome translocation (figures 2g and h).

In some cases it could be established which chromosomes or arms were involved and it could also be seen whether the translocated arms became longer (+) or shorter (−).

III 9 α	: $3^{l(-)} - 5^{s(+)}$	fig. 2c
RA	: $5^{l(+)} - 6^{s(-)}$	fig. 2d
I 31 δ	: $3^{l(+)} - 5^{l(+)} - 6^{l(-)}$	fig. 2e and f
RE	: $3^{l(-)} - X^{(+)}$	fig. 2g and h

In three cases the presence of a translocation was not convincing, because of the bad quality of the slides or insufficient material. In one case, RG, a pericentric inversion appeared to be the reason for the semi-sterility. In some slides it could be seen that the karyotype had also been changed for some other chromosomes.

The RC stock probably carries both a translocation and a pericentric inversion or sometimes the translocation only.

In 13 of the 25 investigated cases no structural rearrangements were found.

Table 2. *Results of different radiation treatments of males and females (comprimated), as measured by the egg hatch in B_1 crosses, and the number of observed rearrangements*

	Dose krad	F_1 fertility	normal		suspected		Cytogenetic analysis		failures	
		n tested	n	%	n	%	n	result	n	%
P ♂ treated	1.8	10 ♂	5	50.0	2	20.0	2	—	3	30.0
X-rays	2.0	10 ♂ + 1 ♀	8	72.7	—	—			3	27.3
	1.5	6 ♂ + 11 ♀	2	11.6	3	17.4	1	—	12	71.0
Group > 1.1		38	15	39.5	5	13.2	3	—	18	47.3
	1.1	16 ♂	6	37.5	4	25.0	3	2	6	37.5
	1.0	22 ♂ + 18 ♀	11	27.5	13	32.5	6	4 + 1?	16	40.0
Group ± 1.0		56	17	30.4	17	30.4	9	6 + 1?	22	39.2
	0.5	37 ♂ + 6 ♀ 43	17	39.6	14	32.5	7	2 + 1?	12	27.9
P ♂ treated	1.0	9 ♂ + 4 ♀	5	38.4	2	15.4	1	1	6	46.2
fast neutrons	0.25	9 ♂ + 9 ♀	5	27.8	4	22.2	—		9	50.0
Group fN		31	10	32.2	6	19.4	1	1	15	48.4
P ♀ treated	1.5	3 ♂ + 5 ♀	1	12.5	1	12.5	—	—	6	75.0
X-rays	1.0	22 ♂ + 21 ♀	5	11.6	8	18.6	4	—	30	69.8
	0.5	10 ♂ + 22 ♀	1	3.1	3	9.4	1	1?	28	37.5
Control group		20 ♂ + 6 ♀ + 10 pairs	14	38.9	7	19.5	—		15	41.6

Results of Different Radiation Experiments

Table 2 gives a summary of the results. The treatments are compressed together, neglecting differences in growth stage, for males with X-rays, males with fast neutrons, and females with X-rays. The X-irradiations of males are summarized in three groups, one with doses above 1.1 krad, one with doses of 1.1 krad together with 1.0 krad and one with a dose of 0.5 krad.

In the fast neutron treatments an irradiation of 0.25 krad is mentioned, which is still being investigated. Values for control crosses are also represented. The tested F_1 was divided into a class with more than 75% egg hatch (normal), a class with an egg hatch of between 60 and 30% or suspect on account of many brown eggs, and a class (failures) with an egg hatch of below 10%, no egg hatch or even no eggs.

For each class the percentage of the total number of tested F_1 for that dose is listed. Some strains of the suspected class have been analyzed, and the number with observed chromosomal rearrangements is noted under results.

Altogether the fertility of 146 F_1 males was tested, of which definite rearrangements were found in seven stocks while in 2 stocks the conclusion is not clear (table 1) 88 F_1 females were also tested, with 2 proved rearrangements and one indistinct case. The total output of the 234 tested F_1 was 4% clearly visible rearrangements. Table 3, in which the percentages are calculated on the numbers of respondents (being the total tested minus failures), is even more compressed.

Conclusions and Discussion

The data obtained so far prove that it is possible to induce translocations and/or inversions in the onion fly, which express themselves in heterozygotes by the reduction of fertility. These results resemble those of Laven *et al.* (1971) for *Culex pipiens* L. To induce these rearrangements, X-rays can be applied to males and females either as late pupae just before eclosion or as young adults (table 1).

As far as semi-steriles in the F_1 are concerned, it makes no difference whether late male pupae or young adult males are irradiated, probably because the early spermatids, expected to be the most sensitive for translocation induction, are already present in late male pupae (Sobels, 1969).

Irradiation of females causes serious reduction of fecundity. When females are irradiated as old pupae or as young adults their ovaries are still developing (Theunissen, 1971). Irradiation with the doses used at these stages results in a loss of fecundity. The whole oogenesis ceases in young females. Apart from lethal mutations in the germ line, the disturbed activity of nurse cells causes egg production to be stopped (Theunissen, 1971). Roughly, it may be stated that irradiation of females is not advisable for the production of semi-sterile stocks because little F_1 is produced and many F_1 individuals are sterile or nearly sterile, whereas the number of visible rearrangements among the respondents is not decisively higher (fig. 1, table 2 and 3). In the backcrosses both sexes can be used, the males being better respondents (fig. 1).

