CHROMOSOMAL REARRANGEMENTS IN THE ONION FLY *HYLEMYA ANTIQUA* (MEIGEN), INDUCED AND ISOLATED FOR GENETIC INSECT CONTROL PURPOSES

Studies on cytogenetics and fertility, with emphasis on an X-linked translocation

Dit *proefschrift met stellingen* van

CORNELIS VAN HEEMERT,

landbouwkundig ingenieur, geboren te Leeuwarden op 28 april 1944, is goedgekeurd door de promotor, dr.ir. J. Sybenga, lector in de erfelijkheidsleer.

De Rector Magnificus van de Landbouwhogeschool

H.A. Leniger

Wageningen, 7 december 1974

C. van Heemert

Chromosomal rearrangements in the onion fly *Hylemya antiqua* (Meigen), induced and isolated for genetic insect control purposes

Studies on cytogenetics and fertility, with emphasis on an X-linked translocation

Proefschrift
ter verkrijging van de graad van
doctor in de landbouwwetenschappen,
op gezag van de rector magnificus, prof. dr. ir. J.P.H. van der Want
hoogleraar in de virologie
in het openbaar te verdedigen
op donderdag 27 maart 1975 des namiddags te vier uur
in de aula van de Landbouwhogeschool te Wageningen

OH CHROMOSOMES

(melodie: O denneboom)

Oh chromosomes, my chromosomes,
How faithful is thy mission.
Oh chromosomes, my chromosomes,
Thou bringest my condition.
You make my eyes look brown or blue.
My blood group, too, depends on you,
Meiosis brings us something new,
Not gained by simple fission.

Oh chromosomes, my chromosomes,
We've learned to know you better,
We know the code of DNA
We can translate each letter.
Our thymine must have adenine,
Our guanine mates with cytosine;
Their messenger, pure RNA
Puts our proteins together.

Oh chromosomes, my chromosomes,
How faithful is thy mission.
Oh chromosomes, my chromosomes,
How sad is my condition.
My grandsire's gift for singing well
Has gone to some lost polar cell.
That's why I sing this doggerel;
I can do no better.

G.L. Stebbins 1969

aan Janny
Judith
Marcel

Voorwoord

Het is voor mij een genoegen dat ik via dit voorwoord in de gelegenheid ben om verschillende personen en instanties te bedanken voor hun steun en bijdrage bij het tot stand komen van dit proefschrift.

In de eerste plaats gaat mijn dank uit naar mijn ouders die mijn studie en studietijd mogelijk maakten.

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Mijn gastheer, Prof. Dr. Ir. J.H. van der Veen, wil ik dankzeggen voor de periode die ik op de afdeling Erfelijkheidsleer verbleef. Ik zal zeer goede herinneringen aan deze tijd overhouden, maar de herinnering loopt nog verder terug. Beste Jaap, als student maakt ik onder jouw supervisie een belangrijke fase door. Jouw geïnspireerdheid in het vak genetica bleek op mij besmettelijk te werken, zoals ondervonden tijdens het mutatieonderzoek in Arabidopsis en de inzichtelijke colleges populatie-genetica. Ondanks mijn vertrek naar het ITAL hoop ik nog regelmatig van de gastvrijheid gebruik te mogen maken om het noodzakelijke contact te behouden.

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To Dr. A.S. Robinson (ITAL) I would like to speak my appreciation for the continuous contact we have since he joined the project. Dear Alan, you

STELLINGEN

I

De kleine acrocentrische chromosomen van de uievlieg *Hylemya antiqua* (Meigen) zijn de geslachtschromosomen.

Dit proefschrift

II

Het induceren van structurele chromosoommutaties voor het gebruik bij de genetische insektenbestrijding dient bij een zo laag mogelijke stralingdosis te gebeuren. De genetische achtergrondschade en het risico van te complexe structurele mutaties worden hierdoor geminimaliseerd.

Dit proefschrift

III

Duplicatie-deficiëntie gameten van translocatie heterozygote of translocatie trisome vaders van de in dit proefschrift beschreven X-gekoppelde translocatie zijn alle in staat tot bevruchting.

Dit proefschrift

IV

Afwezigheid van chiasmata bij mannetjes van de uievlieg wordt bevestigd door het normaal fertiel zijn van (pericentrische) inversie heterozygote mannetjes.

Dit proefschrift

V

De inductie van androgenese bij insekten kan met een groter effect geschieden door middel van een temperatuurschok dan door middel van röntgenstraling.

Heemert, C. van (1973). Nature New Biology 246, 149: 21-22

VI

De somatische synapsis zoals door Halfer en Barigozzi beschreven voor *Drosophila melanogaster* is niet representatief voor de somatische paring in Diptera in het algemeen.

Halfer, C. and Barigozzi, C. (1973). Chromosomes today Vol. 4: 181-186

VII

De fertiliteit van een translocatie heterozygoot uitgedrukt als het percentage "egg-hatch" is alleen een maat voor de "alternate" segregatie indien duplicatie-deficiëntie karyotypen post-embryonaal niet levensvatbaar zijn. De door Curtis en Hill in hun modellen gebruikte factor $\bar{W}$ voor fertiliteit kan hierdoor onderschat zijn.

Curtis, C.F. and Hill, W.G. (1971). Theor.Pop.Biol.2:71-90

VIII

De postulering van Dennhöfer dat een enkele mendelende factor "sg" de verhouding tussen de verschillende oriëntatietypen in een translocatie heterozygoot bepaalt is zeer onwaarschijnlijk.

Dennhöfer, L. (1974). Theor.Appl. Gen. 44:311-323

IX

Eventuele introductie van genetische methoden bij de bestrijding van de uievlieg in Nederland dient van overheidswege te geschieden.

X

Ontwikkeling van de wetenschap hangt niet alleen af van de geboden materiële mogelijkheden en de vakkennis van de onderzoeker, maar tevens van diens intuïtie -door Ramsey beschouwd als "disclosure": het dóórbreken van een nieuw idee- en diens capaciteit om dit idee uit te werken. In het kader van het wetenschapsbeleid dient hieraan meer aandacht besteed te worden.

Ramsey, I.T. (1963). Religious Language, New York, Macmillan Paperback Edition

XI

Het zich te geïsoleerd in zijn specialisme opstellen van de wetenschappelijke onderzoeker is een rem op de ontwikkeling van de geïntegreerde bestrijding van ziekten en plagen in land- en tuinbouw.

Proefschrift van C. van Heemert
Wageningen, 27 maart 1975

made a quick start in a new field and it was surprising how fast you could take over Clary's work. I hope we will soon be able to solve the puzzle of the translocation homozygotes.

Zonder assistentie voor de kweek en de cytologie zou het werk zeer veel langer hebben geduurd. Dankbaarheid ben ik dan ook verschuldigd aan Dorothée Botje (I.A.E.A. budget) en aan Willem van den Brink (T.N.O. budget).

Alle medewerkers van de afdeling Erfelijkheidsleer wil ik gaarne dankzeggen voor de goede contacten die ik de afgelopen jaren mocht hebben. In het bijzonder wil ik de Heer K. Knoop bedanken voor zijn steeds weerkerende fotografische en technische hulp en het vervaardigen van tekeningen en tabellen. Manuscripten en brieven werden door Henriët Boelema met veel typevaardigheid behandeld. De klimatologische beheersing van de kweekruimte door de Heer P.L. Visser werd zeer op prijs gesteld.

De leden van de uievliegclub op het I.P.O., Drs. Thijs Loosjes, Winold Noordink Ing., Jan Noorlander, Ir. Jan Theunissen en Dr.Ir. Jan Ticheler dank ik voor de goede connecties, waardoor nieuwe feiten of ideeën verkregen werden.

De Centrale Organisatie van T.N.O., Sectie Landbouwkundig Onderzoek maakte het mij mogelijk ruim vier en een half jaar aan de uievlieg te werken. Tevens werd ik in de gelegenheid gesteld om regelmatig congressen te bezoeken. Verder dank ik T.N.O. voor de financiële bijdrage in de kosten van dit proefschrift.

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Last, but not least, wil ik mijn vrouw Janny bedanken voor haar bijzondere bijdragen. Ook erg veel dank voor het uittypen van het proefschrift. Graag draag ik dit proefschrift aan jou en de kinderen op.

Contents

Introduction

Mankind is continuously confronted with insects causing considerable damage in agriculture or endangering public health especially in tropical countries. For different reasons alternatives for chemical insecticides were developed. Firstly, as a result of the chronic use of these agents resistance against insecticides appeared in several insect species. Secondly, persistence of the chemicals used in the field is considered by most people to be an important case of environmental pollution. Thirdly, the non-specificity of many insecticides used is an argument for investigations on a more selective method of insect control not affecting beneficial insects and other organisms.

It is for these reasons that entomologists and geneticists have started studies on a number of insect species to investigate if their reproduction can be cut down by the use of a genetic or other autocidal system causing sterility. The onion fly, *Hylemya antiqua* (Meigen) being an important pest species attacking the onion crop in the Netherlands was chosen as an object in this country. Fortunately no other important insects attack the onion crop, otherwise it would be difficult to test the damage caused exclusively by the onion fly after a control program. About ten years ago at the Institute for Phytopathological Research (IPO) a number of studies was started on, among others, mass-rearing, radiation effects, and ecology. The sterile insect release method (SIRM) was the main purpose in the first few years. With this method a recurrent release of a large number of sterilized insects many times per generation and during many years has to be carried out. Some of the most important factors on which success of this method depends are good competitivity and longevity of the released complete sterile insects compared to the native flies in the field. Mass-rearing must be carried out without problems. Further, geographic isolation is very important to prevent fertile immigrants moving into the treated areas. The sterile insect principle is based on the induction of 100% dominant lethality in the gametes of the irradiated parents. As a consequence, the fertilized eggs die in an early stage and do not hatch.

A different approach was started (1969) at the ITAL and the Department of Genetics by developing strains with structural chromosome mutations (trans-

locations, inversions or compound chromosomes) causing "semi"-sterility. By the principle of using chromosomal rearrangements in contrast to the sterile insect method, the sterility of the strain is hereditary and will show up again in later generations. In the first instance it was decided to concentrate on radiation-induced chromosomal rearrangements rather than on other genetic means (cytoplasmic incompatibility, hybrid sterility, meiotic drive, deleterious genes or sex-ratio distortion).

A chromosomal rearrangement can be used in an other way viz. as a genetic transporting mechanism. Negative heterosis or underdominance of the structural heterozygotes can lead to elimination of one of the two homotypes (either the normal or the translocation homozygous karyotype) in the case the population is in a disequilibrium. By linking a conditional lethal gene with the rearrangement one can replace the original population by the modified population and subsequently eliminate the new population by the effect of the introduced gene. Here, this method has not been given further attention.

In absence of data for the onion fly on the optimal radiation dose and conditions for inducing chromosomal rearrangements causing "semi"-sterility, first the methodology was developed. The first three articles of the underlying thesis cover the irradiation work, the selection on the basis of "semi"-sterility after testcrosses and the isolation of suitable strains. Further, a cytological description of the isolated "semi"-sterile strains is presented. This author is responsible primarily for the parts on cytogenetics and fertility.

In the fourth and fifth paper we deal with an unusual X-linked translocation. This translocation appeared to be very suitable for basic studies on meiotic disjunction of the chromosomes of the translocation complex as appeared from observations on M II (males) and young eggs (males and females). Studies on the relationship between embryonic lethality ("semi"-sterility) and the duplication/deficiency karyotypes are of fundamental importance. As the result of meiotic numerical non-disjunction in translocation heterozygous females, several different karyotypes like translocation trisomics, primary trisomics, tertiary trisomics and duplication/deficiency karyotypes occurred. Many of these were viable into the adult stage. The meiotic behaviour and embryonic lethality of these aneuploids is discussed.

For an ordinary X-linked translocation only females can become homozygous. However, we have found both viable translocation homozygous males and females which can reproduce. These had one or two additional sex-chromosomes. This will be further considered in the general discussion.

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

K. J. A. WIJNANDS-STÄB and C. VAN HEEMERT

Institute for Atomic Sciences in Agriculture, Wageningen and Department of Genetics, Agricultural University, Wageningen (The Netherlands)

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 237 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 ø 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.

A 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.

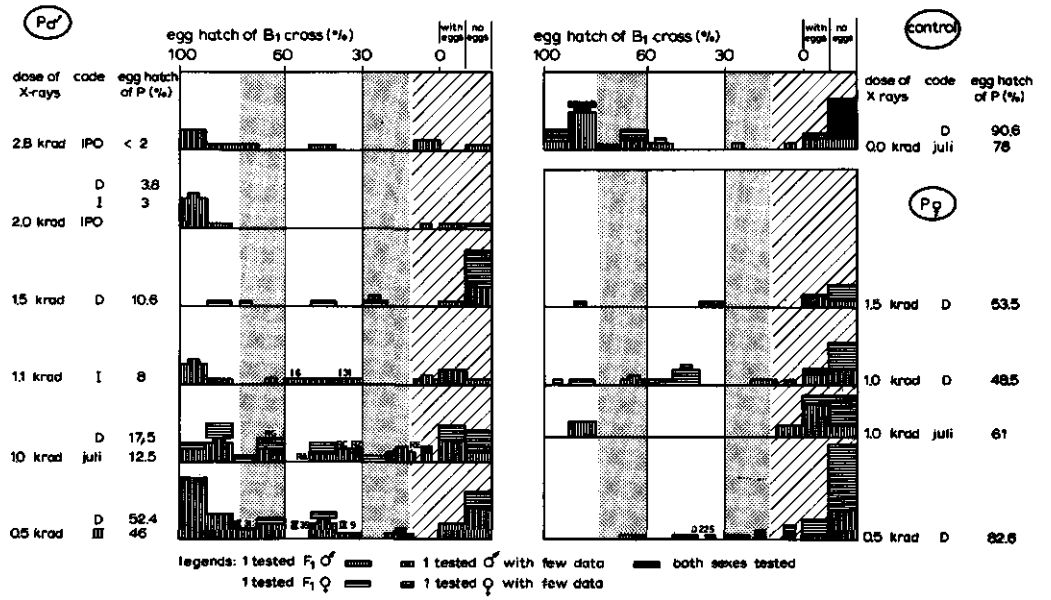

Fig. 1. Diagram of the range of egg hatch of B_1 crosses after different irradiation treatments of $P\,\sigma$ or $P\,\varphi$. Dotted areas and area between 60—30% E. H. contain the F_1 stocks suspected of carrying a chromosomal rearrangement. Shaded area contains the failures

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	5	(1)		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	25		1	2←1	(5)	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	$5^l - 6^s$ ∓
													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

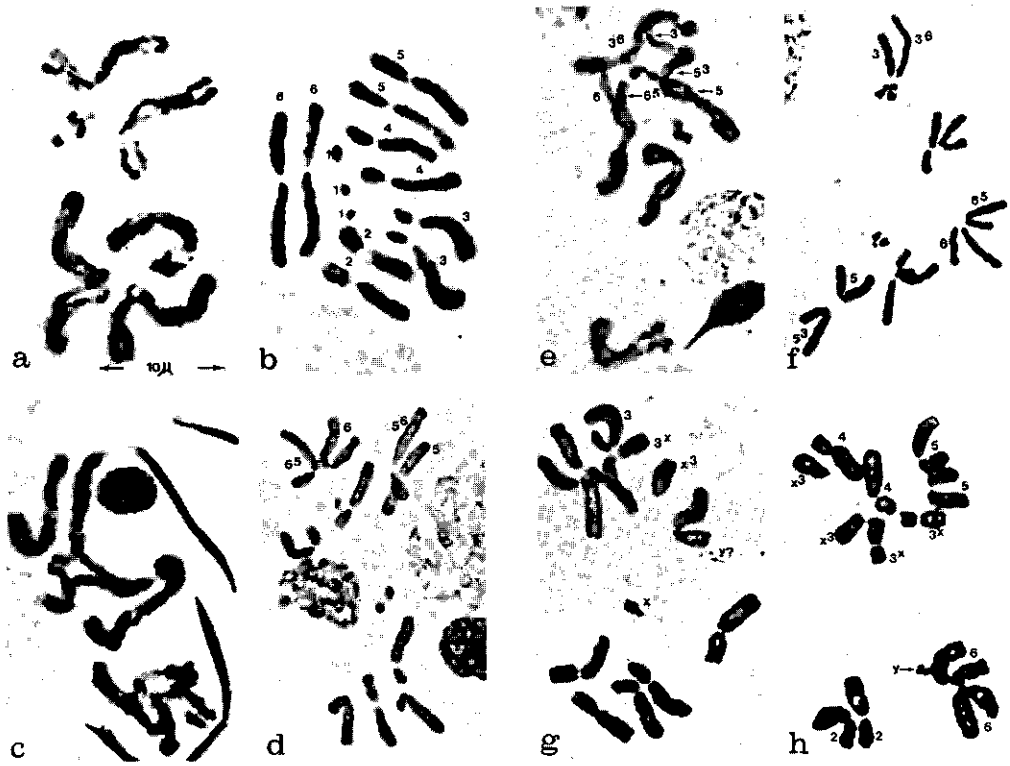

Fig. 2. Photographs of the normal and translocated karyotypes in the onion fly.

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 $5^l - 6^s$;

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	2.8	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 ♂ + 25 ♀	—	—	4	11.4	1	1?	31	88.6
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) 91 F_1 females were also tested, with 2 proved rearrangements and one indistinct case. The total output of the 237 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	%	rearrangement n	%
♂	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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C. van Heemert
Department of Genetics
of the Agricultural University
53 Generaal Foulkesweg
Wageningen (The Netherlands)

K. J. A. Wijnands-Stäb
Institute for Atomic Sciences
in Agriculture
Wageningen (The Netherlands)

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.

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Radiation induced semi-sterility for genetic control purposes in the onion fly *Hylemya antiqua* (Meigen)

II. Induction, Isolation and Cytogenetic Analysis of New Chromosomal Rearrangements

C. van Heemert and K.J.A. Wijnands-Stäb

Department of Genetics, Agricultural University, Wageningen and
Institute for Atomic Sciences in Agriculture, Wageningen (The Netherlands)

SUMMARY

The study of the radiobiological and cytogenetic aspects of induced semi-sterility for the application in genetic control of the onion fly *Hylemya antiqua* (Meigen) has been continued. Doses of 1.5 krad of X-rays or 0.25 krad of fast neutrons were applied to males and 1.0 krad of X-rays or 0.25 krad of fast neutrons to seven days old females. On the basis of semi-sterility (between 60% and 30% egg hatch) in backcrosses to normal flies, eleven strains were suspected of carrying a chromosomal rearrangement. Seven had a reciprocal translocation and two from a 1.5 krad X-ray treatment, showed complex rearrangements. In two strains no rearrangements were found. Combining experimental data of earlier experiments with the new results we concluded that the irradiation of males with low doses, 0.5 krad of X-rays or 0.25 krad of fast neutrons is suitable for the induction of chromosomal rearrangements. Strains carrying rearrangements from such low dose treatments will be further used for the genetic control experiments of the onion fly.

INTRODUCTION

The induction of chromosomal rearrangements for the control of the onion fly, *Hylemya antiqua* (Meigen), has been described by Wijnands-Stäb and van Heemert, (1974). More data were needed which are presented in this report. When males were irradiated with 1.0 krad of X-rays, this dose appeared suitable for the

production of semi-sterile translocation stocks. In order to increase the yield of rearrangements, this time a dose of 1.5 krad of X-rays was decided upon. Females were treated with 1.0 krad of X-rays when seven days old, instead of one day old females which had a very low fecundity after irradiation due to disturbed ovarian development (Theunissen, 1971). Fast neutrons were administered at a lower dose (0.25 krad) than previously (1.0 krad) to both sexes in order to increase the parental fertility.

