Theoretical and Applied Genetics 45, 349–354 (1975)

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 application in the 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 day-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. Two homozygous translocations are described. Combining 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 genetic control experiments on 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 and are presented in this report. When males were irradiated with 1.0 krad of X-rays, this dose appeared to be 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, because the latter 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 parental fertility.

As described in the previous paper, selection for semi-sterility was carried out in the F_1 generation (see Materials and Methods). Crosses with an egg hatch mainly between 60% and 30% were used for cytological analysis. This category of semi-sterile F_1 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). 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 described 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 inbreeding avoided. 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 mass mating. F_1 flies were individually testcrossed in the first backcross (B_1). In this paper, as 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 easy discrimination of brown eggs (late embryonic lethals) from the unfertilized and the hatched (empty) eggs (van Heemert, 1973).

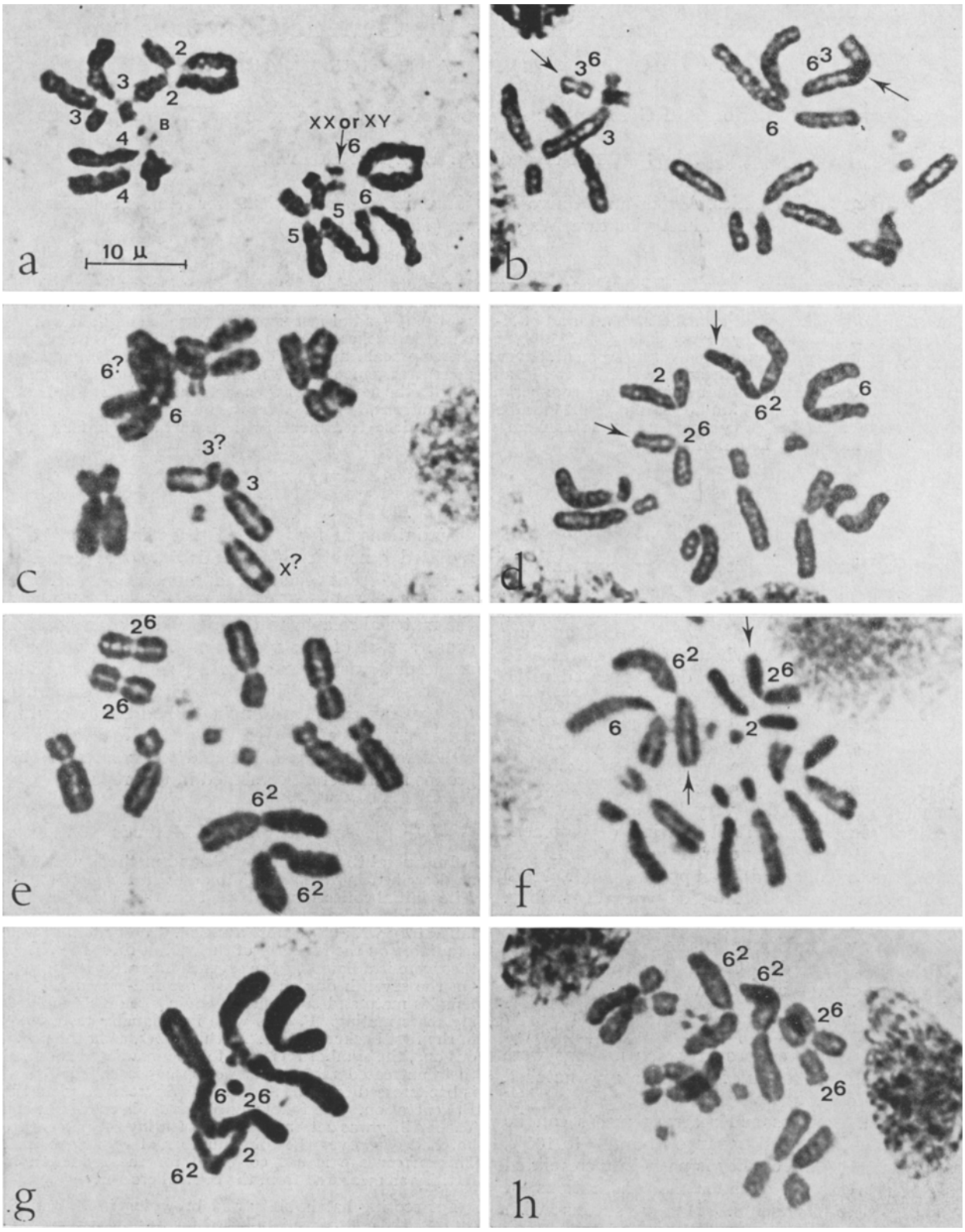

Fig. 1. Photomicrographs of normal and translocated karyotypes of the onion fly (*Hylemya antiqua*)