Irradiation of males with 1.0 krad fast neutrons induces more dominant lethals than 1.0 krad X-rays (table 1, *P* generation % egg hatch). In the smaller F_1-pool that is left, the number of carriers of a structural rearrangement might be higher than for X-rays. The few data represented here do not allow any conclusions to be drawn. All conclusions on the efficiency of the irradiation treatments are given without statistical proof. The variation of the material used and the few data on each treatment are not suited to statistical analysis.

From fig. 1, it is apparent that most of the induced translocations were observed in the suspect class which had an egg hatch of between 60 and 30%, but some, such as RG, III 31 and RE, could be found in the dotted area between 75 and 60% and between 30 and 15%. In the experiments reported, cytological analysis of stocks originating from an F_1 with a normal egg hatch was omitted. In later experiments, some material from stocks with a normal fertility in F_1, and also from stocks of controls with a suspected % egg hatch was analyzed. Chromosomal rearrangements were never found, but this does not prove that rearrangements will not occur in these categories.

	Egg hatch B_1	Egg hatch B_{3-4}
I 31 ♂	40%	45%
RE	20%	60–70%
RA	50%	70%

Table 3. *Number of proved structural rearrangements related to number of semi-sterile F_1 and their percentage from tested F_1 with an egg hatch above 10%, grouped for males treated with X-rays or fast neutrons and females with X-rays, and the control values*

Irradiated sex	Treatment	tested F_1 n	respondent n	suspected n	suspected %	rearrangement n	rearrangement %
♂	X-rays	137	85	36	42.4	8 (+2?)	9.4 (11.7)
♂	fast neutrons	31	16	6	37.5	1	6.35
♀	X-rays	83	19	12	63.1	1?	5.3
♂ + ♀	control	36	21	7	33.3		

In later generations of some of the translocations reared for cytogenetic purposes, a higher fertility has sometimes been achieved. This is probably influenced by a change of incubation method for the eggs: three instead of two days and a temperature of 29 °C instead of 23 °C. This change permits a better distinction between unfertilized eggs and late embryonic deaths. The unfertilized eggs were, from that time, deducted while calculating the % egg hatch. In this way the fertility of the control crosses is also essentially higher, because incomplete fertilization falls out. A natural selection against induced recessive lethals or other effects of irradiation might also have influenced egg hatch. The increase in fertility is favourable for the rearing of the insect in small numbers.

The clear distinction of brown eggs was favourable for cytogenetic investigation. For example, in the case of RE the cross of the translocation heterozygote with the normal mates scored an average of 30% brown eggs. If translocation heterozygotes were intercrossed, 51% brown eggs was observed. In this case, translocation homozygotes were detected for the first time among the progeny (fig. 2h). Experiments will be carried out to establish a stock of homozygotes so that cage experiments may be started with these translocation homozygotes released into a normal population stock.

Several kinds of chromosomal rearrangements were observed. Although the efficiency of induction and selection is not very high (10% of respondents at 1.0 krad X-rays on males), it should be possible to isolate the kind of chromosomal rearrangement desirable for genetic control in the onion fly.

A problem which still has to be solved is the design of a procedure for genetic control in which chromosomal rearrangements at least fit theoretically (Wijnands-Stäb and Frissel, 1973). The combination of rearrangements with conditional lethals should now be studied experimentally.

Another question is the relationship between radiation dose and chromosome-breakage events. Increasing the dose results in a higher chance of breakage so that complicated rearrangements and/or fragments will occur. This was observed in the cases I 31 δ and I 6 α after 1.1 krad of X-rays on late male pupae. In I 31 δ, a clear cyclic translocation was found because of a three-hit-event in which three chromosome pairs were involved. Three rather long chromosome segments were exchanged (figs. 2e and *f*). This stock perfectly resembles the theoretical description by Curtis and Robinson (1971) of a three-chromosome double translocation. I 6 α showed an extra fragment. Doses of about 1.0 krad of X-rays give a reasonable result. At lower doses the proportion of normal individuals in the F_1 rises. This fact increases the amount of work necessary. Improving the mating conditions, which is being studied at present, would increase the efficiency of the selection procedures.

It was observed that most of the chromosomes were involved several times in one of the reciprocal translocations. Chromosome 3 was involved in translocations RE, I 31 δ and III 9 α, chromosome 5 in I 31 δ, III 9 α and RA, chromosome 6 in RA, I 31 δ and in the pericentric inversion of RG. The length of the chromosome is one of the factors which determines the chance of becoming involved in a rearrangement, so it is strange that the small X-chromosome was found in the RE translocation. It is also interesting to note where the breaks occurred, on the chromosomes. In the case of chromosome 3, the long arm 3^l was always involved and there is some indication that the initial breaks were often more or less in the middle of 3^l, as observed in III 9 α, I 31 δ and RE.

In many somatic metaphases one or two tertiary (heterochromatic) constrictions were seen in the middle of chromosome arm 3^l (Boyes, 1954). For example, Whittingham and Stebbins (1969) pointed out that breakage positions in translocations are usually located within or at the end of heterochromatic regions. This agrees with our observations that the breaks in the long arm of chromosome 3 as well as the break in the X-chromosome are close to or inside the heterochromatic region.

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Note added to the proof:

From later experiments in the *X*-linked translocation stock (RE) it appeared that a normal $XY^{(\delta)}/XX^{(\varphi)}$ sex-determination system is involved. The third small (metacentric) chromosome doesn't pair usually with the two acrocentric sex chromosomes. In the control series we could get rid of this chromosome in both sexes and therefore should be considered as a B-chromosome.