As described in the previous paper the selection for semi-sterility was carried out in the F1 generation (see Materials and Methods). Crosses with an egg hatch between 60% and 30% mainly were used for cytological analysis. This category of semi-sterile F1-crosses will be further named suspected. In contrast to the previous paper the crosses in which no eggs were produced and the crosses of which no eggs hatched are called failures.

In general chromosomal rearrangements can be found in the whole fertility range from 0% to 100% (Searle et al, 1974). The fertility is positively correlated with the percentage of alternate orientations of translocation multivalents. Mainly strains with a 60%-30% egg hatch were selected because these can still be reared efficiently and may be useful for genetic control purposes.

Variations in fertility had been found in the irradiated generation (Wijnands-Stäb and van Heemert, 1974) at each irradiation treatment. We assumed that the variation in radiation sensitivity of the material related to the method of rearing might cause a part of this variation. Therefore the same treatment was given to males from a continuous reared stock without inducing diapause and to males from an earlier generation which had been stored for several months as pupae in diapause. No difference in radiosensitivity was found and the results of both groups are therefore combined in this report.

MATERIALS AND METHODS

In general the same materials and methods were used as decribed in Wijnands-Stäb and van Heemert, 1974. After initial collection of larvae from the field, the onion flies had been reared for three to five generations in the laboratory with avoidance of inbreeding. The male flies were irradiated on the first day of their adult life. The female flies were irradiated on the seventh day after emergence. On the seventh day the ovaries are full-grown and the females mate and subsequently oviposite quickly following massmating. F_1 flies were individually testcrossed in the first backcross (B_1). In this paper like in the previous

one the symbol P is used for the irradiated flies and their untreated mates; their offspring is called F_1, and is backcrossed to untreated mates (B_1 cross), to yield the B_1 generation. The following backcross is called B_2, etc. The fertility was determined by measuring the percentage hatched eggs over the total number of eggs deposited. This percentage is not corrected for the reduction in fertility as measured from the control crosses.

For scoring hatchability and browning in B_1 and B_2 crosses the eggs were incubated for three days at 29°C and at a high R.A.H. This permits a good discrimination of brown eggs (late embryonic lethals) from the unfertilized and the hatched (empty) eggs (van Heemert, 1973). The percentage of brown eggs versus empty eggs ($\frac{B}{B+E} \times 100$) is used as another criterion for the selection of semi-sterile stocks.

The offspring of semi-sterile B_1 crosses was preserved for further rearing and/or cytological analysis. If possible five sons and five daughters were test-crossed individually in a second backcross (B_2), to see if any sex-linkage is present. The fertility in the control in general is about 85%, however in a few cases a rather low fertility was found. For cytologic analysis testes were preferred, since primarily due to meiotic pairing the presence of a rearrangement can be established even when small segments are exchanged or a symmetrical exchange is present. Larval brains were useful for cytology as well although difficulties in the analysis may arise when the exanged segments were small and/or similar in size.

RESULTS

A survey of the treatments and most fertility scores and cytogenetic analyses is given in table 1. The fertility of the irradiated parents in crosses with normal mates expressed as the percentage of egg hatch is rather low (4% - 15%). Strains suspected of carrying a chromosomal rearrangement (60%-30% E.H.) are listed with the percentage of egg hatch and the percentage of brown eggs (late embryonic lethals). In a control cross usually the percentage of brown eggs is below 10%. Mostly semi-sterility in the B_1 and the B_2 is correlated with a percentage of brown eggs of 20%-60%. The frequency of rearrangements found in a particular stock is given as the number of individuals with the rearrangement divided by the total number analyzed. A short indication of the kind of rearrangement is given in the table.

Table 1. The effect of different radiations on the onion fly in terms of fertility and structural mutations in the B_1 and B_2. Only the strains which are analyzed cytologically are mentioned.

P generation				B_1 cross tested cytologically				B_2 cross tested cytologically					
Dose in krad	sex	E.H.	tested on fertility	B_1 code	sex	E.H.	B.E.	B_2 code	sex	E.H.	B.E.	Structural mutations*	Comments
1.5 X-rays	♂	4	33										
				Fe 1	♂	31	43	B_2 a	♂	47	49	3/6	$3^{1(-)}$ - $6^{s(+)}$
								B_2 b	♂	49	42	3/6	
								B_2 c	♀	57	31	3/6	
				Fe 2	♂	25	60	B_2	♀	32	15	1/6	$3^{1(-)}$ - $6^{1(+)}$
				Fe 3	♀	54	20	-				15/23	three pairs in complex
				Fe 4	♀	30	67	-				6/8	chrom. 3,6 and X or Y in complex
				Ma 4	♀	44	47	B_2 a	♀	31	53	1/1	4^1 - 6^1
								B_2 b	♀	35	37	0/3	
								B_2 c	♂	74	15	1/6	
								B_2 d	♂	26	61	2/3	
				Ma 5	♂	63	7	B_2	♀	27	55	0/1	
1.0 X-rays	♀	15	24	Ma 1	♂	57	18					2/6	$2^{1(-)}$ - $6^{s(+)}$
0.25 fast neutrons	♂	10	25										
				Ma 7	♀	38	43	B_2 a	♀	65	26	4/12	$2^{1(-)}$ - $6^{s(+)}$
								B_2 b	♀	37	38	0/3	
				Ma 8	♀	39	53	B_2	♀	51	50	0/9	
				Ma 10	♂	58	18	-				1/1	recipr. transl.
				Ma 11	♂	34	47	-				2/2	3^1 - 6^1
0.25 fast neutrons	♀	8	12										
control		85											

Legends table 1

E.H. % egg hatch

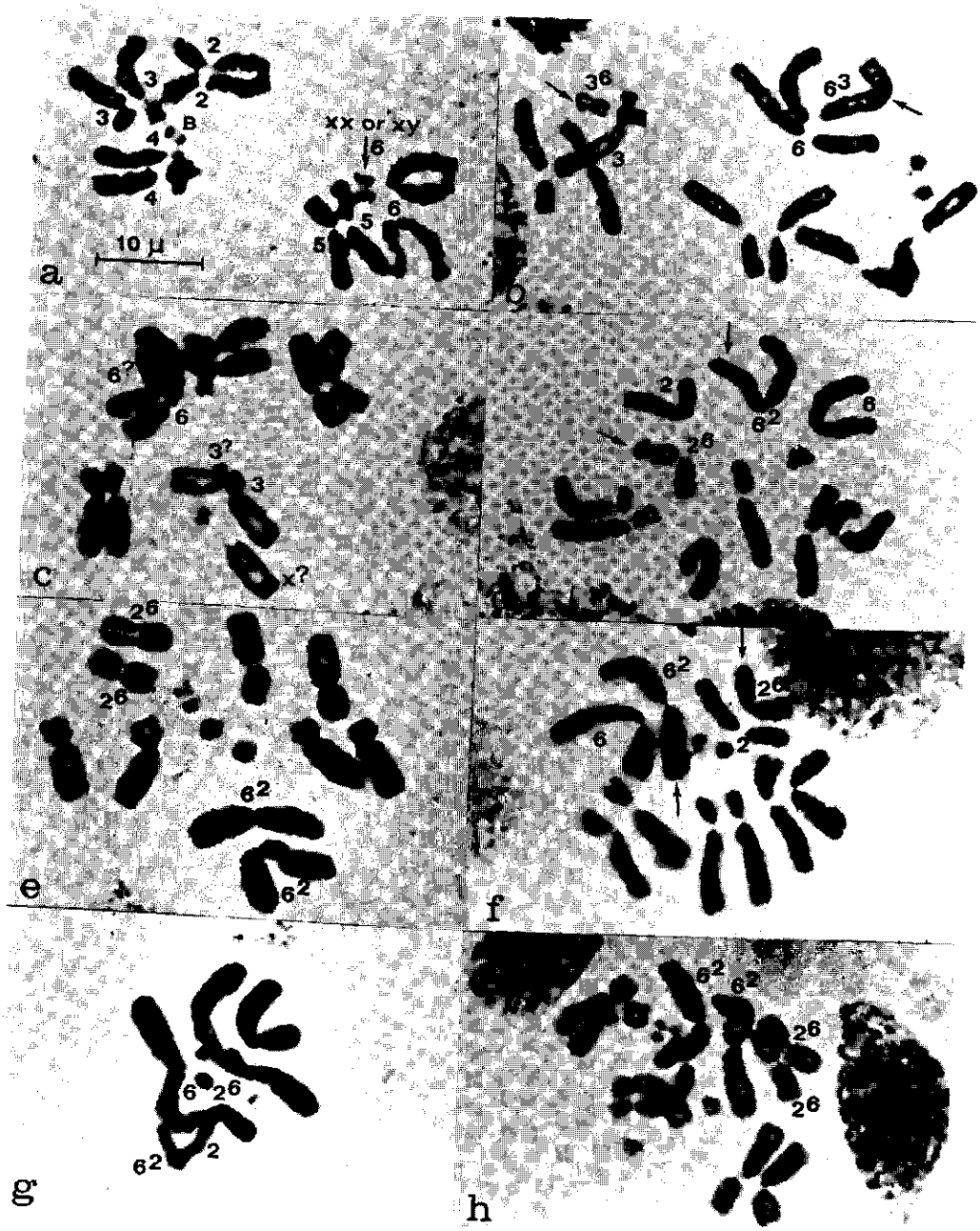
B.E. % brown eggs

⌒ sib strains

*ratios are numbers of individuals with rearrangements divided by total number of individuals from each stock, which were checked cytologically (in B_1 and/or B_2).

Eleven strains suspected of having a rearrangement were analyzed cytologically for the presence of chromosomal rearrangements and in nine of these a rearrangement was found. Five F_1 stocks which were suspected were lost during the rearing before cytologic analysis was carried out.

In fig. 1a the normal karyotype is shown. In the progeny of backcrossed flies from the Fe 1 stock, a reciprocal translocation was found between chromosomes 3 and 6 in mitotic as well as meiotic stages. The long arm of chromosome 3 had become shorter and the short arm of chromosome 6 had gained in length: $3^{1(-)}$ - $6^{s(+)}$. Fig. 1b shows a mitotic metaphase in a larval brain cell of a heterozygote for this translocation. Among larvae from B2 crosses of Fe 2 in one case out of six a rearrangement between $3^{1(-)}$ and $6^{1(+)}$ was found. The other five appeared to be normal. Complex rearrangements were found in the offspring of Fe 3 and Fe 4. Three pairs of chromosomes were involved (Fe 4 see fig. 1c) and even trisomy for chromosome 3 could sometimes be observed in a few larvae. One reciprocal translocation was found in the progeny of B_2 crosses (Ma 4). Testes pre-


a
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parations showed that the interchanged segments of this tranlocation (4^{l}-6^{l}) are of an equal length. After irradiation with X-rays of seven days old females, one translocation was found (Ma 1) between chromosomes 2 and 6: $2^{l(-)} - 6^{s(+)}$, fig. 1d. This is the first clear case in which we have obtained a translocation in the progeny of an irradiated female. Two different translocations, Ma 10 and Ma 11, were found in the B_1 cross progeny of a male irradiated with fast neutrons, 0.25 krad. Although only three testes preparations could be analyzed, we were able to establish that the chromosome arms 3^{l} and 6^{l} are involved in Ma 11. In two B_2 crosses of a male treated with fast neutrons (0.25 krad) only for Ma 7 a rearrangement ($2^{l(-)} - 6^{s(+)}$) was found (figs. 1f and 1g) in the testes of the male progeny. Data on sibcrosses involving translocations Ma 1 and Ma 7 will be discussed below. No individuals suspected to have a rearrangement were found in the progeny of females irradiated with fast neutrons (tabel 1).

In fig. 2 we have presented the data in a slightly different way as presented in fig. 1 of the previous paper. As mentioned above only the B_1 crosses in which no eggs or no hatching eggs were scored were considered as failures. The sterility area most relevant for genetic control with translocation stocks (60%-30%) is indicated. The range of percentages of egg hatch in the B_1 crosses with progeny is shown for the various treatments (table 1). The rearrangements which were found are marked in the figure. As seen in fig. 2, the fertility of the B_1 crosses after the 1.5 krad X-ray treatment of males has values mainly in the middle of the scale between 60% and 30%. Strains in this fertility range almost all had structural rearrangements. One strain with a translocation isolated after this treatment (Fe 2) was found aside of the 60%-30% suspected area. In the case of males treated with 0.25 krad of fast neutrons the B_1 fertility scores are spread over the whole scale. In the progeny of the suspected B_1 crosses chromosomal rearrangements were seen in three cases. Irradiation of females (X-rays or fast neutrons) resulted in B_1 crosses showing a rather high fertility. In one case after 1.0 krad of X-rays on females,

Figure 1. Photomicrographs of normal and translocated karyotypes of the onion fly (*Hylemya antiqua*). a. Normal karyotype. Spermatogonial metaphase. 2n = 12 + B. The chromosome designations have been indicated aside the centromeres. b. Fe 1. Translocation heterozygote $3^{l(-)} - 6^{s(+)}$. Larval brain cell. Mitotic metaphase. c. Fe 4. Complex rearrangement. $3^{l} - 6^{l} - X$. Mitotic metaphase. Duplicated for a large segment of the long arm of chromosome 3. d. Ma 1. Translocation heterozygote $2^{l(-)} - 6^{s(+)}$. Larval brain cell. Mitotic metaphase. e. Ma 1. Translocation homozygote. Spermatogonial metaphase. f. Ma 7. Translocation heterozygote $2^{l(-)} - 6^{s(+)}$. Spermatogonial metaphase. g. Ma 7. Translocation heterozygote $2^{l(-)} - 6^{s(+)}$. Diakinesis/Prometaphase ♂. Crossfigure. h. Ma 7. Translocation homozygote. Larval brain cell. Mitotic metaphase. The arrows indicate the translocated chromosome arms.

a B_1 cross with 57% egg hatch had a translocation (Ma 1). There is about an equa distribution of the rearrangements over the F_1 males and females. Five of the 55 backcrossed F_1 males (9%) and four of the 39 F_1 females (10%) had a chromosomal rearrangement.

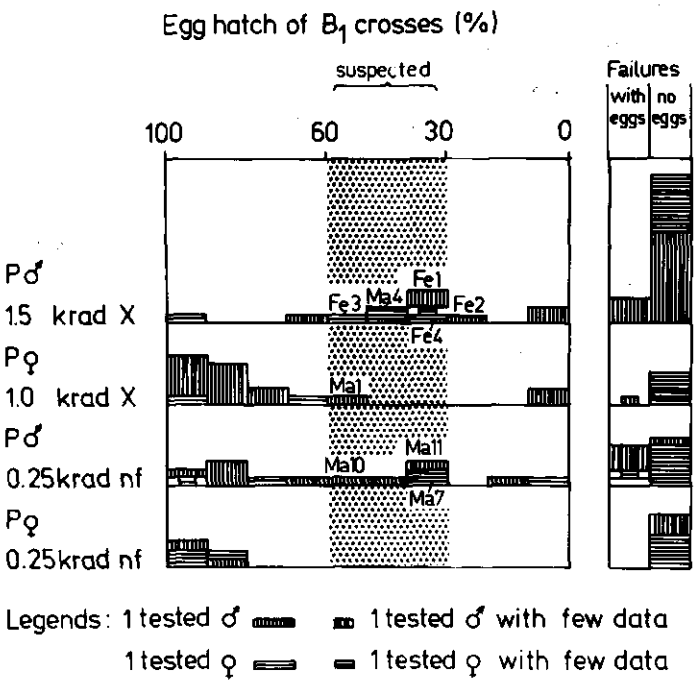

Fig. 2. Diagrammatic summary of B_1 data. The marked area covers the region between 60% and 30% E.H. suspected of carrying chromosomal rearrangements which are checked cytologically (compare tabel 1).

DISCUSSION

In table 2 the most relevant data of the reported experiments are combined with comparable data published previously (Wijnands-Stäb and van Heemert, 1974). Although sample size per treatment is relatively small, a few comments can be made. At a dose of 1.5 krad of X-rays (on males) 34% of the B_1 crosses produced progeny and many were failures (66%). About half of these fell in the fertility range of 60%-30% and generally carried a chromosomal rearrangement. At doses of 1.0 (and 1.1) krad of X-rays (on males) the percentage of reproductive F_1-flies

Table 2. Combined results from irradiation experiments and cytogenetic analysis, as published by Wijnands-Stäb and van Heemert (1974) I, and from experiments decribed in this report (II).

P generation: radiation dose (krad)	type	sex	report	B_1 cross: total number tested	number and percent with progeny: n	%	60%-30% E.H.: n	%**	Cytogenetic*** analysis: n	rearr.	Failures: n	%*
1.5	X-rays	♂	I+II	50	17	34	8	47	5	4	33	66
1.1 and 1.0	X-rays	♂	I	56	39	70	8	20	6	(4-)5	17	30
0.5	X-rays	♂	I	43	31	72	6	19	2	(1-)2	12	28
1.0	fast neutrons	♂	I	13	9	69	2	22	1	1	4	31
0.25	fast neutrons	♂	II	25	14	56	5	36	4	3	11	44
1.0	X-rays	♀ 1 day old	I	43	16	37	5	31	4	0	27	63
1.0	X-rays	♀ 7 days old	II	24	17	71	1	6	1	1	7	29

* Of total number tested in B_1 cross

** Of the number with progeny

*** Cytogenetic analysis of the 60%-30% E.H. category

is considerably higher (70%). Only twenty percent of these is suspected and again in most of those analyzed structural mutations were present. At 0.5 krad of X-rays (on males) 72% of the B_1 crosses produces progeny. Although the picture has changed in favour of the class with a normal fertility (> 75% E.H.), still 19% is suspected.

In general it can be stated that for the irradiation of males with X-rays a decrease of the dose will considerably enlarge the percentage of B_1 crosses with a normal fertility and the percentage of suspected crosses decreases. The high percentage of 47 after 1.5 krad X-rays may look acceptable, but gives more complicated rearrangements and many other deleterious effects. The high percentage of failures compared to all other treatments points in the same direction.

The data on the fast neutron treatments of males with a dose of 1.0 krad resemble most those of the 1.0 (and 1.1) krad X-ray treatment of males. The treatment of males with 0.25 krad of fast neutrons even seems to give more strains which carry chromosomal rearrangements.

Comparing the results of B_1 crosses for the treatment with 1.0 krad of X-rays of females on the first day after emergence (Wijnands-Stäb and van Heemert, 1974) with the same treatment of females on the seventh day of their adult life, a large difference can be noticed. Young females apparently are very sensitive for

the induction of mutations. Although many failures (63%) were observed the percentage of suspected strains is not low (31%). However, the cytological analysis of four strains was negative. For the treated seven days old females a much lower percentage (29%) of failures was scored, but a very low percentage (6%) of suspected B_1 crosses was found. Only one translocation could be traced in this case Ma 1, fig. 1d). The egg production of the P females irradiated on the seventh day after emergence is essentially better than of females irradiated on the first day. Nevertheless the egg production is reduced to about 1/5 of the normal production of a control series. After ten days oviposition ceases, while normally it may go on for a month.

In two cases (Ma 4 and Fe 1) males as well as females were backcrossed in a second backcross (B_2). No sex-linkage of the semi-sterile strains was found as can be seen in table 1.

We have used unirradiated flies from the control stock as if they were irradiated. They were mated in small groups as usually was done in B_1 crossings. At the start of oviposition the females were separated. Rather a lot of these control crosses were failures. Their egg hatches predominately were between 100% and 75%. A few strains revealed semi-sterility, even accompagnied by about 25% of brown eggs. However no chromosomal rearrangement could be found. This fact must be taken into account in the interpretations of all egg hatch data. Onion flies massmated in larger numbers and not separated before oviposition score a much better average egg hatch of about 85%.