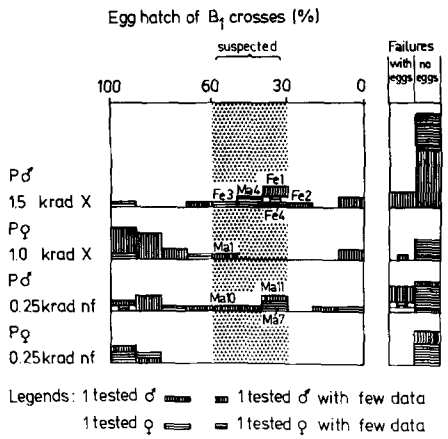

Fig. 2. Diagrammatic summary of B_1 data. The marked area covers the region between 60% and 30% E. H. suspected of carrying chromosomal rearrangements which are checked cytologically (compare table 1)

The percentage of brown eggs versus empty eggs $\left(\frac{B}{B+E} \times 100\right)$ 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 testcrossed individually in a second backcross (B_2) to see if any sex-linkage is present. The fertility in the control was generally about 85% but in a few cases a rather low fertility was found. For cytologic analysis testes were preferred, since, primarily because of 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 also useful for cytology, although difficulties in analysis may arise when the exchanged segments are 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 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, the percentage of brown eggs is usually below 10%. Semi-sterility in the B_1 and the B_2 is correlated mostly 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 rearrengement is given in the table.

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 could be carried out. Fig. 1 a shows the normal karyotype. 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: $3^{l(-)}-6^{s(+)}$. Fig. 1 b shows a mitotic metaphase in a larval brain cell of a heterozygote for this translocation. Among larvae from B_2 crosses of Fe 2, in one case out of six a rearrangement between $3^{l(-)}$ and $6^{l(+)}$ 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. 1 c) 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 preparations showed that the interchanged segments of this translocation (4^l-6^l) were of equal length. After irradiating seven day old females with X-rays, one translocation was found (Ma 1) between chromosomes 2 and 6: $2^{l(-)}-6^{s(+)}$, fig. 1 d. 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 two 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 also of males treated with fast neutrons (0.25 krad), only for Ma 7 was a rearrangement ($2^{l(-)}-6^{s(+)}$) found (figs. 1 f and 1 g) in the testes of the male progeny. Data on sibcrosses involving translocations Ma 1 and Ma 7 will be discussed below. No individuals suspected of having a rearrangement were found in the progeny of females irradiated with fast neutrons (Table 1).

In fig. 2 we have presented the data in a slightly different way, as presented in fig. 1 of the previous

a) Normal karyotype. Spermatogonial metaphase. 2n = 12 + B. The chromosome designations have been indicated beside 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 σ. Crossfigure;

h) Ma 7. Translocation homozygote. Larval brain cell. Mitotic metaphase.

The arrows indicate the translocated chromosome arms

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					B_2 cross					
Dose in krad	sex	E. H.	tested on fertility	tested cytologically				tested cytologically				Structural mutations*	Comments
				B_1 code	sex	E. H.	B. E.	B_2 code	sex	E. H.	B. E.		
1.5 X-rays	♂	4	33										
				Fe 1	♂	31	43	B_2 a	♂	47	49	3/6	$3^{l(-)} - 6^{s(+)}$
								B_2 b	♂	49	42	3/6	
								B_2 c	♀	57	31	3/6	
				Fe 2	♂	25	60	B_2	♀	32	15	1/6	$3^{l(-)} - 6^{l(+)}$
				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	$4^l - 6^l$
								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	$2^{l(-)} - 6^{s(+)}$
0.25 fast	♂	10	25	Ma 7	♀	38	43	B_2 a	♀	65	26	4/12	$2^{l(-)} - 6^{s(+)}$
neutrons								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	$3^l - 6^l$
0.25 fast neutrons	♀	8	12										
control		85											

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)

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 treatments 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 outside 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, a B_1 cross with 57% egg hatch had a translocation (Ma 1). There is about equal 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.