We wish to emphasize that the output of strains with a structural mutation is a minimum score. In the first place only a small sample of the produced F_1 is backcrossed to measure fertility. Secondly for several reasons carriers of a structural mutation can be lost. For instance the scoring for rearrangements is done on B_1 crosses with severely reduced fertility and/or a high percentage of late embryonic lethals. Due to rearing difficulties at the larval stage some suspected strains were lost. As can be seen in table 1 some samples for cytological analysis were rather small (e.g. Ma 5). As expected in the case of a translocation carrier, half of the larval offspring will be normal and half will have the translocation. For statistical reasons rearrangements may go undetected, although in general wherever possible at least 6 individuals were taken for analysis to obtain about a 98% probability for at least finding one translocation carrier. In addition we may have overlooked translocations with a very small or a symmetrical exchange.

With translocations Ma 1 and Ma 7 (both between 2^1 and 6^5) we have started a sibcross programme to isolate homozygous flies. These two translocations are

very similar in respect to the segments exchanged. We have obtained a few homozygous translocation flies in the adult stage (fig. 1e) in the Ma 1 stock, but in Ma 7 only in the larval stage (fig. 1b). The fertility of both is rather high in backcrosses and consequently the fertility of a T/+ x T/+ cross is still high enough to obtain sufficient offspring for the isolation of translocation of homozygotes. The work on the isolation of homozygotes of Ma 1 and Ma 7 will be continued.

It is striking that as in the case of Ma 1 and Ma 7 in nearly all rearrangements we have found, the largest chromosome (6) is involved. Both arms of this chromosome are involved in an equal frequency. The long arm of chromosome 3 is involved rather often as well. Like we have found before (Wijnands-Stäb and van Heemert, 1974) the length of the chromosome (-arms) probably plays a role in the chance of becoming involved in a rearrangement.

Finally we can conclude that for the induction and isolation of semi-sterile strains carrying a chromosomal rearrangement, males are more suitable than females. A dose of 1.5 krad of X-rays on males seems to induce too much genetic damage to obtain fully viable semi-sterile strains and there is a good chance of getting rearrangements which give too many complications (Searle at al, 1974) to be used for genetic control purposes. The large class of failures (66%) in B_1 crosses probably contains many sublethal mutations. At a dose of 1.0 (and 1.1) krad of X-rays a much lower percentage (30%) of failures and a relatively high number of rearrangements was found. The results of 0.5 krad of X-rays on males seems rather similar to the irradiation with 1.0 (and 1.1) krad. Nevertheless we believe that a dose of 0.5 krad must be advised, because it can be assumed that the obtained semi-sterile strains have less genetic damage from the irradiation (Robinson and van Heemert, 1975).

For the study of the performance of semi-sterile strains a translocation which can simply be recognized cytologically is important, to monitor the frequency of the translocation in cage experiments. For application in genetic control programmes we hope that strains with a low fertility of the translocation heterozygote, but a normal competitiveness can be obtained, and isolated as a homozygote. Release of one or more translocation stocks will then be carried out to establish the effect on the natural population, singly or in combination. Since single translocations only can be made homozygous if the fertility is not too low (> 60%), combinations of two or more stocks each with a moderate sterility should be used, to reach a sufficient sterility for genetic control.

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Preliminary radiobiological studies on *Hylemya antiqua* (Meigen) and data on three radiation induced (0.5 krad) chromosomal rearrangements

A.S. Robinson and C. van Heemert

Association Euratom-ITAL, Wageningen and
Department of Genetics, Agricultural University, Wageningen (The Netherlands)

ABSTRACT

Using varying doses of X-rays (500-3000 rad), seven day old adult male onion flies, *Hylemya antiqua*, were irradiated and mated to virgin females. In eggs from individual females the % egg hatch and the % of late enbryonic lethals was assessed. Late embryonic lethality could be observed by the brown appearance of the eggs. The sperm of the onion fly is relatively sensitive to the radiation-induction of dominant lethal mutations. Three Krad gave almost complete sterility. The % of late embryonic lethals showed an initial rise with dose to a peak at 1 Krad, thereafter there was a decline. Arguments are put forward as to the merits of using a low radiation dose for the induction of chromosomal rearrangements for insect control. To test this males were irradiated with 500 rad and the F_1 progeny were screened for reduced fertility. Out of a total of 74 test-crossed F_1 males and females three rearrangements have been isolated and confirmed cytologically to the present time, two reciprocal translocations and one pericentric inversion. The duplication/deficiency gametes from such rearrangements following fusion with normal gametes lead to late embryonic lethality and hence produce brown eggs. However, in the two translocation stocks viable duplication/deficiency larvae (7 - 9 days old) have been observed. The fertility and cytology of the three rearrangements are described. Only the female carriers of the inversion showed reduced fertility. The fertility of both translocations was in excess of 50% of the wild-type value. Preliminary inbreeding has been undertaken but as yet no homozygous stock has been established.

I. INTRODUCTION

Hylemya antiqua Meigen is the main insect pest attacking the onion crop in the Netherlands. In 1972, 7361 ha. were planted with onion and the export value of the crop was $ 28.7 M.Mass rearing (1), ecology (Loosjes, unpublished data), radiobiology and cytology (2) and population modelling (3) of the insect have already been well studied thus providing an excellent base for a possible genetic control method using radiation induced chromosomal rearrangements.

The use of chromosomal rearrangements for insect control, specifically translocations, first proposed by Serebrovskii (4), was developed independently by Curtis (5), since when a host of publications both theoretical (6, 7,8,9) and practical (10,11,12,13) have indicated the potential of the technique. Chromosomal translocations can function in two interrelated ways in an insect control programme.
Firstly, by subjecting the natural population to a high genetic load and secondly, as a transport/replacement system for the incorporation of conditional lethal factors or mutants which render the replacements innocuous.

The degree of genetic load necessary to produce an actual decrease in population numbers is influenced by many factors including density dependent regulation, immigration and emigration and the reproductive capacity of the natural population.

The work reported here is a continuation of the experiments begun by Wijnands-Stäb and van Heemert (2) and the short term aim is the construction of a strain of *Hylemya antiqua* through the use of homozygous rearrangements which will generate a high genetic load when released either as a multiple homozygote or heterozygote into a native population.

II. RELEVANT LABORATORY DATA OF *HYLEMYA ANTIQUA*

The generation interval in the laboratory is 4 - 6 weeks. Mated females can produce eggs over a period of 3 - 4 weeks and up to 600 eggs have been re-

corded from one female. Following emergence there is a pre-oviposition period of about one week. In control matings the eggs from fertilized females, after incubation at 29°C and 80% humidity for three days, can be differentiated by three biological end points: a) empty hatched eggs, b) unhatched 'brown' eggs i.e. late embryonic lethals and c) unhatched white eggs i.e. unfertilized eggs or early embryonic lethals. Following mass mating of control males and females, individually egged females gave the following percentages of eggs in the three categories: 93.4 ± 3.9%, 3.0 ± 1.3% and 3.5 ± 2.9%, respectively.

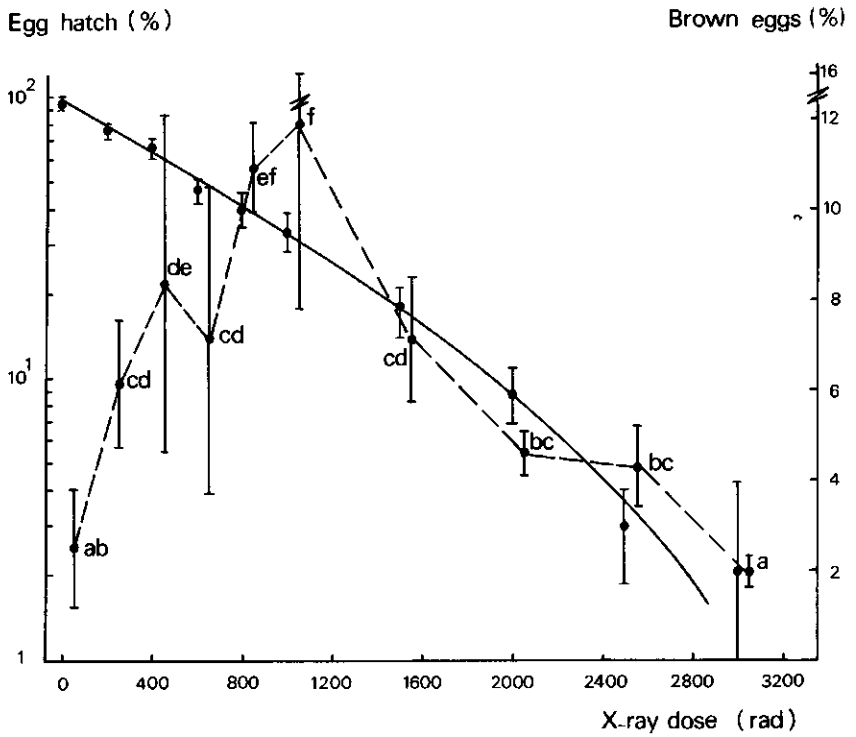


Figure 1. Dose response curves for dominant lethality (●——●) and late embryonic lethality[x] (●– – –●) induced in mature sperm of *Hylemya antiqua*.
[x]Values followed by the same letter are not significantly different from each other at the 5% level as determined by Duncan/s multiple range test.

III. DOSE RESPONSE CURVES FOR DOMINANT LETHALITY AND LATE EMBRYONIC LETHALS INDUCED IN X-IRRADIATED SPERM

As mature sperm provides a homogeneous cell sample and is sensitive to the induction of chromosomal rearrangements, seven day old adult males were

irradiated. The males were treated with varying doses of X-rays at a dose rate of 300 rad/min and mated in mass to an equal number of ten day old virgin females for three days. The males were then discarded and females caged individually. In the eggs from these females the % egg hatch and the % brown eggs were measured. The results are shown in Fig. 1: the sperm of *H. antiqua* is relatively radiosensitive compared with other Diptera i.e. a dose of three Krad caused almost complete sterility, it was possible to rear and mate the few surviving F_1 progeny from the males given three Krad. (The F_1 generation is defined as the progeny of irradiated males following outcrossing to control females.) The virtual straight line plot of the graph, log % egg hatch against dose, indicated that single hit kinetics were involved for the majority of the dose range. The dose-response curve for the % of brown eggs i.e. late embryonic lethals showed an initial rise with increasing dose to a peak at one Krad, thereafter there was a decline because at higher doses it became increasingly probable that an egg had at least one early acting dominant lethal, which could forestall the expression of later acting embryonic dominant lethals. There were significant differences in the % of brown eggs for the different doses ($F = 5.71^{xxx}$ d.f. 9 and 40). Von Borstel and Rekemeyer (14) recorded a similar dose response curve for late embryonic lethals following irradiation of *Habrobracon* sperm, although the peak was at four Krad. The brown appearance of the eggs is probably produced by the tyrosinase system which in *Drosophila* is active in embryos older than six hours (15).

IV. THE CHOICE OF A RADIATION DOSE FOR THE INDUCTION OF CHROMOSOMAL REARRANGEMENTS

Radiobiological data indicate that the higher the dose of radiation given to the parental generation the higher the frequency of rearrangements recovered in the F_1 generation. However, for insect control purposes quality is of more importance than quantity and one of the most important aspects (for genetic control) of quality is the fitness of the rearrangement as a homozygote. In most insect species so far studied a large proportion of induced translocations are either lethal when made homozygous or show severe fitness reductions e.g. *Drosophila* (16,17), *Aedes aegypti* (18) and *Lucillia cuprina* (19). However, on the positive side there are reports in pest insects of viable translocation homozygotes (20,21,22). The reduced viability of homozygous translocations can be due to position effects or damage at the translocation

breakpoints or to radiation induced or naturally occurring recessive lethals. Sobels (17) has calculated the relative importance of these different aspects with translocations in *Drosophila*. It has been claimed that by a series of backcrosses, radiation induced recessive lethals can be removed but it is highly improbable that genetic engineering will remove the effect due to a true position effect or damage at the translocation breakpoint. Relevant to this argument is the observation that in maize (23) and *Drosophila* (24) crossing-over is reduced in the region of a translocation breakpoint and it is conceivable that the absence of close pairing during meiosis in the region of the breakpoint would make extremely difficult the removal, by backcrossing, of recessive lethals within this particular chromosomal segment. The higher the dose of radiation used the higher is the probability that a recessive lethal would be included in the non-crossover region. Two other points indicated that at least as far as the onion fly is concerned, the removal of recessive lethals by backcrossing would not be a worthwhile procedure.

a) In *H. antiqua* males there is no recombination and in females there are as yet no data on the frequency of recombination.
b) With a generation time of six weeks, backcrossing is an extremely laborious and time consuming process.

In a second experiment with these considerations in mind a very low radiation dose, 500 rads X-rays given to seven day old males, was used; it was considered that this dose would give a detectable frequency of translocated F_1 individuals and that it was rather improbable that the same individuals would carry many induced recessive lethals. From Fig. 1, 500 rads would lead to about 50% egg hatch in the eggs fertilized by sperm from the irradiated males.

V. RECOVERY OF REARRANGEMENTS IN THE F_1 GENERATION

There are no marker genes available in *Hylemya antiqua*, so initially the presence of rearrangements is ascertained by reduced fertility in outcrosses of F_1 individuals to control insects. It is also known that the duplications and deficiencies from translocations can act as late embryonic lethals and hence produce brown eggs (25).

Since single pair matings are not very successful in *Hylemya antiqua* the following mating techniques were used; F_1 females were confined in mass with control males and subsequent to mating they were placed in individual cages;

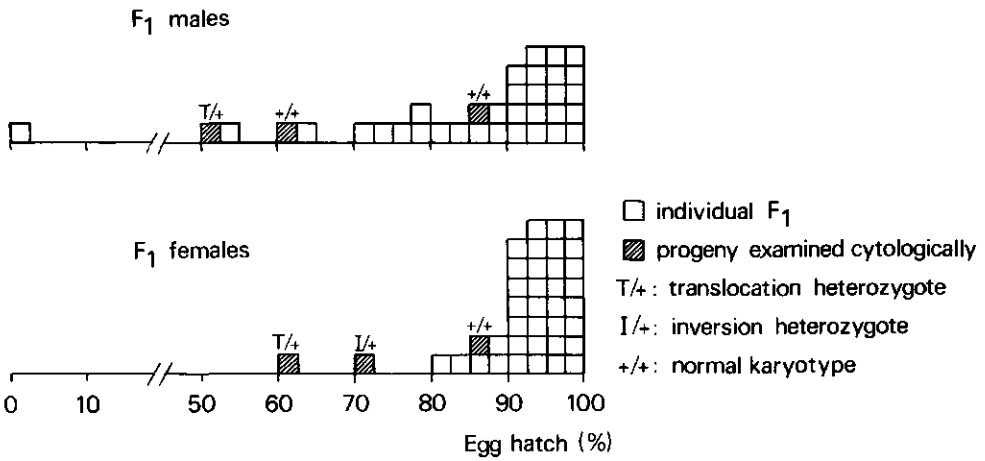


Figure 2. Fertility of test-crossed male and female *Hylemya antiqua* F_1's following radiation of parental males, with 500 rads of X-rays.

F_1 males were placed individually in separate cages with three control females. Using these techniques it is possible that the females do not always receive a full supply of sperm, (especially when F_1 males are tested) and therefore a varying proportion of unfertilized eggs may be laid. Consequently, if the % egg hatch is calculated from the total number of eggs laid then large variations can occur. To reduce the variation the white unhatched eggs were deducted from the total before calculating the egg hatchability. Fig. 2 shows the distribution of the % egg hatch for 74 F_1's testcrossed to control insects. Larvae from six stocks were examined cytologically for the presence or absence of rearrangements in mitotic preparations.

As a routine procedure larval brains of 7-9 day old larvae were used for cytological analysis. Techniques were as described in a previous paper (2). Somatic pairing makes it easy to identify homologous chromosomes (Fig. 3a). It also facilitates the detection of differences in length between the normal and the rearranged chromosomes. Mitotic prophases, because of their pronounced telomeric pairing, were also used. Meiotic pairing was studied in diakinesis/prometaphase stages in the testes of young males in order to obtain the critical evidence for the presence of rearrangements. The identification of rearrangements in mitotic preparations greatly increases the efficiency of the screening process. It would also be valuable in field experiments.

As indicated in Fig. 2, three rearrangements were identified, two reciprocal translocations and one pericentric inversion. However, the progeny of one semi-sterile male showed no visible aberration. It is possible in this case that the exchanged segments were symmetrical and/or that the exchanged segments were very small. Both these conditions make it impossible for the translocations to be observed in mitotic preparations.

VI. DATA ON THE THREE REARRANGEMENTS

The fertility and the segregation ratio of the three rearrangements are shown in Table I.

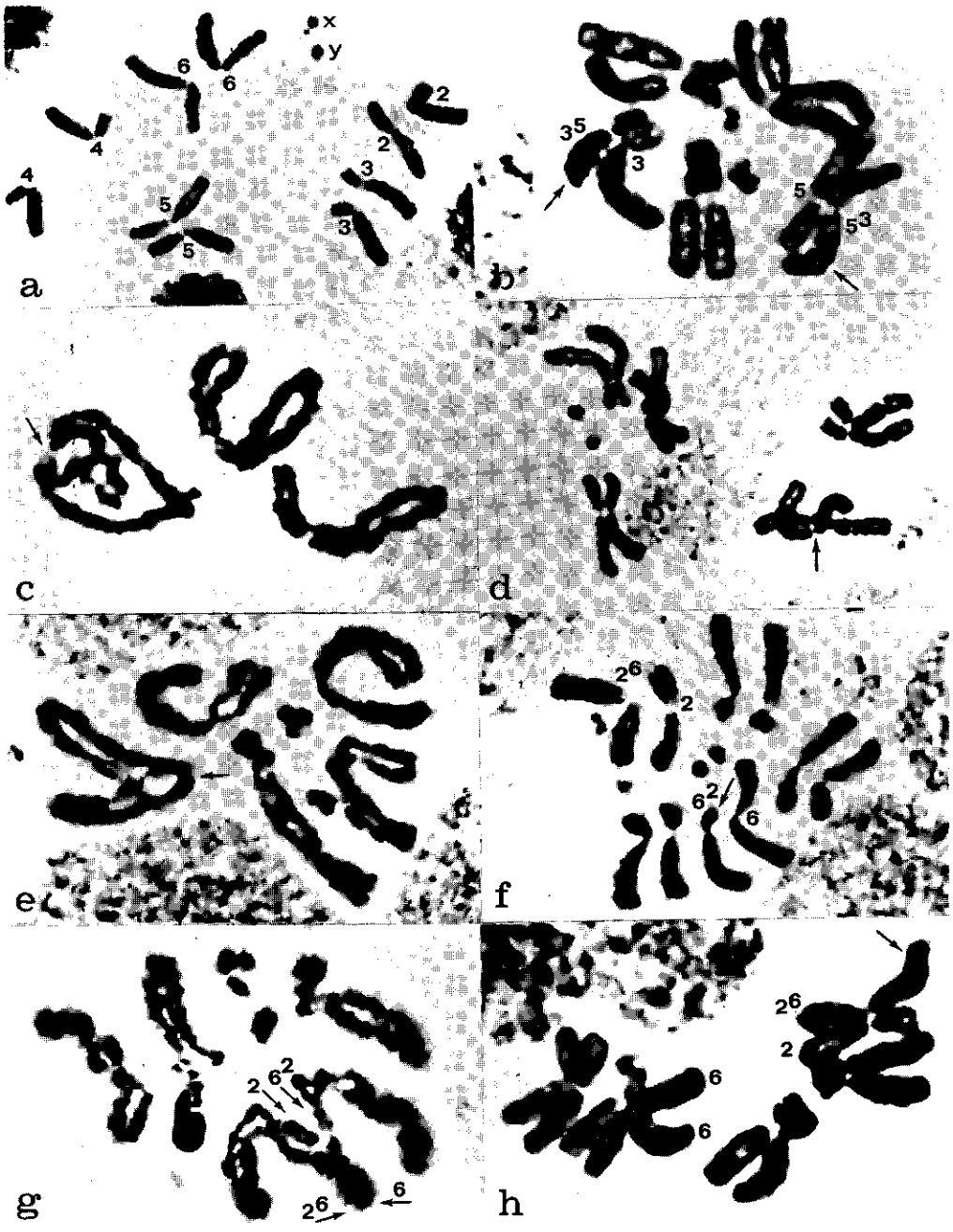
a) T (3, 5) 6

It was isolated in the progeny of an F_1 female. The long arm of chromosome 3 lost a large segment and gained a short segment from the short arm of chromosome 5 (Fig. 3b). The arm ratio in a normal chromosome 5 is 1.4 and the arm ratio of the translocated chromosome 5^3 is 1.0. The relative small new chromosome 3^5 was used as a marker in an inbreeding programme for the isolation of homozygotes. In this stock duplication deficiency karyotypes (3 3 $5^3$5) were regularly found in the larval stage.

Table I. Fertility and Segregation Ratio of 3 Radiation Induced Rearrangements in *H. antiqua* when test-crossed to Control Insects.

	FEMALE		MALE	
	FERTILITY (% EGG HATCH)	SEGREGATION RATIO +/+ : HET	FERTILITY (% EGG HATCH)	SEGREGATION RATIO +/+ : HET
T (3, 5) 6	62.2 ± 9.2	10 : 12	61.7 ± 7.1	11 : 6
T (2, 6) 5	81.9 ± 10.9	9 : 12	74.9 ± 5.6	11 : 13
In (6) 1	73.9 ± 6.7	30 : 31	97.4 ± 2.8	49 : 48
Control	96.7 ± 3.1			

There was no difference in the fertility of males and females carrying this stock when they were outcrossed to control insects. The fertility was in excess of 50% of the control value that would be expected following random alternate and adjacent segregation from a translocation heterozygote. It in-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dicates that there was either preferential alternate segregation as found in *Blatella germanica* (26) and in males of *Cochliomyia hominivorax* (27) or there was survival to the larval stage of a large proportion of the duplication/deficiency karyotypes. In *Glossina austeni* (21) and *Musca domestica* (28) all translocations appear to have a fertility very close to the expected value of 50%. The reasons for these species differences are not clear.

Preliminary inbreeding work has been carried out in order to produce a homozygous stock. Following inbreeding in mass of a translocation stock it is expected that a proportion of the matings will be between heterozygotes and consequently translocation homozygotes can be generated. If the only viable zygotes from these matings are produced from genetically balanced sperm and eggs and if the translocation homozygote is not egg-lethal then the expected fertility of such crosses (from Table 1) would be $\frac{62.2 \times 61.7}{100} = 38.4\%$. However, the preliminary data show a much higher fertility (50-55%) of such sib-crosses indicating that a proportion of unbalanced sperm and eggs complement each other's duplications and deficiencies and so produce viable genotypes (29). Using the translocated 3rd chromosome as a marker, the translocation homozygote karyotype has been observed in the larval stage but as yet not in the adult stage. This fact together with the observation that the % pupation in such crosses is reduced might indicate larval lethality of the translocation homozygous genotype. Only a small number of sib-crosses have yet been tested and a much larger inbreeding programme is underway.

b) In 6(1)

This inversion was isolated in the progeny of an F_1 female showing a % egg hatch of 76%. It is a pericentric inversion i.e. the centromere is inclu-

Figure 3. a. Normal karyotype of the onion fly. Mitotic metaphase (2n = 12) in a larval brain cell. b. Mitotic metaphase of a translocation heterozygous larva of T (3,5)6. Arrows indicate the translocated arms. c. Mitotic prophase of an inversion heterozygote of In (6)1 in a larval brain cell. Arrow indicates position of the centromeres of chromosome pair 6. Cell incomplete. Note the loop. d. Mitotic metaphase of an inversion heterozygous larva. Arrow indicates the position of the two centromeres of chromosome pair 6. Note the disturbed somatic pairing in this pair (arms curved). e. Diakinesis/prometaphase in the testes of an inversion heterozygous male. Arrow indicates the position of the centromeres of chromosome pair 6. Note the loop. f. Spermatogonial metaphase of a translocation heterozygous male of T (2,6) 5. Arrows indicate the translocated arms. g. Multivalent in a diakinesis/prometaphase stage of a translocation heterozygous male of T (2,6) 5. Arrows indicate the paired arms involved in the translocation. h. Mitotic metaphase of a larva with a large duplication (see arrow) in chromosome 2.

ded within the inverted segment.

In late mitotic prophases a ring configuration can be seen in chromosome pair 6 (Fig. 3c). Mitotic metaphases showed the presence of a typical inversion configuration in chromosome pair 6 in that both arms of one of the pair always have a curve in about the middle (Fig. 3d). An explanation of these observations can be given by considering the way somatic pairing is acting during the mitotic cycle. Centromeric pairing is of equal strength in prophase and metaphase. However, telomeric pairing is much stronger in the prophase. During the transition from prophase to metaphase the homologous areas around the centromeres proximal to the inversion breakpoints stay paired. Subsequently the chromosome ends distal to the breakpoint begin to loose their pairing because of the breakdown of telomeric attraction. In achiasmate male meiosis, pairing is rather complete over the total chromosome length and as expected a loop is observed in diakinesis/prometaphase stages (Fig. 3e).

Two observations would indicate that the inversion breakpoints are equidistant from the centromere. Firstly, the arm ratio of the normal and inverted chromosome 6 is the same and secondly, the centromere is positioned in the middle of the inversion loop (Fig. 3c,e). Rough estimations indicate that the breakpoint positions are at the middle of the short arm and at 1/3 of the length of the long arm from the centromere.

As indicated in Fig. 3e linear pairing during the meiotic sequence is achieved by the formation of an inversion loop. Crossing-over within the loop leads to the formation of duplication/deficiency gametes (30) and hence to a reduction in fertility. Such cross-over products have been cytologically observed in the eggs from test-crossed females of this inversion stock. Fertility reductions implicating inversions have also been observed in *Aedes aegypti* (31), *Culex pipiens* (32), *Culex tritaeniorhynchus* (33) and *Musca domestica* (34).

With In (6)1 only the female carriers of the inversion exhibit reduced fertility, the male inversion heterozygotes have normal fertility (Table 1). We conclude that this is strong evidence for the absence of crossing-over in the male of the onion fly: this is in agreement with data for other Cyclorrhaphid Diptera (35,36) and confirms the previous observation of achiasmate male meiosis (37). Both sexes showed the expected 1 : 1 segregation of the inversion and wild-type gametes as determined by cytological analysis of the larvae from test-crosses. Inbreeding has been carried out with this stock in an attempt to obtain the inversion as a homozygote. However, there are three difficulties apparent. Firstly, as the inversion heterozygous male does

not show reduced fertility it is impossible to differentiate the matings between the inversion females and wild-type males from those between inversion males and females. However, if the inversion homozygote is egg lethal then the fertility of the latter matings would be reduced by an additional 25%. Secondly, as the only gametes which are recovered from an inversion female are non-crossover types it is impossible to remove radiation induced or naturally occurring recessive lethals within the inverted segment.
Thirdly, as indicated above, the inversion homozygote cannot be differentiated cytologically from the wild-type karyotype.

c) T (2, 6) 5

This was isolated in the progeny of an F_1 male and the shortest and largest autosomes are involved. The short arm of chromosome 6 lost a large piece and received a small piece from the short arm of chromosome 2. Both of the translocated chromosomes can be easily differentiated from the other chromosomes in the karyotype (Fig. 3f,g). Translocation homozygotes, if viable will be very easy to discriminate from translocation heterozygotes and normal karyotypes by looking for the presence of two, one or none translocated chromosomes 6.

Further evidence for the asymmetry of the exchange can be obtained by the occurrence of duplication karyotypes $22^{6}66$ in the larval stage (Fig. 3h). Such individuals have a duplication for chromosome 6 and a very small deficiency for chromosome 2. The survival of such duplication types is perhaps the reason why the observed % egg hatch of this translocation is high (see Table I). Using the translocated chromosome 6 as a marker, this translocation could be observed in the late larval stage as a homozygote, but as yet not in the adult stage.

VII. CONCLUSIONS

Several tentative conclusions can be drawn from these preliminary studies.

a) Chromosome rearrangements useful for genetic control can be induced by low doses of radiation. However, because of the small number of rearrangements so far tested for homozygous viability a conclusion as to the merits of a low v.s.a high dose of radiation has still to be established.

b) Cytological observation of mitotic chromosomes in larval preparations great-

ly reduces the time involved in the isolation of rearrangements. In order to use this technique a translocation involving the exchange of asymmetrical pieces is necessary in order to differentiate between the translocation homozygote, the translocation heterozygote and the wild-type karyotype.

c) Because of the survival of duplication/deficiency karyotypes to the late larval stage, it is important for control of the onion fly that translocations are used which involve the exchange of large segments of the chromosomes.

d) The use of inversions for exerting a genetic load in a wild population would appear to be limited as the males do not show reduced fertility because of the absence of crossing-over.

e) When inbreeding to produce homozygotes as large a number of individuals as possible should be used in order to maximize the size of the inevitable genetic bottleneck through which the homozygotes must pass.

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Meiotic Disjunction, Sex-Determination and Embryonic Lethality in an X-linked "Simple" Translocation of the Onion Fly, *Hylemya antiqua* (Meigen)

C. van Heemert
Department of Genetics, Agricultural University, Wageningen

Abstract. It was shown that the translocation in study is X-linked. After test-crossing translocation heterozygous males they generally only produce translocation heterozygous daughters and normal sons. The small acrocentric chromosomes involved in the translocation appeared to be the sex-chromosomes. The X-chromosome has a secondary constriction which is missing in the (male determining) Y-chromosome. Meiotic orientation was studied in translocation heterozygous males and females. The alternate and adjacent I orientations were found in about equal frequencies. Further, numerical meiotic non-disjunction (two types) occurred in translocation heterozygous males (about 2%), but is much higher in females (18.7%). In (achiasmate) males the homologous centromeres predominantly regulate meiotic pairing, coorientation and disjunction, apparently independently of the chromosomal rearrangement. Disturbed telomere pairing in particular leading to reduced chiasma frequency most probably explains the high numerical non-disjunction in chiasmate females. A rather good relationship exists between the percentage "semi"-sterility (28%), scored as late embryonic lethals (eggs, 72 hrs.) and the percentage karyotypes (20%) in young eggs (8–16 hrs.) with a large chromosomal deficiency. The remaining sterility (8%) can be explained by the somewhat decreased viability of tertiary trisomics and duplication karyotypes at the end of the egg stage. This translocation behaves like a "simple" one.

Introduction

One out of a series of translocations recently induced in the onion fly, *Hylemya antiqua* (Meigen) (Wijnands-Stäb and van Heemert, 1974) differed from the others in genetic behaviour. In test-crosses, heterozygous males generally produced only normal sons and translocation-heterozygous daughters, whereas heterozygous females produced both normal and translocation sons as well as daughters. This suggests X-linkage. Because of this linkage, the translocation offers a good opportunity to check Boyes' (1954) assumption that the pair of small acrocentric chromosomes in the onion fly is the sex-chromosome pair.

In general at meiosis in a translocation heterozygote a multivalent is formed, which may show irregularities in chromosome disjunction.

With random orientation about half of the gametes are expected to receive an unbalanced set of chromosomes, leading to lethality in the progeny, usually during embryogenesis (semi-sterility). Some abnormal karyotypes are viable even in the adult stage and a few can even reproduce. One of the purposes of this study was to relate the frequencies of the different balanced and unbalanced embryonic types in the onion fly to meiotic orientation and disjunction, as well as to the degree of sterility.

Materials and Methods

The translocation studied here, was induced by irradiating newly emerged males with 1.0 Krad of X-rays (Wijnands-Stäb and van Heemert, 1974). It was selected from backcross material after screening for semi-sterility. In the embryonic, the larval as well as the adult progeny of suspect B_1 and B_2 crosses we could easily recognize the translocation chromosomes cytologically. For testing males for the presence of the translocation we placed one male in a small cage with three normal virgin females. The onion fly hardly breeds in single pairs (10% success). Virgin females to be tested were mass mated with normal males. About 15 females from the suspected translocation stock and 15 males from the control stock were usually put in one cage. Females were separated in small cages when they started producing eggs. When a sufficient number (50) had been laid, the eggs were incubated for three days at 29° C and nearly 100% r.a.h. The eggs were classified as white (unfertilized), empty (hatched) and brown (late embryonic lethals) with a stereomicroscope (12 ×). In general half of the males and females tested appeared to be normal: almost all the eggs hatched (95%; 2.5% were white, 2.5% brown). The other half had the translocation and about 25%–35% of the eggs did not hatch and turned brown and again only 2%–3% remained white. These eggs are non-fertilized as we have observed cytologically and the same percentage was found in the control.

We have made cytological observations on the translocation among the embryonic, larval and adult offspring grown from testcrossed "semi"-sterile parents. Young eggs (8–16 hours), larvae (9–11 days), young males and females (1 day) were used for cytological analysis. After completion of the progeny test the karyotype of the male was determined from spermatogonial metaphases. Lack of suitable tissues makes it difficult to analyzé the karyotype of the females after eggproduction. Females which carried the translocation could therefore only be distinguished from their normal sibs by analyzing the offspring.

Eggs were placed into a drop of lacto acetic orcein (LAO) and dechorionated with a pair of fine needles. The vitelline membrane was ruptured and the embryonic tissue was stained at least one hour. We have used 2% LAO as a fixing-staining medium made according to the following procedure: dissolve 1.0 g of natural orcein in 10.42 ml lactid acid (90% pure), add 24.38 ml glacial acetic acid and 15.21 ml distilled water. Boil gently with a reflux cooler for one hour, cool slowly and filtrate. *Larval brains* from well fed larvae were dissected in Levy's saline solution. The composition is: 90.0 g NaCl, 7.08 g KCl and 4.58 g $CaCl_2$ dissolved in 1 liter of distilled water. The brains were fixed and stained directly in LAO. *Testes* from newly emerged males were dissected in Levy and an excess of distilled water was added. After five minutes the swollen testes were then put in LAO. *Ovaries* from newly emerged females were put in LAO directly.

After staining for a maximum of two days the tissues were squashed in 45% acetic acid. Cytological analysis was carried out immediately after squashing.

Photographs were made from temporary preparations with a Zeiss photomicroscope on a high contrast Agfa-Gevaert ortho negative film (12 DIN) or on an Ilford pan film (18 DIN).

The flies were reared at 23° C, appr. 70% r.a.h. and 16 hours of light a day. The translocation stock used in the testcrosses (B_1 and B_2) as presented here, had passed trough six generations after induction. The control stock used has been reared in our laboratory for over ten generations. We have taken care to avoid inbreeding. Originally the stock came from a Dutch field population and a sufficient number of pupae was introduced into the laboratory.

In the stocks used, B-chromosomes were occasionally observed. They have about the same size as the small acrocentric chromosomes. In the testcrosses we have consistently used control mates without B-chromosomes.

Results

The translocation is between chromosome 3 and one of the small acrocentric chromosomes. The exchange resulted in a translocated chromosome 3^-, which lost about half the length of the long arm. It seems not to have gained any chromosomal material from the small (2–3 μ) acrocentric chromosomes. The size of the ten large chromosomes is about 9–12 μ, as based on mitotic measurements. The translocated acrocentric chromosome X^3 gained considerably in length although the presence of a tiny end segment of the acrocentric chromosome at the breakage end of 3^- can not be excluded. Fig. 1a diagrammatically shows the four chromosomes involved. Two submetacentric and two acrocentric chromosomes, all different in size, can easily be recognized in mitotic and some meiotic stages. In Fig. 1b it can be seen how they might pair in a typical pachytene stage. However, in the testes of males which are achiasmate we have never seen this type of pairing, but usually the translocation complex during diakinesis/prometaphase took the shape of Fig. 1c. We have never seen any pairing between chromosome 3^- and the small acrocentric chromosome.

This meiotic pairing behaviour in combination with the very asymmetrical exchange as observed at mitosis allow two different explanations. Firstly, the translocation is a simple one and there is no terminal segment of the acrocentric chromosome attached to the breakage end of chromosome 3^-. The free breakage end of 3^- becomes stable. Secondly, a tiny segment of the acrocentric chromosome, not identifiable at mitosis or meiosis, has been exchanged with the large segment of chromosome 3. This tiny terminal segment apparently is unable to pair with the unaltered acrocentric chromosome. In practice this translocation can be considered as a "simple" one.

According to Boyes the small acrocentrics presumably are the sex-chromosomes, mainly because they sometimes appear to be heteromorphic. In testes of normal onion flies it can hardly be seen if the small

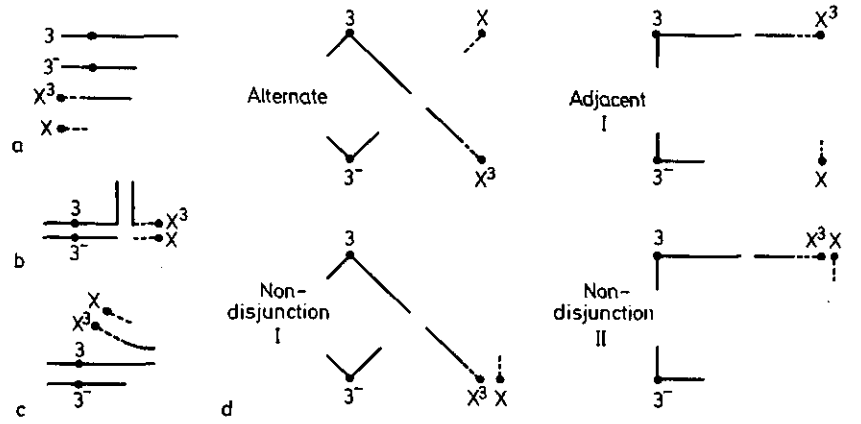

Fig. 1. (a) Idiogram of the chromosomes involved in the translocation complex of a TN female carrying the T(ranslocation) and N(ormal) genomes. (b) Cross-figure as expected in early meiotic prophase I, diagrammatically. (c) Meiotic pairing as usually observed in diakinesis/prometaphase. (d) The four different orientation types (A I) (see Tables 1–3). In the case of TN males replace X by Y

pair of acrocentric chromosomes is heteromorphic. Eggs and larvae appeared to be the most suitable for studying chromosome morphology, due to a more pronounced spreading of the chromosomes and the chromatids. Unfortunately we were not able to sex the individuals at these developmental stages. About half of the eggs and larvae in most of the cells analyzed have one of the acrocentrics with a secondary constriction in the vicinity of the centromere, while in the other half most cells analyzed have both the acrocentrics with a secondary constriction. One of the two acrocentrics in Fig. 2d can be seen to have a secondary conconstriction, which is missing in the other one. In Fig. 3f, *e.g.*, both such a constriction.

An extra very small chromosome present in many individuals could generally be distinguished from the two acrocentric chromosomes, because it is metacentric and is not somatically paired with the acrocentrics. This chromosome most probably is a B-chromosome (see Fig. 2d, f and g). If two B-chromosomes were present they exclusively paired with each other somatically or meiotically and not with the acrocentrics.