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 was considerably higher (70%). Only twenty percent of these are 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 produced progeny. Although the picture has changed in favour of the class with a normal fertility (> 75% E. H.), still 19% are suspected.

In general it can be stated that for the irradiation of males with X-rays a decrease in dose will considerably enlarge the percentage of B_1 crosses with a normal fertility, while 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

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				B_1 cross								
radiation		sex	report	total number tested	number and percent with progeny		60% −30% E. H.		Cytogenetic analysis***		Failures	
dose (krad)	type				n	%	n	%**	n	rearr.	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

effects. The high percentage of failures compared with 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 seems to give even 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 to the induction of mutations. Although many failures (63%) were observed, the percentage of suspected strains was not low (31%). However, the cytological analysis of four strains was negative. For the treated seven day-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 was essentially better than that of females irradiated on the first day. Nevertheless egg production was 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.

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

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, rearrangements are scored in B_1 crosses with severely reduced fertility and/or a high percentage of late embryonic lethals. Because of 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 is 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 at least 6 individuals were taken for analysis wherever possible to obtain about 98% probability of finding at least one translocation carrier. In addition we may have overlooked translocations with a very small or symmetrical exchange.

With translocations Ma 1 and Ma 7 (both between 2^L and 6^S) we have started a sibcross programme to isolate homozygous flies. These two translocations are very similar in the segments exchanged. We have obtained a few homozygous translocation flies in the adult stage (fig. 1e) in the Ma 1 stock, but only in the larval stage in Ma 7 (fig. 1h). The fertility of both is quite high in backcrosses and consequently the fertility of a T/+ × T/+ cross is still high enough to obtain sufficient offspring for the isolation of translocation of homozygotes. The work on the isolation of Ma 1 and Ma 7 will be continued.

It is striking that, as in 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 equally frequently. The long arm of chromosome 3 is involved quite often as well. As 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 et 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 seem similar to those following 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 semi-sterile strains obtained 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 be simply 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 low fertility of the translocation heterozygote, but normal competitiveness, can be obtained and isolated as the homozygote. One or more translocation stocks will then be released, singly or in combination, to establish the effect on the natural population. Since single translocations can only be made homozygous if the sterility is not too high ($< 40\%$), combinations of two or more stocks, each with moderate sterility, should be used to reach a sufficient sterility for genetic control.

Acknowledgements

We thank Dr. Ir. J. Sybenga for his critical reading and discussing of the manuscript. Dr. A. S. Robinson made some valuable suggestions. The technical assistance of Mr. J. Eikelenstam, Mr. K. Knoop and Mr. G. Schelling and the typing by Miss H. Boelema are also much appreciated.

Literature

Heemert, C. van: Isolation of a translocation homozygote in the onion fly *Hylemya antiqua* (Meigen) with a cytogenetic method in combination with the determination of the percentage late embryonic lethals. Genen en Phaenen **16**, 17–18 (1973).

Robinson, A. S., Heemert, C. van: Preliminary radiobiological studies on *Hylemya antiqua* (Meigen) and data on three radiation induced (0.5 krad) chromosomal rearrangements. Proc. Symp. Innsbruck, 1974. Vienna: I.A.E.A./F.A.O. 1975 (In press).

Searle, A. G., Ford, C. E., Evans, E. P., Beechey, C. U., Burtenshaw, M. D., Clegg, H. M.: The induction of translocations in mouse spermatozoa. I. Kinetics of dose response with acute X-irradiation. Mut. Res. **22**, 157–174 (1974).

Theunissen, J.: Radiation pathology in *Hylemya antiqua* (Meigen). In: „Sterility principle for insect control or eradication" (Proc. Symp. Athens, 1970) p. 329–340. Vienna: I.A.E.A./F.A.O. 1971.

Wijnands-Stäb, K. J. A., Heemert, C. van: Radiation induced semi-sterility for genetic control purposes in the onion fly *Hylemya antiqua* (Meigen). I. Isolation of semi-sterile stocks and their cytogenetical properties. Theor. Appl. Genetics **44**, 111–119 (1974).

Received August 28, 1974

Communicated by H. Stubbe

C. van Heemert and
K. J. A. Wijnands-Stäb
Institute for Atomic Sciences
in Agriculture
6 Keyenbergseweg
Wageningen (The Netherlands)