Table 1 shows M II and testcross segregations of TN males [carying a T(ranslocation) and a N(normal) genome] with the chromosomes $33^{-}X^{3}Y$ (Fig. 1a). Cytological observations of the progeny include egg, larval and adult stages (B_1 generation). Among 143 young eggs from testcrosses involving heterozygous males 5 different karyotypes ($33^{-}X^{3}X$, 33XY, $33^{-}XY$, $33X^{3}X$ and $33^{-}X$) were observed. In 62 larvae descended from three males, three different karyotypes ($33^{-}X^{3}X$, 33XY and $33X^{3}X$)

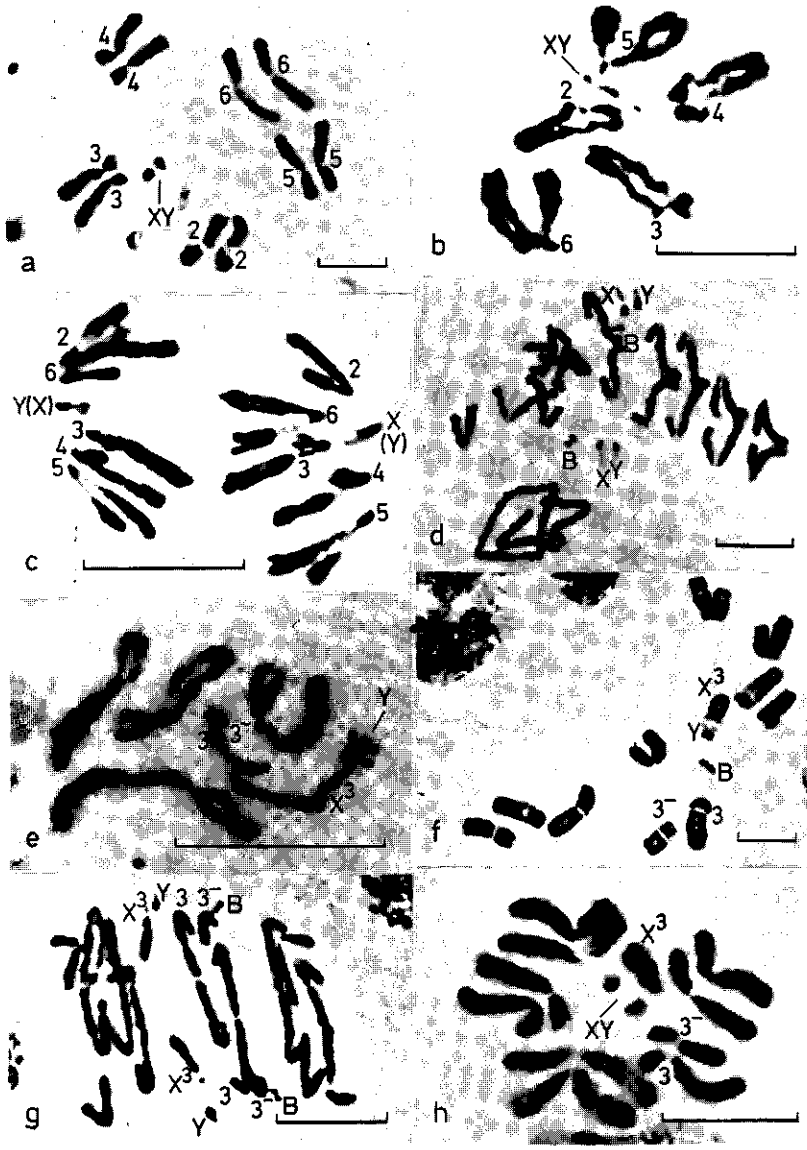

Fig. 2[1]. (a) Normal (NN) karyotype (33XY) of the onion fly. Spermatogonial metaphase. Note strong somatic pairing. (b) NN(33XY) karyotype. Diakinesis/prometaphase ♂. (c) NN(33XY) karyotype. AI ♂. X(Y) means X or Y. (d) NN karyotype + a metacentric B-chromosome. Mitotic anaphase from a larva, presumably male. (e) TN(33−X^{3}Y) karyotype. Diakinesis/prometaphase ♂. (f) TN karyotype + B-chromosome. Mitotic metaphase from a larva, presumably male. (g) TN karyotype + B-chromosome. Mitotic anaphase from a larva, presumably male. (h) TN + X (33−X^{3}XY) karyotype. Spermatogonial metaphase.

1 The bars on all photomicrographs represent 10 μm.

were observed. The 33⁻XY karyotype observed at a young egg stage has a large deficiency for about half the length of the long arm of chromosome 3, and most probably dies in a late embryonic stage. This embryonic lethality will be considered again in the discussion more extensively.

In the column of the B_2 generation are indicated the results of individual testcrosses of B_1 males and females. This constitutes an additional check on the B_1 karyotypes. The presence of TN(33⁻X^3X) females and normal (33XY) males in the B_1 generation was confirmed. Table 1 further shows that the daugthers of TN fathers either have the translocation (TN) or an abnormal duplication karyotype $33X^3X$ which is probably viable in the adult stage, but not fertile. In the larval stage we have seen many karyotypes with $33X^3$ plus one normal small acrocentric chromosome. Since no $33X^3Y$ males were ever found after analysis of a sufficient number of adult males, such larvae must have been females with a $33X^3X$ karyotype. On the basis of expected segregations these females should not have late embryonic lethals among the progeny (brown eggs). However, all the testcrossed B_1 females showed "semi"-sterility (brown eggs) and must have been TN(33⁻X^3X). Therefore we conclude that the $33X^3X$ karyotypes if reaching the adult stage can not reproduce.

Out of 38 B_1 sons of TN males 37 were normal (33XY); one was a TN + X (33⁻X^3XY). This trisomic karyotype results from numerical meiotic non-disjunction: Chromosomes Y and X^3 go to the same pole. The numerically deviant karyotypes as observed in the M II of TN males can also be concluded to be due to non-disjunction (Table 1). A 33⁻X^3XX (TN + X) female is not expected from a TN father unless spontaneous meiotic non-disjunction for the X-chromosome occurred in the 33XX mother.

Table 2 gives the results of the screening of eggs, larvae and adult progeny from TN 33⁻X^3X females. The second metaphase was not accessible. In 118 eggs from 5 testcrossed TN mothers, 8 different karyotypes were observed. In 72 larvae from two backcrossed females we have scored 6 different karyotypes. Apparently the 33⁻XX or 33⁻XY (Fig. 3f) and 33⁻X or 33⁻Y (Fig. 3e) karyotypes observed in young eggs are lethal in the larval and late embryonic stages. Adult males (110 from 6 females) but no females were analyzed and 6 different karyotypes were observed, as in the larval stage. Besides NN, TN and TN + X sons we have scored $33X^3Y$, $33X^3XY$ and 33Y sons. In particular the viability of the 33Y (Fig. 3b and c) karyotype is striking, such males even produce sperm.

We came to the conclusion after M II analysis of TN males and analysis of young eggs of TN males and females (Tables 1 and 2), that 4 different meiotic orientation types of the translocation complex occur

Table 1. The segregations at M II and in testcross progenies of 33-X³Y (TN) males (compare Fig. 1). The control females were 33 XX(NN). Scoring of adult females (B_1) is difficult and was not carried out

Type of orientation	M II of P ♂♂		B_1 generation					B_2 generation	Figures
	Type	Score	Karyotype	Eggs	Larvae	Sex	♂ adults		
Alternate	3-X³	49	33-X³X	33	21	♀	0	+	
	3 Y	55	33 XY	38	19	♂	37	+	2a, b
Adjacent I	3-Y	38	33-XY	37	—		—	—	
	3 X³	42	33 X³X	35	22	♀	0	0	
Non-disjunction I	3-X³Y	2	33-X³XY	0	0	♂	1	0	1h
	3	1	33 X	0	0	♀	0	0	
Non-disjunction II	3-	1	33-X	1	—		—	—	3e
	3 X³Y	2	33 X³XY	0	0	♂	0	0	
Total		190		144	62		38		
Nr. of testcrossed male parents		6		4	3		4		

0 = not observed, — = deficient karyotype, absent due to late embryonic lethality; + = cytological result of the progeny (B_2, egg stage) of the tested B_1 in accordance with the expectation.

as presented in Fig. 1d. We did not quantitatively analyze the orientation types during metaphase I of TN males due to the very low number of M I and A I cells. For technical reasons female meiosis was not accessible. The alternate and adjacent I orientations occur in about an equal frequency as judged from the classification of young eggs of testcrossed TN males and females (Tables 1 and 2). On the basis of observations at M II in TN males it could be concluded that somewhat more alternate orientation occurred (Table 1). No indications for adjacent II or other orientations were observed in these experiments. It is shown in Fig. 1d that non-disjunction I can be compared with the alternate orientation because of the similar way of disjunction of the three major chromosomes 3,3- and X³. The role of the small acrocentric X or Y is considered as secondary in respect to orientation. In the same way non-disjunction II can be considered as an alternative for the adjacent I configuration. In Table 3a we have compared the sum of alternate and non-disjunction I with the sum of adjacent I and non-disjunction II. Statistically we can accept that (Alt + ND I) : (Adj I + ND II) = 1 : 1, ($0{,}10 < p < 0{,}25$; $n = 452$) Table 3b shows the total percentage of non-disjunction as observed from M II cells of TN males and from young eggs of testcrossed TN males and females. It is obvious that there is quite a difference between

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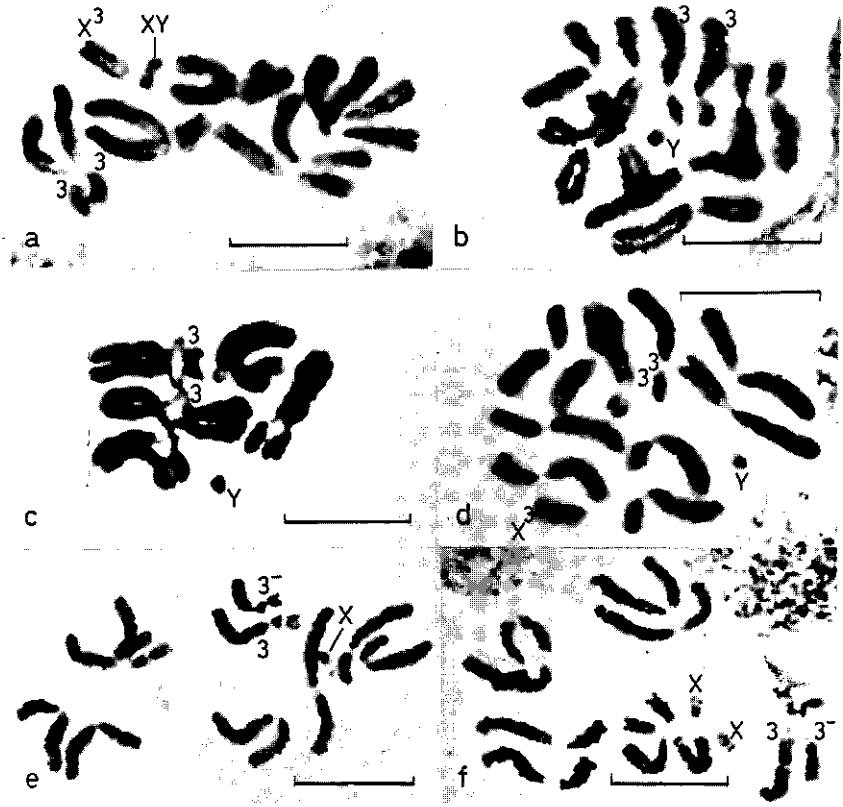

Fig. 3. (a) 33X³XY karyotype. Spermatogonial metaphase. (b) 33Y karyotype. Spermatogonial metaphase. (c) 33Y karyotype. Diakinesis/prometaphase ♂. (d) 33X³Y karyotype. Spermatogonial metaphase. (e) 33−X karyotype. Mitotic metaphase from a young embryo, presumably female. (f) 33−XX karyotype. Mitotic metaphase from a young embryo, presumably female on the basis of chromosome morphology. The bars on the photomicrographs represent 10 μm

TN males and females. In TN females 18.7% of the orientation types are non-disjunction types. Both types (I and II) occur in about equal frequencies. TN (33⁻X³Y) males showed 3.2% and 0.8% non-disjunction (I + II) as judged from M II cells and young eggs respectively. In (viable) 33X³Y males, with a duplication for half the length of the long arm of chromosome 3, we have scored 3.8% non-disjunction (only one type possible) from M II cells (Table 3b). This corresponds with the percentage of 3.2 for TN males.

"Semi"-sterility scored as brown eggs of TN males and females in testcrosses is between 25% and 35%. In 3 TN females we have compared

Table 2. The segregations in testcross progenies of 33-X^{3}X (TN) females (compare Fig. 1). The control males were 33XY (NN). Scoring of adult females (B_1) is difficult and was not carried out. The B_2 generation was not investigated. See further Table 1

Type of orientation	M II type P ♀♀	B_1 generation Karyotype		Eggs	Larvae	Sex	♂ adults	Figures
Alternate	3-X^3	33-X^3	X + or Y	25	21	♀		
						♂	28	2e, 2f
	3 X	33 X	X + or Y	26	24	♀		
						♂	38	2a, 2b
Adjacent I	3-X	33-X	X + or Y	22	—			3f
							—	
	3 X^3	33 X^3	X + or Y	23	13	♀		
						♂	21	3d
Non-disjunction I	3-X^{3}X	33-X^{3}X	X + or Y	5	7	♀		
						♂	12	2h
	3	33	X + or Y	8	5	♀		
						♂	6	3b, 3c
Non-disjunction II	3-	33-	X + or Y	4	—			3e
							—	
	3 X^{3}X	33 X^{3}X	X + or Y	5	2	♀		
						♂	5	3a
Total				118	72		110	
Nr. of testcrossed female parents				5	2		6	

the percentage "semi"-sterility or late embryonic lethality (eggs, 72 hours) with the percentage of cytologically determined deficient karyotypes in young (still viable, 8–16 hours) eggs (Table 4). Simultaneous tests were carried out for cytology (a total of 80 eggs) and for "semi"-sterility (397 eggs). The "semi"-sterility scored was about 28%, which is 8% higher than the percentage deficient karyotypes (20%) as scored in young eggs, and this 8% may be due to a reduced viability of deviant other karyotypes.

Table 3. a. The number of gametic karyotypes as scored at M II or in young eggs. Alternate and non-disjunction I combined, and adjacent I and non-disjunction II combined, average ratio 1.15:1.00

b. Numerical non-disjunction as scored on TN males and females and $33X^3Y$ males. Y (X) means: Y or X

a

Orientation	Gametic karyotypes	33^-X^3Y (TN ♂)		33^-X^3X (TN ♀)	
		M II	Young eggs	Young eggs	←stage
Alt. + ND I	$(3^-X^3+3Y)+(3^-X^3Y+3)$	107	71	64	
Adj. I + ND II	$(3^-Y+3X^3)+(3^-+3X^3Y)$	83	73	54	

b

	33^-X^3Y (TN ♂)		33^-X^3X (TN ♀)	$33X^3Y$ (♂)
	M II	Young eggs	Young eggs	M II
Alt. + Adj. I	184	143	96	149
ND I + ND II	6	1	22	6
% Numerical non-disjunction (I + II), i.e. (X^3 + Y (X) to the same pole)	3.2%	0.8%	18.7%	3.8%

Table 4. Relation between percentage late embryonic lethality ("semi"-sterility) as scored in eggs of 72 hours and the percentage deficient karyotypes in young eggs (8–16 hours)

Karyotype tested	Nr. of crosses	Nr. of eggs for cytology	% deficient karyotypes in young eggs (8–16 hours)	Nr. of eggs for sterility score	% sterility (eggs, 72 hours)	Average % sterility from other crosses	Nr. of eggs	Nr. of crosses
TN ♀	3	80	20%	397	28%	31%	> 1000	6
Control	10	100	0%	500	2–3%	2–3%	> 2000	> 20

Conclusions and Discussion

Boyes (1954) advanced four arguments for the view that the acrocentric chromosomes are the sex-chromosomes. Only one seems relevant: the acrocentric chromosomes sometimes differ in morphology, a phenomenon normally considered as characteristic for the sex-chromosomes. The acrocentric chromosomes in some related *Hylemya* species may be rather different in morphology and size. In *Hylemya fugax*, *e.g.*, the acrocentric chromosomes appeared to be involved in a multiple sex-determination system, $X_1X_2Y/X_1X_1X_2X_2$.

From our experiments it can be concluded rather definitively that the small acrocentric chromosomes are the sex-chromosomes. The acrocentrics were seen to be different in morphology, due to the presence (in the X) or absence (in the Y) of a constriction in the vicinity of the (terminal) kinetochore (Figs. 2d, 3e and f). Secondly it was shown that this translocation between chromosome 3 and one of the acrocentrics is sex-linked. We can conclude that the acrocentrics are the sex-chromosomes, because no sex-linkage could be found for translocations in which chromosome 3 but not one of the acrocentrics in involved. Further it is clear that the translocation is X-linked and not Y-linked, as in that case it could not occur in the female sex. A XY (♂)/XX(♀) sex-determination system is considered as the most likely. Some more information on the sex-determination can be extracted from data presented elsewhere (van Heemert, 1974) where it is shown that 33XXY males appear after test-crossing translocation trisomic males ($33^{-}X^{3}XY$). No 33XXX karyotypes were expected and indeed no normal females with an extra X-chromosome were observed. This strongly suggests that the Y-chromosome as such is male determining. Several authors, *e.g.* Ullerich (1963), Ullerich *et al.* (1964) and Hiroyoshi (1964) stated that in many *Diptera* the Y-chromosome is male-determining as in mammals, in contrast to *Drosophila* in which the X/A balance determines the sex. The distinct secondary constriction is located in the vicinity of the centromere of the X-chromosome but not in the Y-chromosome. It can also clearly be seen in the translocated acrocentric chromosome X^{3} (Fig. 2g).

Table 2 shows that karyotypes monosomic for the sex-chromosomes could be derived from TN females. Six adult males (33Y) were seen which only had one sex-chromosome, presumably a Y-chromosome (Fig. 3b, c). In the adult stage it could not be proven cytologically, that this chromosome is the Y-chromosome. There was no certainty in respect to the presence or absence of a secondary constriction. The X-chromosome that is missing in this karyotype probably is genetically inert, and it may have only a function in the female. Males of the 33Y constitution produce spermatozoa but no information about fertility is available.

The presence of very small metacentric B-chromosomes which do not pair somatically nor meiotically with the sex-chromosomes was not mentioned by Boyes (1954). However, the Canadian material we investigated and which Boyes used as well, possessed the same type of B-chromosomes. For the backcross experiments we have used a control series in which the B-chromosome was absent, to exclude misinterpretations, which could arise from the superficial similarity of the sex- and B-chromosomes.

Concerning the coorientation and disjunction in this translocation we can see from Tables 1 and 3a that alternate, adjacent I, nondisjunc-

tion I and II, but no adjacent II were found. Nor were the nondisjunction types corresponding to adjacent II observed. As appeared from the literature adjacent II is considered a relatively infrequent event in animals. In mice *e.g.*, Searle *et al.* (1971) indicated that the percentage of adjacent II in three different translocations is 13% on the average. Jost and Laven (1971) showed in *Culex pipiens* that in ten different translocations studied, adjacent II is the least frequent orientation. John and Hewitt (1963) have shown in *Chorthippus brunneus* that in an asymmetrical translocation between a small acrocentric and a large metacentric chromosome adjacent II only occurred in a few cases. The results of Jaylet (1971) from two translocations in the newt show that no adjacent II was found and that the homologous centromeres always disjoin in the first anaphase. Curtis *et al.* (1972) investigated 5 translocations in the tsetse fly and there were no indications for the adjacent II orientation. La Chance *et al.* (1964) assumed adjacent II to be absent in TN males as well as in TN females. John and Lewis (1965) in their review of the results of La Chance, could agree with this if translocation heterozygous males are concerned and considered it to be a result of achiasmate meiosis. In such an achiasmate situation homologous chromosomes have a prolonged pairing up to the first metaphase, and as Jaylet (1971) assumed, homologous centromeres disjoin in the first anaphase and no adjacent II occurs.

John and Lewis (1965) have argued that the results of La Chance in the case of translocation heterozygous females should fit the expectations better when adjacent II (25%) is assumed. Lewis and John (1963) even showed in one spontaneous translocation of *Chorthippus brunneus* that about 50% adjacent II might occur.

In general, however, when there is no preference for alternate orientation, a 1:1 ratio of alternate: adjacent (I + II) is expected, adjacent II being relatively infrequent. Apparently in this translocation there is no preference for alternate orientation (Tables 1 and 3a). From the data by Brink and Cooper (1932) on a (presumably) "simple" translocation in maize we could conclude that there were only two orientation types, alternate and adjacent I. These occurred in a ratio of 1:1, corresponding to what we have found in our translocation in the onion fly. There is no certainty that the fact that these translocations are "simple" is the cause of this 1:1 ratio. Only chains of four (Fig. 1) occur and no coorientation of the centromeres of 3$^-$ and X or Y is possible. We conclude that primarily homologous centromeres disjoin in this translocation and that therefore no adjacent II coorientation was found. From Table 3a it can be seen that even when alternate and non-disjunction I are combined and adjacent I with non-disjunction II, the 1:1 ratio has not changed. The two non-disjunction types can be considered as modifications of

alternate and adjacent I. This is particularly clear in the case of TN females where ND I and ND II occur in about equal frequencies (Table 2). This indicates that both the X^3 and the X orientate randomly, which can be explained by different mechanisms.

It is rather peculiar that there is such a difference in numerical non-disjunction between TN males and females (Table 3 b). In the case of TN females 18.7% was found, in the case of TN males about 2%. Ullerich (1964) has found numerical non-disjunction in *Phormia regina* (males achiasmate). About 1% was found in a Y-linked translocation as the result of a modified alternate orientation (like ND I in our case). The Y-chromosome is very small and acrocentric, while the translocated Y is much longer than the normal Y. This is analogous to our translocation in which the X^3 is much longer than the X-chromosome. In fact Ullerich's translocation can be considered as a "simple" translocation as well. Of course, numerical non-disjunction could only be observed in males, because females do not have the translocation. Their score of about 1% is in agreement with our result of 2%. In general sex-chromosomes show a somewhat higher numerical non-disjunction than autosomes (Würgler and Lütolf, 1972).

Several explanations may be given for the higher numerical non-disjunction in TN females than in TN males. One explanation might be disturbed meiotic pairing, which occurs at the time the X^{3-} and the X-chromosomes start their pairing (in TN females). Very frequently meiotic pairing starts from the telomeric end of the chromosome arms. In this translocation the X^3 and X do not usually find each other probably because the X-telomere at the place of the breakpoint in X^3 in case of a true simple translocation does not function. In the case that a tiny end segment of X is attached to 3^- there is no place where X and X^3 can start pairing. It seems that in achiasmate male meiosis predominantly centromere pairing is responsible for the association and disjunction of the homologous chromosomes. The pairing and disjunction of the X^3- and Y-chromosomes will not differ from the pairing and disjunction of the X- and Y-chromosomes in normal males. An other explanation can be the reduced chiasma frequency in the "interstitial" segment of the X^3- and X-chromosomes in the female. This reduction may be due to the proximity of the breakpoint to the telomere of the X and to the centromeres of the X^3 and X (Fig. 1 b). In males chiasmata were never found and therefore these factors do not operate here.

Several authors have observed numerical meiotic non-disjunction in certain translocation stocks of different animal species. These translocations had in common that a relatively small chromosome is involved, as in our X-linked translocation. Jost and Laven (1971) have reported numerical non-disjunction in chiasmate males of *Culex pipiens* in several

translocations in frequencies up to 30%. They argued that this is due to early separation of a relatively small sub-metacentric chromosome from the pairing complex. In these cases trivalents and univalents predominated, resulting in the formation of (n + 1) and (n —1) gametes. In mice Eicher (1973) and de Boer (personal communication) found numerical non-disjunction in translocation stocks where one of the translocated chromosomes was relatively small. In these translocations of *Culex* and mice which have a chiasmate meiosis, the small chromosome may have only a low chiasma frequency and, as a result the coorientation of the chromosomes in the translocation complex may be disturbed. In contrast to these translocations the relatively small chromosome in TN females of the onion fly is not a translocated one, but the original X-chromosome. It may be assumed to have a reduced chiasma frequency as a result of pairing problems in the critical telomere area where the small X-chromosome pairs with the rest of the translocation complex. The small size of the X-chromosome as such is not the cause of the low chiasma frequency, but it does contribute to a reduced probability of chiasma formation once one of the fundamental conditions (pairing) is disturbed.

Table 4 shows that there is good correspondence between the percentage of embryonic lethals ("semi"-sterility) and the percentage of deficient karyotypes as cytologically determined in young eggs. The karyotypes with a long segment of the long arm of chromosome no. 3 missing, without any chromosomal compensation by an X^3 chromosome, were not observed in the larval stage even in very young larvae. These karyotypes are 33^-X or 33^-Y and 33^-XX or 33^-XY in the progeny of testcrossed TN females (Table 2). They may cause 20% of brown eggs while the total percentage of brown eggs is 28%. From the literature it can be noted that in several other insect species these so called hypoploids die off in the middle stages of the embryonic development and cause semi-sterility (von Borstel and Rekemeyer, 1959; Imaizumi, 1962; Wright, 1971). The time of death of these hypoploids is very regular (von Borstel, 1963).

The explanation of the remaining 8% can be given in two ways. Firstly, we have found 2.5% brown eggs in the control but no cytologic anomalies were observed in the eggs. This percentage must always be taken into account. The remaining 5.5% of brown eggs is probably caused by the reduced viability of some specific deviant karyotypes. The number of $33X^3X$ or $33X^3Y$ and $33X^3XX$ or $33X^3XY$ karyotypes in the larval and adult stages (Table 2) is much lower than expected from the egg-scores. We have indications that $33X^3X$ or $33X^3Y$ individuals pupate poorly. Some may die at late embryonic stages. Less viable $33X^3X$ or $33X^3Y$ karyotypes of Table 2 might in fact be mainly $33X^3Y$, because the $33X^3X$ karyotypes in the larval progeny of TN males appeared to be as viable as the other karyotypes in the larval stage

(Table 1). A comparable situation was found by Curtis *et al.* (1972) who found that a certain unbalanced combination originating from a normal gamete and a gamete from an adjacent I orientation can stay alive, even into the adult stage. Such so called hyperploids (duplication karyotypes) most probably contribute considerably to the late embryonic lethality, as also suggested by Poulson (1940), Imaizumi (1962) and Scriba (1967). These authors stated that so called hyperploids sometimes also produce post-embryonic lethality. This we found in our experiments too. It is not surprising that in this asymmetrical translocation the viability of so called hyperploid karyotypes is relatively high. They mainly carry duplications and no deficiencies as normally is the case in reciprocal translocations. The (monosomic) 33X or 33Y karyotype, deficient for an X-chromosome (Table 2), might contribute as well to the browning due to lower viability in the egg stage. No investigations were carried out on the embryological aspects of the lethal syndromes.

A surprising observation was that the duplication types $33X^3XX$ or $33X^3XY$ and $33X^3X$ or $33X^3Y$ apparently were not able to fertilize mates, unlike the other viable aberrant karyotypes. We never found any progeny of these types although in the case of males, spermatozoa were found in the testes. In the asymmetrical translocation of maize (Brink and Cooper, 1932) one duplication (hyperploid) type was able to produce offspring through the eggs and not the pollen. However this type had a rather low fertility.

This paper precedes a second one (van Heemert, 1974) about different trisomic types derived from translocation heterozygous parents. There the same aspects as presented here will be discussed.

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C. van Heemert
Department of Genetics
Agricultural University
53 Generaal Foulkesweg
Wageningen
The Netherlands

Meiotic Disjunction and Embryonic Lethality in Trisomics Derived from an X-linked Translocation in the Onion Fly, *Hylemya antiqua* (Meigen)

C. van Heemert

Department of Genetics, Agricultural University, Wageningen

Abstract. Translocation- and tertiary trisomics (for the X-chromosomes) were obtained after testcrossing translocation heterozygous females of an X-linked "simple" translocation stock. Meiotic disjunction as judged from segregations at M II (males) and in young eggs of testcrosses (males and females) in translocation trisomics was studied. No progeny of tertiary trisomic males and females was found, but male M II could be studied. Six different orientation types appeared in translocation trisomic ($2n+1$) males and these were present in equal frequencies. No adjacent II configurations were found. The small X- and Y-chromosomes and the large translocated X-chromosome of the translocation complex disjoin at random (n and $n+1$ gametes) in both translocation- and tertiary trisomic males. In translocation trisomic females four different orientation types appeared. From the high frequency of two of these (together, 94.5%) it is concluded that the two normal X-chromosomes show preferential pairing and disjunction, while the translocated X-chromosome moves to either one of the two poles at random. Primary trisomic (for the X-chromosome) males (XXY) and females (XXX) were obtained from testcrossed translocation trisomics. Cytological analysis of adult male progeny of testcrossed XXY males showed that no random orientation for the X-, X- and Y-chromosomes occurred because half of the sons was disomic (XY) and half of them trisomic (XXY). A possible mechanism is discussed. Analysis of young eggs of testcrossed XXX females indicated a segregation of $2X:1X = 1:1$. The level of "semi"-sterility as scored from testcrosses of translocation trisomics appeared to be as in translocation heterozygotes. Here again a close relation exists between "semi"-sterility and deficiencies in eggs for a large chromosomal segment. The possible use of this translocation for genetic control of insect pests is discussed.

Introduction

In an earlier article an X-linked translocation in the onion fly ($2n = 12$) was described (van Heemert, 1974). A large submetacentric chromosome (no. 3) and the small acrocentric X-chromosome are involved in this translocation. The translocated chromosome 3^- lost about half the length of the long arm. It seemed not to have gained any chromosomal material from the X-chromosome. The translocated acrocentric chromosome X^3 gained considerably in length. This translocation is considered as a "simple" translocation but may also be a highly unequal reciprocal translocation.

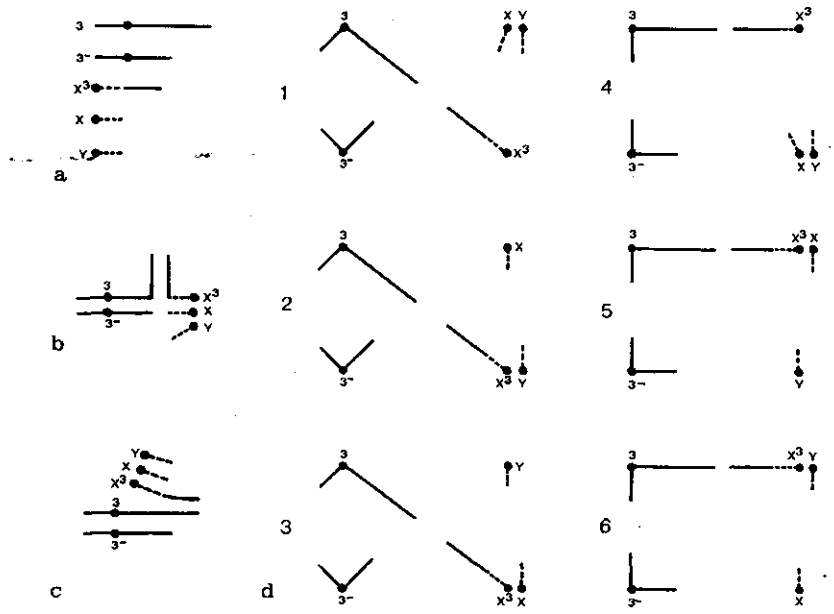

Fig. 1. (a) Idiogram of the chromosomes involved in the translocation complex of a TN + X male. (b) Cross-figure as expected in early meiotic prophase I, diagrammatically. (c) Meiotic pairing as usually observed in diakinesis/prometaphase. (d) Six different orientation types (A I). In the case of TN + X females replace Y by X. Orientation types 2 and 3 become identical as do types 5 and 6 in the case of TN + X females

It appeared that in addition to the alternate and adjacent I orientations, numerical non-disjunction for the X-chromosome occurred. As a result, translocation trisomics, tertiary trisomics, monosomics and duplication karyotypes were produced, besides the normal (NN) and translocation heterozygous individuals (TN). The terminology used is explained in Sybenga (1972). All these aberrant karyotypes reached the adult stage, but only the translocation trisomics were fertile.

Translocation trisomics (TN + X) although having an additional X-chromosome compared to translocation heterozygotes (TN), showed the same percentage (25%–35%) of "semi"-sterility in both sexes. Five chromosomes are involved in one complex at meiosis. In Fig. 1a we have drawn them diagrammatically for a TN + X male: 3,3$^-$,X^3,X and Y. In the case of a TN + X female the Y-chromosome should be replaced by an X-chromosome.

It has been shown (van Heemert, 1974) that the pattern of inheritance of the translocation heterozygotes (TN) is typically X-linked. Trans-

location heterozygous males after testcrossing produce normal sons (except in one case, where a TN + X son appeared) and "semi"-sterile daughters exclusively. Translocation heterozygous females produced normal as well as translocation heterozygous males and females.

However, we discovered that several "semi"-sterile males after testcrossing produced translocation sons as well as normal sons.

We found another, indirect, indication for this. When flies obtained from an individual testcross of certain "semi"-sterile males were sibcrossed, many matings (about 25%) showed normal fertility. This is not expected in the case of a normal X-linked translocation, because all the sons of a testcrossed translocation-heterozygous father (TN) will be normal and all the daughters will have the translocation. Therefore all matings between sons and daughters of such a father should show "semi"-sterility. Cytological analysis showed these unusual fathers to be translocation trisomics (TN + X).

The progeny of testcrossed translocation trisomic (TN + X) males and females has been investigated in the egg, larval and adult stages. M II cells of TN + X males were analyzed as well. Disjunction at meiosis of the X^3, X and Y chromosomes of TN + X males and the X^3, X and X chromosomes of TN + X females with homologous centromeres was studied and compared with the disjunction of X^3Y or X^3X in TN males and females respectively (van Heemert, 1974). Only insufficient numbers however, of analyzable M I and A I cells were available for quantitative analysis.

Fig. 1 b shows how the five chromosomes of the translocation trisomic might pair at pachytene. Since the onion fly has achiasmate males, regular diplotene stages are not expected. We have observed diakinesis/prometaphase pairing in spermatocytes as indicated in Fig. 1 c. Fig. 1 d diagrammatically shows the 6 different orientation types the presence of which was demonstrated by segregations at M II and in young embryos. There were no indications for adjacent II or other orientation types. Orientation types 1, 2 and 3 in TN + X males, have in common that the major chromosomes 3^- and X^3 go to the same pole and chromosome 3 to the opposite pole. The X- and Y-chromosomes can be found together in one pole or in different poles. In the case of orientation types 4, 5 and 6, chromosomes 3 and X^3 go to the same pole while chromosome 3^- goes to the other pole. Again the X- and Y-chromosomes can be found together in one pole or in different poles.

Only four different orientation types could be concluded to be present in TN + X females, and are analogous with the types 1, 2 (3), 4 and 5 (6) as shown in Fig. 1 d.

Primary trisomics for the X-chromosome were found among the offspring of testcrossed TN + X flies. The disjunction of the X, X and Y

Table 2. The segregations in testcross progenies of 33-X³XX (TN + X) females (compare Fig. 1). The control males were 33XY (NN). Scoring of adult females (B_1) is difficult and only six could be analyzed. No larvae were investigated. For further explanation see Table 1

Type of orientation	M II type parental females	B_1 generation Karyotype		Eggs	Larvae	Sex	Adults ♂	Adults ♀
1			X			♀		0
	3-X³	33-X³	+ or	2				
			Y			♂	0	
			X			♀		1
	3 XX	33 XX	+ or	5				
			Y			♂	1	
2			X			♀		2
	3-X³X	33-X³X	+ or	34				
			Y			♂	6	
			X			♀		2
	3 X	33 X	+ or	27				
			Y			♂	6	
3			X					—
	3-XX	33-XX	+ or	0	—			
			Y				—	
			X			♀		1
	3 X³	33 X³	+ or	0				
			Y			♂	1	
4			X					—
	3-X	33-X	+ or	30	—			
			Y				—	
			X			♀		0
	3 X³X	33 X³X	+ or	29				
			Y			♂	3	
Total				127			17	6
Nr. of testcrossed female parents				3			3	3

the 33XX mother occurs. Indeed no 33X³Y males were observed even though a sufficient number was analyzed. In the same way karyotypes of eggs with 33 + three small acrocentric chromosomes were 33XXY (males) exclusively (Table 1).

Table 2 gives the results of the analysis of the progenies of testcrossed females which were TN + X as could be demonstrated by the presence of the 33XXX or 33XXY karyotypes in young eggs. Six different karyo-

types were observed in the egg stage. The karyotypes 33-XXX or 33-XXY and 33 X^3X or 33 X^3Y both resulting from orientation type 3 (Fig. 1) were not observed in the eggs. The karyotypes 33-X^3X or 33-X^3Y and 33 XXX or 33 XXY were present only in a small number. No larvae were scored but young adult flies of both sexes were analyzed. In 17 young newly emerged males we observed five different karyotypes. Four different karyotypes were found in six young females. Although in young eggs the products of orientation type 3 were not observed, yet one 33 X^3X adult female and one 33 X^3Y adult male were found, which can only arise as the result of this orientation type 3. Apparently the frequency of this orientation type is very low.

In Table 3a the frequencies of gametic types corresponding to specific orientation types as scored at M II and as derived from eggs of testcrosses involving TN + X males and females are combined. The sum of orientation types 1, 2 and 3 were compared with the sum of orientation types 4, 5 and 6 in the case of TN + X males, while the sum or orientation types 1 and 2 and the sum of orientation types 3 and 4 were compared in the case of TN + X females. There was no significant deviation from a 1:1 ratio in TN + X males nor females. As indicated in Table 3b, the X- and Y-chromosomes of TN + X males go to the same pole and the X^3 to the opposite pole in 32.7% (eggs)—34.7% (M II) of the cases observed. In tertiary trisomic males (33 X^3XY) a corresponding value (30.7%) was observed at M II. In TN + X females both X-chromosomes go to the same pole and the X^3-chromosome to the opposite pole in only 5.5% (eggs) of the cases.

Table 4 shows the percentages of disjunction types for the XXY and XXX trivalents in primary trisomic (NN + X) males and females respectively. These trisomics were originally obtained from testcrossed TN + X parents (Tables 1 and 2). From testcrossed NN + X (33 XXX) females we have found half of the eggs with an additional X-chromosome and the other half with the normal number (2) of sex-chromosomes. This clearly demonstrates that the XXX trivalent always disjoins as: 2 X/1 X. Here the segregation of the sex-chromosomes was analyzed in the testcross progeny (60 eggs) of the homogametic sex (♀) and it is not necessary to distinguish between the sexes in the progeny. When, however, as in the case of NN + X males (33 XXY) segregation is analyzed in the testcross progeny of the heterogametic sex, the sex of the progeny is relevant, and in the onion fly can only be determined in the adult stage, 52.4% of the sons (101) appeared to be 33 XXY and 46.6% 33 XY.

We have investigated the relationship between late embryonic lethality in eggs (72 hours) and deficiency in young eggs (8–16 hours) for a large chromosomal segment. Ninety eggs (8–16 hours old) from testcrossed TN + X males were used for cytology, and 18% had the large deficiency.

Table 3a. The number of gametic karyotypes as scored at M II and in young eggs from testcrosses. Orientation types 1, 2 and 3 combined and 4, 5 and 6 combined (see Fig. 1 d). Replace Y by X in the case of TN + X females. Numbers gathered from Tables 1 and 2. — b. Disjunction percentage of X^3XY in translocation trisomic males and in tertiary trisomic males and the disjunction percentage of X^3XX in translocation trisomic females

$\frac{X}{Y}-X^3$ means: X and Y go to one pole and X^3 to the other. X(Y) means: X or Y.

a Orientation type	Gametic karyotypes	33^-X^3XY(TN + X ♂)		33^-X^3XX(TN + X ♀)
		M II	Young eggs	Young eggs
1 + 2 + 3	$(3^-X^3 + 3XY) + (3^-X^3Y(X) + 3X(Y))$	47	31	68
4 + 5 + 6	$(3^-XY + 3X^3) + (3^-Y(X) + 3X^3X(Y))$	51	24	59

b Disjunction	Transl. tris. males 33^-X^3XY(TN + X)		Tert. tris. males $33X^3XY$	Disjunction	Transl. tris. females 33^-X^3XX(TN + X)
	M II (98 cells)	Young eggs (55)	M II (52 cells)		Young eggs (127)
$\left.\begin{matrix}X^3\\X\end{matrix}-Y\right\}$ $\left.\begin{matrix}X^3\\Y\end{matrix}-X\right\}$	65.3%	67.3%	69.3%	$\frac{X}{X^3}-X$	94.5%
$\frac{X}{Y}-X^3$	34.7%	32.7%	30.7%	$\frac{X}{X}-X^3$	5.5%

Table 4. Disjunction percentages of XXY and XXX in primary trisomics (for the X-chromosome). In the case of males we have analyzed adult sons from testcrosses. Disjunction in females was established by analyzing young eggs (8–16 hrs.) from testcrosses

$\frac{X}{Y}-X$ means: X and Y go to one pole and the second X to the other pole.

33XXY (♂)		33XXX (♀)	
$\frac{X}{Y}-X$	52.4%	$\frac{X}{X}-X$	100%
$\frac{X}{X}-Y$	47.6%		
101 sons		60 young eggs	

Table 5. Relation between the percentage late embryonic lethality ("semi"-sterility) as scored in eggs (72 hours) and the percentage deficient karyotypes in young eggs (8–16 hours)

Karyotype tested	Nr. of crosses	Nr. of eggs for cytology	% deficient karyotypes in young eggs (8–16 hours)	Nr. of eggs for sterility score	% sterility (eggs 72 hours)	Average % sterility from other crosses	Nr. of eggs	Nr. of crosses
TN + X ♂	2	90	18	328	27	29	> 1000	7
Control	10	100	0	500	2–3	2–3	> 2000	> 20

Another 328 eggs (72 hours old) from the same batch were scored for the percentage "semi"-sterility (late embryonic lethality): 27%. The remaining 9% may be due to a reduced viability of other deviant karyotypes. The average percentage of "semi"-sterility in other experiments is about 29% (Table 5).

Conclusions and Discussion

The meiotic behaviour of the chromosomes in translocation heterozygous (TN) males and females could be reasonably well established by analyzing M II and young eggs (van Heemert, 1974). Particularly the meiotic disjunction of the translocated X-chromosome (X^3) and the normal X-chromosome in TN females and the meiotic disjunction of X^3 and Y in TN males was studied. It appeared that 18.7% numerical non-disjunction (X^3 and X to the same pole) occurred in TN females and only about 2% in TN males (X^3 and Y to the same pole; Table 3, van Heemert, 1974).

Translocation trisomic (TN + X) males and females have an additional X-chromosome compared to TN males and females. Here we compared the coorientation and disjunction of the three chromosomes with homologous centromeres in TN + X males (X^3, X and Y) with the behaviour of X^3, X and X in TN + X females. The data on disjunction in tertiary trisomic males, 33 X^3XY (Table 3b), show that the coorientation and disjunction of the X^3-, X- and Y-chromosomes is similar to that in translocation trisomic (TN + X) males. This similarity suggests that it does not make any difference whether 33 or 33$^-$ is present in combination with the chromosomes X^3, X and Y. Further it was concluded from segregations (M II and eggs) of tertiary trisomic (33 X^3XY, 2n + 1) males and of translocation trisomic (TN + X, 2n + 1) males and females, that only (n) and (n + 1) gametes are produced and no (n + 2) or (n—1) gametes. Therefore we assumed that for TN + X males orientation types 1, 2 and 3 (Fig. 1)

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can be combined, as well as orientation types 4, 5 and 6. Similarly, types 1 and 2, and types 3 and 4 can be combined in the case of TN + X females. It was shown statistically that the sum of orientation types 1, 2 and 3 is not different from the sum of 4, 5 and 6 ($0.50 < p < 0.75$; n = 163) in TN + X males and that 1 + 2 is not different from 3 + 4 ($0.25 < p < 0.50$; n = 127) in TN + X females (Table 3a). This corresponds to the 1:1 ratio of (Alt + ND I):(Adj I + ND II) as observed in TN males and females (Table 3a, van Heemert, 1974). In both TN and TN + X for males as well as for females there is an equal chance for X^3 to go to the same pole with chromosome 3 as with chromosome 3^-, independently of the presence of one or two normal sex-chromosomes.

Looking at the behaviour of the chromosomes X^3, X and Y in TN + X males, we see that in 33.7% of the cases observed (Table 3b) the X- and Y-chromosomes go to the same pole (orientation types 1 and 4, Fig. 1). In about $^2/_3$ of the cases X and Y disjoin and it is plausible that the two types of gametes with X^3Y or X (orientation types 2 and 6) and gametes with X^3X or Y (orientation types 3 and 5) occur in equal frequencies, $^1/_3$ each. It can be concluded from these results (M II and young eggs) that random coorientation and disjunction (n/n + 1) occurs for the X^3-, X- and Y-chromosomes in TN + X (2n + 1) males. Orientation types (1 + 4):(2 + 6):(3 + 5) = 1:1:1. An explanation for this random process might be that the three different chromosomes (X^3, X and Y) have homologous centromeres, and because the males have no chiasmata the association of the homologous centromeres determines the coorientation. In spite of the same size of the X- and Y-chromosomes, distributive pairing apparently does not occur, as otherwise we would have found mainly X^3Y (X^3X) and X (Y) gametes.

In TN + X females a completely different situation was found. In the X^3XX group of chromosomes, the two identical X-chromosomes disjoin in 94.5% of the cases observed (Table 3b) and in only 5.5% of the eggs of testcrossed TN + X females both X-chromosomes from the TN + X female parent are present. Distributive pairing (size-dependent) may be an explanation for this phenomenon, but preferential (homologous) pairing of the two normal X-chromosomes can explain their almost 1:1 disjunction as well. In the latter case chiasmata can occur between the two X-chromosomes and probably are absent between the X^3 and X. Apparently the X^3-chromosome moves at random to either one of the two poles at the first anaphase (Table 2). As a consequence the gametes 3^-X^3 and 3XX (orientation type 1, Fig. 1) are formed and when fusion with a 3X or 3Y gamete of the normal male testcross parent takes place, 33^-X^3X or 33^-X^3Y and 33XXX or 33XXY karyotypes will be found in the eggs (Table 2). No eggs originating from orientation type 3 were found as these would have had the 33^-XXX (or 33^-XXY) or 33 X^3X (or 33 X^3Y)

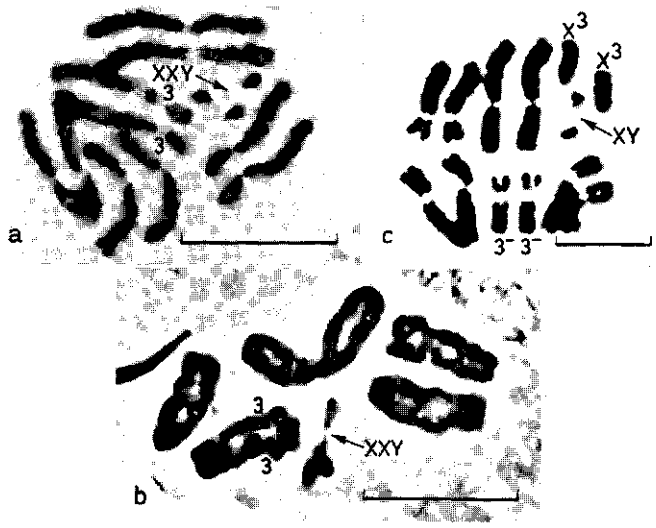

Fig. 2. (a) NN + X (33 XXY) karyotype. Spermatogonial metaphase. XXY means X + X + Y. (b) NN + X (33 XXY) karyotype. Diakinesis/prometaphase ♂. (c) TT + XY ($3^-3^-X^3X^3XY$) karyotype. Translocation homozygous male (tetrasomic). Spermatogonial metaphase. The bars on the photomicrographs represent 10 μm

karyotype. However, after cytological analysis of B_1 adults (Table 2) we could conclude that this orientation type must have occurred occasionally.

Primary trisomic males (NN + X) appeared in the progeny of TN + X males and females and not in that of TN. NN + X females were found among the progeny of TN + X females exclusively. NN + X males and females were both very viable and completely fertile. Primary trisomics (NN + X, Fig. 2a and b) and translocation trisomics (TN + X) have in common that only (n + 1) and (n) gametes occur and that their ratio is 1:1. Table 4 shows that when two X-chromosomes and one Y-chromosome are present there is no random disjunction. In a random situation twice as many XY and X gametes as XX and Y gametes would be expected. However, equal frequencies of XY (52.4%) and Y (47.6%) were found. In diakinesis/prometaphase in males both X-chromosomes usually are paired very intimately and the Y-chromosome, though paired, can be seen more apart (Fig. 2b). Both X-chromosomes might act as a "couple" in 50% of the cases and disjoin together from the Y-chromosome as normally one X disjoins from the Y in 33 XY males. In the other 50% the two X-chromosomes disjoin from each other and the Y goes with either one of the two X-chromosomes into a pole. No Y- nor X-chromosome seems to get lost.

females and TN + X males show about the same percentage (28% and 27% respectively) of brown eggs (72 hours) and about the same percentage of deficient karyotypes (20% and 18% respectively), as scored cytologically in young eggs (8–16 hours). TN males and TN + X females were not tested. Testcrossed primary trisomic (NN + X) males and females did not show any "semi"-sterility and no deficiencies in young eggs were observed.

McDonald and Rai (1971) suggested the use of a sex-linked translocation for genetic control purposes. They concluded from computer simulation studies, that X-linked translocations are more useful than autosomal translocations for release experiments. The use of double heterozygotes which are heterozygous for two different sex-linked translocations seemed even more successful for genetic control in *Aedes aegypti*. For translocations in general, important limiting factors are competitive ability, degree of the "semi"-sterility and population growth rate. For normal X-linked translocations the theoretical impossibility of homozygotes in both sexes is an additional limitation for mass rearing. We were able to isolate homozygotes for this X-linked translocation in the onion fly even in the male sex. This rather unexpected result can be explained as follows. When in a cross between a TN + X male and a TN female $3^{-}X^{3}Y$ and $3^{-}X^{3}$ gametes are formed respectively, we may expect some $3^{-}3^{-}X^{3}X^{3}+Y$ karyotypes, which are translocation homozygous males (TT + Y). A TN + X male crossed with a TN + X female can give translocation homozygous (TT + Y) males as well. Even TT + XY males are present rather often (Fig. 2c). Translocation homozygous females could be detected by analysis of their oogonia. They can be $3^{-}3^{-}X^{3}X^{3}$ or $3^{-}3^{-}X^{3}X^{3}$ + one or two additional X-chromosomes. We maintained the stock in which translocation homozygous flies were observed by full sibmating. Morphologically the translocation homozygotes appeared to be normal and completely competitive with NN individuals. Their fertility was about 75% (control: 94%). The stock was kept homozygous for three generations but unfortunately probably became contaminated with normal flies. Attempts to make it homozygous again are under way. Disjunction of the X^{3},X^{3},X and X or X^{3},X^{3},X and Y chromosomes of tetrasomic TT + 2X females or TT + XY males respectively will then be studied as well and compared with the disjunction of the X^{3},X and Y, and of the X^{3},X and X chromosomes of the translocation trisomics presented here.

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C. van Heemert
Department of Genetics
Agricultural University
53 Generaal Foulkesweg
Wageningen
The Netherlands

General discussion

From the results of the first three articles (I, II and III) a few comments can be made which may be relevant for future isolations of "semi"-sterile stocks carrying chromosomal rearrangements. Comparison of the different doses used on males (all below the 100% sterilizing dose of 3 krad of X-rays) led to the conclusion that a low dose of 0.5 krad of X-rays on males is recommendable for the induction of chromosomal rearrangements to be used for genetic control purposes. The reasons are firstly, that a few or no complex chromosomal rearrangements causing "semi"-sterility are induced at such low doses. Secondly, the genetic background damage (such as recessive lethals) will be reduced, which is important for homozygosing the translocations. Ytterborn (1970) has shown with Drosophila that translocations induced with lower radiation doses have a greater chance to be viable as homozygotes. On the other hand more initial screening in the F_1 has to be carried out to find a "semi"-sterile strain, because the frequency of rearrangements is low. Originally, 13 days old pupae instead of adult males were irradiated because these seemed to be more manageable. A disadvantage was the ignorance of the precise developmental stage and also the fact that half of the emerged flies (the females) had to be discarded. As far as the output of "semi"-sterile stocks is concerned, no difference between irradiating 13 days old pupae or young adult males was found. This can be understood when one considers that early spermatids, expected to be the most sensitive for the induction of translocations (Sobels, 1969), are already present at late pupal stages. Lateron it was concluded (article III) that irradiation of about seven days old males might be more useful because of the presence of a more homogeneous sample of mature sperm. In this case, of course, probably most chromosomal rearrangements are induced at a less sensitive stage, resulting in a lower yield of chromosomal rearrangements per unit of dose.

Females appeared to be very sensitive to X-rays, compared to males. Glass (1956) has shown the same in Drosophila. In particular just emerged females in which the ovaries still have to develop (Theunissen, 1971), are sensitive as appeared from the high infecundity in the parental and even the F_1 genera-

tion, and many F_1 crosses as far as they could be made were sterile. A minority of the irradiated females (P generation) had a relatively high fertility compared to males irradiated with the same dose, probably because eggs which were laid were rather free of radiation damage and therefore could hatch. Seven days old females gave much better results, because the fecundity was higher, although the egg production is still reduced to about 1/5 of the normal production of a control series and stopped earlier. A reasonable number of F_1 crosses produced offspring, but most of them had an about normal fertility and were not suitable for our purpose.

The use of fast neutrons on females even at a low dose is probably not advisable, due to high infecundity in the P and F_1 generation. However, irradiation of young males with fast neutrons gives results comparable to those of the use of X-rays. At a low dose of 0.25 krad a few reciprocal translocations were found in a rather small sample. Fast neutrons, having a higher track density, probably cause less genetic background damage than X-rays.

Table A. Distribution of the initial breakpoints (21) of 10 different translocations (table B) over the 11 different chromosome arms (See table B). Armlength at mitotic metaphase in larval brain cells according to Boyes (1954).

14	13	12	11	10	10	8	7	6	5	4	Armlength (in order; microns)
6^l	3^l	4^l	5^l	2^l	6^s	5^s	2^s	4^s	3^s	1	Chromosome arms
1	4	1	3	4	5	1	1	-	-	1	Distribution of breakpoints

In the first article (1) we have made a few comments on the positions of the initial breakpoints of the translocations. We stated that the length of the chromosome arms probably is one of the factors which determines the chance of becoming involved in a chromosomal rearrangement. From table A giving the chromosome arms in which the initial breakpoints of the ten translocations (table B) are located it can indeed be seen that the arm length plays a role. The conclusions of Burnham (1964) in his review of break positions in maize and Drosophila are in general in agreement with our results. However, in those organisms the smaller chromosome arms mostly were usually somewhat more involved in the translocations than expected on the basis of mitotic length. Most (18) of the initial 21 breakpoints are located in the six of the eleven chromosome arms which are longer than or equal to 10 μ. It is not clear why 6^l and 4^l each are involved only once, although the armlengths are among the greatest. Chromosome arms 3^s and 4^s are not involved in any of the

translocations. However, in (3) 2 is a pericentric inversion, thus a break must have occurred in the short arm of chromosome 3. Apparently no preferential participation of particular chromosome arms during the induction of translocations could be demonstrated. In the first paper we thought it plausible that the initial translocation breakpoints preferentially were located in achromatic areas of the chromosomes or in secondary constrictions. We can see now, that there is no relationship between the occurrence of breakpoints and these achromatic (heterochromatic?) areas. Of course, small heterochromatic areas may be present, not discernable with the convential staining techniques we have used.

For the recognition and isolation of translocations genetic markers are generally employed in insect genetics. These are not available in the onion fly but the use of cytological techniques can be applied without problem. The karyotype of the onion fly consists of six pairs of chromosomes. Due to somatic pairing exchanged non-homologous segments of reciprocal translocations can readily be distinguished, unless these are too short and/or equal in size. In cases of doubt meiotic analysis (males) as a rule could give evidence.

A standard method of brooding the eggs followed by the scoring of brown eggs (van Heemert, 1973) proved to be more succesfull for the screening of the "semi"-sterility than the use of egg hatch. Originally, the egg hatch was measured (at a temperature of 24°C) by dividing the empty eggs by the total number of eggs: unhatched white eggs + empty eggs. By brooding the eggs at 29°C a part of the unhatched eggs turned brown in colour in the case of a translocation. Late embryonic lethality (brown eggs) was shown to be related with duplication/deficiency karyotypes resulting from adjacent orientation in the translocation heterozygote.

In crosses between two translocation heterozygous (TN) individuals as expected, we scored higher percentages of brown eggs in comparison with the testcross, as both parents instead of one produce unbalanced gametes. In most of the translocation stocks which were sibcrossed we could distinguish three levels of sterility in spite of a sometimes considerable variation. These correspond with the sterility from NNxNN, NNxTN and TNxTN crosses. When the testcross fertility of a translocation is rather high (for instance 85%) or low (for instance 15%) the difference between the average fertility of a testcross and of a cross between two heterozygotes is small (in this example 13%). The maximum difference of 25% occurs in the case the testcross fertility is 50%. With a moderate variation a difference of 13% is too small to dis-

Table B. Review of the different chromosomal rearrangements induced in the onion fly in relation to the induction dose, chromosome arms involved, egg hatch, homozygosity and larval duplication/deficiency karyotypes.
? not yet analyzed; - not found; + observed; T translocation; In inversion.

Code	Original dose	Chromosomes involved	Egg-hatch % test cross	Homozygous as larvae/adults	Larval dupl./ def. types	Comments
T 1	1.0 Krad X ♂	$3^{l(-)}$-X$^{(+)}$	± 72	L A	+	X-linked
T 2	1.1 Krad X ♂	$3^{l(+)}$-$5^{l(+)}$-$6^{l(-)}$	± 55	-		cyclic
T 3	1.0 Krad X ♂	$5^{l(+)}$-$6^{s(-)}$	± 70	-		
T 4	0.25 Krad fN ♂	$2^{l(-)}$-$6^{s(+)}$	± 80	L	+	resembles T 10
T 5	0.5 Krad X ♂	$2^{s(+)}$-$6^{s(-)}$	± 79	L	+	
T 6	0.5 Krad X ♂	$3^{l(-)}$-$5^{s(+)}$	± 62	L A	+	
T 7	1.0 Krad X ♂	$2^{l(-)}$-$4^{l(+)}$	± 60	?		
T 8	"spontaneous"	2^{l} - 5^{l}	± 50	?		symmetrical
T 9	1.5 Krad X ♂	$3^{l(-)}$-$6^{s(+)}$	± 55	-		
T 10	1.0 Krad X ♀	$2^{l(-)}$-$6^{s(+)}$	± 80	L A	+	resembles T 4
In 1	0.5 Krad X ♂	Chr. 6	± 74 ♀ ± 97 ♂	-	+	pericentric, symmetrical
In 2	"spontaneous"	Chr. 3	± 80 ♀ ± 94 ♂	?	+	pericentric, asymmetrical

criminate between the two fertility levels. The fertility in the control (NN) crosses normally has little variation.

From the experiments as described in the first three articles we could definitively recognize 17 different translocations and two pericentric inversions. Ten translocations and the two inversions were kept for further research (Table B). The rest was lost or not maintained because of rearing difficulties or because the translocation was not suitable for more detailed cytological analysis. Three out of these ten translocations (T2, T3 and T9) were removed from the sib program for the following reasons.

Translocation T2 is rather complex. It is a cyclic translocation (three breakpoints) in which three pairs of chromosomes are involved. No translocation homozygotes were ever seen, even after sibcrosses for at least 4 generations. This translocation produced very good egg rafts with a sterility of about 50% and had a good competitiveness with normal flies. These characteristics were the reason why this particular stock was successfully used in a preliminary field experiment. From a cage in which thousend normal and thousend translocation heterozygous flies were released, after three weeks we have

recaptured 15 females and these were allowed to oviposite in the laboratory. An average sterility of 36% was scored, while the control cage showed no sterility.

Analysis of T3 indicated that the translocation heterozygous karyotype could not unequivocally be discriminated from the normal karyotype.

In the case of T9 a rather high dose of 1.5 krad of X-rays was used and therefore it was assumed that inbreeding would give homozygotes carrying detrimental factors (recessive lethals) in a homozygous condition. As a check one sibcross was studied and indeed no homozygote was found in the larval stage.

Five of the seven remaining translocations have been sibcrossed and the larval progeny (6 larvae per caged female) was analyzed cytologically for the presence of translocation homozygotes (TT) in the case a relatively high sterility was found. In theory $\frac{1}{4}$ of the sibcrosses between randomly taken sibs out of testcross progenies (NN+TN) will show this high level of sterility. If the presence of translocation homozygotes could be cytologically proven in the progenies of individually caged females, both parents should have been heterozygous (TN) for the translocation. Surprisingly in all five translocation stocks which were sibcrossed translocation homozygotes were found cytologically. In three of these, T1, T6 and T10, they were viable into the adult stage (Table B). In the translocations T4 and T5 homozygotes could be found only as older larvae, but further analysis is needed. Of T1 and T10 we know that the translocation homozygotes are fertile and can produce offspring. In both cases some of the individual sibcrosses with flies from the first sibcross progenies were found to have approximately an about normal fertility and had a progeny consisting of 100% translocation heterozygotes. These must have been the combination of a normal and a translocation homozygous parent.

The difficulty of obtaining a homozygous stock most probably is due to the small scale on which sibcrosses could be made. Theoretically about one out of sixteen crosses with flies from the progeny of a cross between translocation heterozygous parents will be between two translocation homozygous individuals. It obviously requires large scale experiments to be able to isolate a homozygous translocation stock.

An important feature we have observed is the occurrence of duplication/deficiency karyotypes at the larval stage. Surprisingly these karyotypes were found only in those translocations of which translocation homozygotes were observed. In general it is rather uncommon that such karyotypes reach the

latest larval stages. In the case of T1 even the adult (with sperm) stage. We have no explanation for this and further investigation is needed. In spite of the many references on translocations in insects it is peculiar that only in a few occasions such viable duplication/deficiency karyotypes were described. Curtis et al (1972) and Ved Brat and Rai (1974) mentioned the presence of such deviant karyotypes in the larval stage of Tsetse fly and *Aedes aegypti* respectively. Most probably other workers have paid insufficient attention to the larval cytology. Sometimes the distinction of a duplication/deficiency karyotype from a translocation heterozygous karyotype was difficult. When one of the two marker (translocated) chromosomes of the translocation heterozygous karyotype is similar to one of the other chromosomes of the karyotype and when at the same time somatic pairing is somewhat less close, confusion with a duplication/deficiency karyotype is possible. Normally only one of the two duplication/deficiency karyotypes originating from an adjacent 1 orientation (possessing the larger duplications and the smaller deficiencies) were found at the larval stage. However, in the case of T5 we have found both possible duplication/deficiency karyotypes from adjacent 1 among the larval offspring of a testcrossed translocation heterozygote.

Sibcrosses of both pericentric inversion stocks have been started. In a few among a large number of sibcrosses of In 1 a lower fertility was found compared to the testcross fertility of inversion heterozygous females. Since inversion heterozygous males are completely fertile (article III) these crosses must have been between inversion heterozygous parents and the inversion homozygotes of the progeny most probably die in the embryonic stage. This assumption is supported by the observation of a higher frequency of inversion heterozygotes (larvae) among the progeny than expected. We do not yet have sufficient data from inbreeding experiments of the In 2.

Detailed studies on the X-linked translocation T1 are reported (IV and V). The presence of an extra X-chromosome in the translocation trisomic karyotypes (TN+X) caused a completely different meiotic behaviour compared to translocation heterozygotes (TN) and different composition of the offspring. In our first attempts to isolate homozygous flies (TT), carried out concurrently with the experiments described in articles IV and V, we have found translocation nomozygotes in both sexes, with one or two additional sex-chromosomes. We now know that these must have arisen from crosses between two translocation trisomic parents (TN+X). Crosses between translocation heterozygotes (TN), as can be concluded from tables 1 and 2 from paper IV, will

give TT or TT+X females (in a ratio of about 4:1) but no TT males. Crosses between TN+X males and females do give translocation homozygotes for both sexes and these possess one (or two) extra sex chromosomes: The male is TT+Y (or TT+X+Y), the female TT+X or TT+2X and occassionaly TT.

In the first step of the homozygosing program we have testcrossed TN+X females. The progeny has a higher frequency of TN+X sons and daugthers than testcrossed TN+X males or TN females. Subsequently 63 sibcrosses were made, of which 32 had an increased sterility (40%-60%) compared to the testcross sterility (25%-35%). The progenies of these were analyzed cytologically (at least six larvae per progeny) with the aim of finding TT+2X (or TT+X+Y) karyotypes. NN was present as well. Five out of these 32 sibcrosses contained TT+2X (or TT+X+Y and sometimes TT+X or TT+Y) among their progeny and an additional three had TT+X (and no TT+2X). The presence of TT+2X (or X+Y) proves that both parents were TN+X. In exceptional cases they could have been TN and TN+X. The progeny of these five crosses were again sibcrossed in the next generation (52 crosses) with the aim of finding only TT+1 or 2 sex-chromosomes. Progenies with NN karyotypes were not maintained. Out of these 52 crosses 38 were analyzed cytologically and in five out of these we have found only TT+1 or 2 sex-chromosomes or TN+X (or TN) karyotypes and no NN types among the six tested larvae per progeny. In one completely fertile cross all the larvae (nine) were TN+X, which indicates that one parent was NN and the other TT+2X (or X+Y). Unfortunately no crosses between translocation homozygotes could be demonstrated. Theoretically in this translocation the chance of finding a cross between translocation homozygotes is approximately 1%, while in autosomal translocations (without complementation) this is appr. 6%. It is planned to continue with the progeny expected to be from crosses between a translocation homozygote and a TN+X karyotype. Finally we hope to end up with an unique strain of translocation homozygotes for this X-linked translocation. In earlier experiments we were able to isolate a homozygous stock, but unfortunately this was lost. Perhaps this stock when again homozygous will turn out to be one with unexpected advantages for genetic control of the onion fly. When not all the individuals carry the two extra sex-chromosomes there is the risk that the stock will backslide into a stock with only TT female individuals, which cannot persist.

In two more translocation stocks (T6 and T10) we found adult translocation homozygotes. In the case of T10 we know already that the TT karyotype is fertile because we have observed one cross (with a normal fertility) and all the larval offspring (12) analyzed was TN.

Little is known about the best way of infiltrating a noxious insect population with translocation flies. It is generally assumed that in order to introduce sterility into the population a single translocation stock is not sufficient. Normally the reproductive capacity of an insect population can be enormous and therefore the use of one translocation, which normally has a moderate "semi"-sterility can not cut down the population density below an acceptable level. A moderate sterility on the other hand is essential for the rearing and homozygosing of the translocation. In the case a single translocation would be sufficient, most probably the release of translocation heterozygotes, synthesized after combining a NN and TT strain, would be advisable rather than the release of translocation homozygotes. The advantages of releasing only heterozygotes are: sterility occurs at once and possibly there may be heterosis of the heterozygotes. If homozygotes are released, sterility does not occur before the next generation and these may be less viable due to inbreeding. With both methods it must be further investigated if only males or both sexes have to be released and how many releases are necessary each generation. The highest sterility will be obtained with a maximum number of translocation heterozygotes in the population or when the frequency of N- and T-gametes is at the equilibrium of 0.5 (Curtis and Hill, 1971). However, a Hardy-Weinberg ratio can not be obtained mainly due to the complementation of "adjacent" gametes. Further, due to the reproductive negative heterosis of the structural heterozygotes the equilibrium is unstable. If the 1:1 ratio of normal to translocation gametes is slightly changed and the TT karyotypes are as fit as the NN types a rapid shift (frequency dependent selection) will occur either in the direction of a complete TT or NN (original) population and the fertility will increase concurrently (Li, 1955). To counterbalance this one must be very keen on translocation heterozygotes with a superior competitiveness in order to maintain the sterility in the population as long as possible. In the case the population becomes homozygous (TT) this is a way to change the population by incorporation of a particularly useful gene linked to the translocation.

The release of double translocation heterozygotes as suggested e.g. by Curtis and Robinson (1971) gives the opportunity of a considerably increased sterility compared to the use of single translocations. Further population genetic studies and simulation studies are needed. The future application of the translocation method to achieve population suppression will depend on cooperative efforts among geneticists, entomologists and ecologists.

duator in the selection for "semi"-sterility.

Adult fertile translocation trisomics and adult sterile tertiary trisomics were obtained (both sexes) after meiotic numerical non-disjunction. In translocation trisomic males the X-, Y- and translocated (extra) X-chromosome were shown to disjoin at random. In females the two normal X-chromosomes almost (95%) preferentially disjoin, while the translocated X-chromosome goes to either one of the poles. Primary trisomic males (XXY) and females (XXX) were obtained from testcrossed translocation trisomic parents. XXY males produced four types of gametes XY, X, Y and XX in equal numbers. XXX females only gave XX and X gametes in an equal number. Succesfull attempts to obtain homozygotes for this X-linked translocation are reported. The theoretical background of genetic insect control is discussed.

Samenvatting

Het beschreven onderzoek, gepubliceerd in vijf artikelen (I-V), had tot doel het isoleren van structurele chromosoom mutaties die "semi"-steriliteit veroorzaken waardoor zij voor de genetische bestrijding van de uievlieg, *Hylemya antiqua* (Meigen) gebruikt kunnen worden. In de eerste drie artikelen worden de resultaten gegeven van het onderzoek naar de inductie met behulp van straling, en de selectie, isolatie en cytologische analyse van "semi"-steriele translocaties en inversies. X-stralen en snelle neutronen werden gebruikt in verschillende doses bij mannelijke en vrouwelijke vliegen (of poppen) van verschillende leeftijden. Het eerste artikel (I) beschrijft o.a. de wijze van bestralen, het testen van de fertiliteit (ei-uitkomst) in de bestraalde- (P) en terugkruisings- (F_1) generatie, de criteria voor "semi"-steriliteit en cytologisch onderzoek van "semi"-steriele stammetjes. In het tweede (II) en derde (III) artikel wordt ingegaan op de resultaten van bestraling bij hogere zowel als lagere doses dan gebruikt in het begin-onderzoek (I). Tevens werd de leeftijd der te bestralen vliegen als variabele ingevoerd.

Er kon vastgesteld worden (I + II) dat het gebruik van hogere doses X-stralen (1.5 krad) op mannetjes relatief veel translocaties maar ook veel complexe chromosoom mutaties oplevert. Uit het oogpunt van het risico van genetische achtergrondschade (b.v. recessief (sub-) lethale factoren) is het gebruik van hogere doses af te raden. Een lage dosis van 0.5 krad (X-stralen op mannetjes) lijkt het meest aanbevelenswaard in dit stadium van onderzoek (II + III). Er moet echter relatief veel screening op "semi"-steriliteit in de F_1 uitgevoerd worden om toch nog een aantal translocaties en inversies te kunnen isoleren. Vrouwtjes die 1 dag oud zijn, zijn zeer gevoelig voor bestraling (1.0 krad X-stralen). Zeven dagen oude vrouwtjes (1.0 krad X) zijn zowel wat de fecunditeit (P en F_1) als wat de F_1 reproductie betreft gunstiger (II), maar in vergelijking met mannelijke vliegen (1.0 krad X) is de opbrengst aan structurele mutaties te laag. Snelle neutronen op mannetjes geven bij 0.25 krad minstens zulke goede resultaten als bij 1.0 krad gezien het percentage gevonden translocaties. Verder onderzoek naar het gebruik van neutronen is nodig.

De door ons ontworpen broedtechniek der eieren, gevolgd door het scoren van bruine eieren (laat-embryonaal lethaliteit als gevolg van duplicatie/deficiency karyotypen), bleek goed te functioneren in combinatie met de cytologische analyse van nakomelingschappen van "semi"-steriele ouders. In totaal werden 17 verschillende translocaties en twee pericentrische inversies gevonden. Bij vijf van de acht reciproke translocaties die onderworpen werden aan een sibcross programma bleken translocatie homozygoten in het larvale stadium voor te komen en in drie gevallen van deze vijf zelfs in het imaginale stadium. Twee van deze drie bleken als homozygoot reproductief te zijn. Verder was het opvallend dat slechts bij de vijf genoemde translocaties in de nakomelingschap (larven) van toetskruisingen duplicatie/deficiency karyotypen afkomstig van "adjacent 1" oriëntaties optraden. Een reden hiervoor is niet bekend. Van de uit "adjacent 1" ontstane duplicatie/deficiency karyotypen (twee typen) weten we dat vooral de typen die een grote duplicatie en een geringe deficiency bezitten tot ver in het larvale stadium levensvatbaar zijn.

De meeste "breukpunten" bleken op de 6 (van de 11) langste chromosoomarmen te liggen. Géén preferentiële deelname van bepaalde chromosoomarmen aan reciproke translocaties werd waargenomen. Uit toetskruisingen van inversie heterozygote ouders bleek dat alleen vrouwtjes "semi"-steriel zijn vanwege het voorkomen van chiasma(ta) in de inversielus van de pericentrische inversie. Inversie heterozygote mannetjes waren even fertiel als de controle mannetjes, hetgeen het achiasmatisch zijn van de mannetjes onderstreept.

De laatste twee artikelen (IV en V) bevatten de studies van de meiotische disjunctie en de embryonaal lethaliteit van een X-gekoppelde translocatie en van de daarvan verkregen trisome karyotypen. Deze studies zijn van belang voor de homozygotering van deze translocatie. Er kon overtuigend aangetoond worden dat de kleine acrocentrische chromosomen de geslachtschromosomen zijn. Van de translocatie heterozygote karyotypen (IV) werd het gedrag van de chromosomen van het complex tijdens de meiose geanalyseerd aan de hand van M II (mannetjes) en jonge eieren (mannetjes en vrouwtjes). In beide geslachten treedt even vaak de "alternate" als de "adjacent 1" oriëntatie op, terwijl numerieke non-disjunctie van het kleinste chromosoom van het translocatiecomplex vaak bij vrouwtjes (18.7%) en weinig bij mannetjes (2%) geconstateerd werd. Vermoedelijk is verstoorde telomeer paring, waardoor verminderde chiasmavorming optreedt, de oorzaak voor de hoge frequentie numerieke non-disjunctie bij vrouwtjes. Bij de mannetjes wordt zeer waarschijnlijk het meiotisch gedrag der chromosomen door de homologe centromeren bepaald. Cytologische analyse van jonge eieren (8-16 uur) van toetskruisingen van translocatie heterozygote

vrouwtjes wees uit dat ongeveer 20% een grote deficiëntie bezit, hetgeen redelijk goed overeenkomt met de gevonden "semi"-steriliteit (laat-embryonale lethaliteit, bruine eieren).

Als gevolg van de numerieke non-disjunctie bij translocatie heterozygote vrouwtjes werden translocatie- en tertiaire trisomen (voor het X-chromosoom) verkregen (V). De tertiaire trisomen, waarbij een groot chromosoom extra voorkomt, bleken niet reproductief te zijn hoewel de meiose (bij mannetjes) nog wel bestudeerd kon worden. De meiotische disjunctie in "semi"-steriele translocatie trisome mannetjes en vrouwtjes, waarbij slechts een klein X-chromosoom extra voorkomt, werd (bij de vrouwtjes indirect via analyse van jonge eieren) bestudeerd. Evenals bij de translocatie heterozygote karyotypen werd géén "adjacent II" gevonden. In translocatie trisome mannetjes blijken de twee normale geslachtschromosomen (X en Y) en het getransloceerde (extra) X-chromosoom volledig volgens het toeval te segregeren. Dit werd ook in tertiaire trisome mannetjes (M II) gevonden. In translocatie-trisome vrouwtjes, waarin naast twee normale X-chromosomen een getransloceerd X-chromosoom aanwezig is, blijken de twee normale X-chromosomen in 95% van de gevallen elk naar één van de twee polen te gaan. In 5% van de gevallen gaan beide normale X-chromosomen naar één pool, terwijl het getransloceerde X-chromosoom naar de andere pool gaat. Het percentage "semi"-steriliteit van toetskruisingen blijkt redelijk goed overeen te komen met het percentage eieren met een grote chromosomale deficiency.

Primaire trisome karyotypen (voor het X-chromosoom) werden gevonden na toetskruising van translocatie trisome karyotypen. De fertiliteit is gelijk aan die van de controle. De XXY mannetjes leveren vier typen gameten: XY, X, Y en XX in een verhouding van 1:1:1:1. XXX vrouwtjes leveren twee typen gameten: XX en X (1:1).

Tot slot is in de algemene discussie het verloop van de homozygotering van de X-gekoppelde translocatie besproken en wordt kort ingegaan op de theoretische achtergrond van de genetische bestrijding.

Curriculum vitae

Cornelis van Heemert
Geboren: 28 april 1944 te Leeuwarden

Eindexamen H.B.S.-B 1962 te Groningen
Landbouwhogeschool Wageningen van september 1962 - september 1970
Studierichting (Hoofdvak): Plantenveredeling
Keuzevakken: Erfelijkheidsleer (verzwaard)
Algemene Plantenziektekunde

In dienst van de Centrale Organisatie TNO, Sectie Landbouwkundig Onderzoek CO-TNO, Den Haag, van 15 juni 1970 - 31 december 1974.
In deze periode als gastmedewerker verbonden aan het laboratorium voor Erfelijkheidsleer van de Landbouwhogeschool, Wageningen.
Vanaf 1 januari 1975 verbonden aan het I.T.A.L. te Wageningen.

Ik kan alleen nog regels schrijven
die net als jullie
vliegen blijven.

(Judith Herzberg uit: *Vliegen*)