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Isolation of a Homozygous X-linked Translocation Stock with two Additional Sex-Chromosomes in the Onion Fly *Hylemya antiqua* Meigen

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Summary. The onion fly, *Hylemya antiqua* Meigen, was subjected to irradiation and selection based on observations of fertility and cytogenetics, in order to isolate structural chromosome mutations which might be used for genetic control of this species. To the present time, only a "simple" X-linked translocation could be obtained as a homozygous stock. Sibcrossing was carried out using translocation trisomics (TN + X) obtained from test-crossed translocation heterozygous females (TN) showing numerical nondisjunction. A homozygous stock was obtained with two additional sex-chromosomes. This is a unique case because normally an X-linked translocation can not be made homozygous in the male sex, which normally only carries one X-chromosome.

Introduction

Mankind is continually confronted with noxious insects, causing damage to agricultural crops and products, or functioning as vectors of diseases, especially in the tropics. Because of the adverse side-effects of chemicals, attempts are being made to develop biological (including genetical) was of controlling insect pests, by methods which cause less environmental pollution and which avoid the problem of insecticide resistance (Woods 1975). The onion fly, *Hylemya antiqua* Meigen, an important pest insect, was chosen in the Netherlands in 1965 as the "target" for a genetic control project. The sterile insect release method was given the main emphasis in the first few years and recently field releases of fully sterilized insects on a small scale (1 ha) appeared to be successful (Loosjes 1974). Since 1969 the induction, isolation and cytogenetic analysis of structural chromosome mutations in the onion fly has become part of the genetic control project (van Heemert 1975). The use of chromosomal translocations (and inversions (Robinson and van Heemert 1975, Robinson 1975)) has been considered in several other insect pests (Baker and Sakai 1974, Curtis 1971, Foster and Whitten 1974, van Heemert 1975, Laven et al. 1971, Lorimer et al. 1972, McDonald and Overland 1973a, Rai et al. 1974, Wijnands-Stäb and van Heemert 1974, van Zon and Overmeer 1972). Such chromosomal rearrangements could be applied in two different ways. Firstly, "semi-sterility", that is associated with translocation heterozygotes, could be used to depress the population fertility. "Semi-sterility" is caused by duplication-deficiency gametes originating from adjacent I or II orientations of the translocation multivalent during meiosis. Following fusion of such duplication-deficiency gametes with normal gametes, unbalanced karyotypes occur which usually die during the embryonic stage, thus decreasing egg hatch (van Heemert 1973). This partial sterility is conventionally referred to as "semi-sterility" but, in fact, most of the translocation stocks which we have isolated, had sterilities greater or less than 50%. The combination of different translocations can give much higher sterilities. Secondly, a translocation could be used as a genetic transporting mechanism. By linking a deleterious gene close to one of the translocation breakpoints, a population could be completely replaced by the modified population after release of a sufficient majority of this type. Both methods require the production of homozygous translocation strains, to reproduce the translocation in large numbers without the occurrence of "semi-sterility", and to produce heterozygotes in any desired proportion of the population to be controlled. However several authors have found that a certain degree of sterility still occurs in most homozygous translocation stocks probably due to inbreeding, position effects and deleterious genes linked to the translocation (LaChance et al. 1964, Lorimer et al. 1972, McDo-

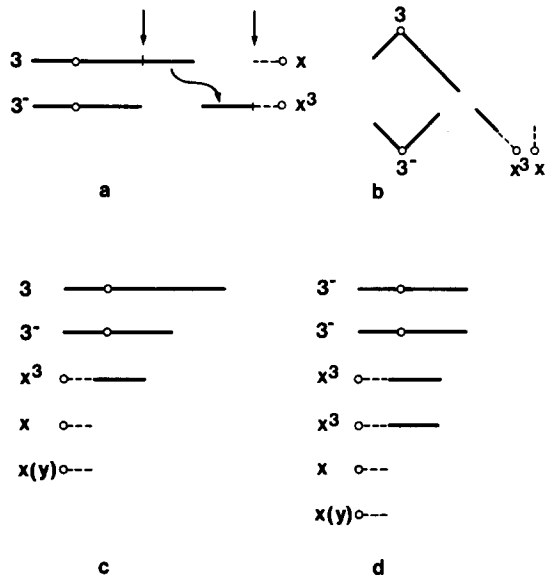

Fig. 1a) Diagram of the "simple" translocation between the X-chromosome and chromosome 3; b) Numerical non-disjunction (type I) in the translocation heterozygous female ($33^{-}X^{3}X$) (see van Heemert 1974a); c) Chromosomal formula for the TN + X karyotype; d) Chromosomal formula for a translocation homozygote with two extra sex-chromosomes

nald and Overland 1973a, Ross and Cochran 1975). Several attempts have been carried out in the onion fly to isolate translocation homozygous strains after sibmating of translocation heterozygotes. In five out of eight different translocation lines, translocation homozygotes were obtained as larvae, and in three out of these five as adults. One of these three, an X-linked translocation, was studied extensively (van Heemert 1973, 1974a, 1974b, 1975). Although this translocation is sex-linked we were able to isolate a homozygous stock (both sexes) in which the presence of two additional sex-chromosomes appeared to be the crucial point. Due to numerical nondisjunction, in translocation heterozygous females (TN) translocation trisomics (TN + X) were obtained which carried one extra X-chromosome. T stands for translocated genome and N for normal genome. These translocation trisomics allowed the isolation of translocation homozygous flies with two extra sex-chromosomes, as will be reported in this paper.

Materials and Methods

The translocation used in this work was induced by irradiating young adult males with 1.0 krad of X-rays (250/25 deep therapy apparatus, operating at 250 kVp and 15 mA, with a dose rate of 200 rad/min) (Wijnands-Stäb and van Heemert 1974). It was selected after backcrossing females and screening for "semi-sterility". The chromosomes involved in the translocation can be recognized easily at several developmental stages (eggs, larvae and male adults). A normal karyotype of the onion fly consists of 10 submetacentric autosomes and two small acrocentric sex-chromosomes (Fig. 1, A-D). In the translocation, about half of the long arm of chromosome 3 is attached to the most distal segment of the X-chromosome. Apparently none, or only a very thin piece, of the small sex-chromosome is translocated to chromosome 3 and only chain quadrivalents were observed in male meiotic prophase 1 (Fig. 2E). From earlier studies we concluded that it is most probably a simple (i.e. nonreciprocal) translocation (van Heemert 1974a). It was proved that the sex-chromosome involved in the translocation is the X-chromosome because, in testcrosses of translocation heterozygous males, only normal sons and translocation heterozygous daughters occur, whereas heterozygous females produce both normal and translocation sons and daughters. The karyotype of TN (♀) may therefore be symbolized $33^{-}X^{3}X$. Figure 1a shows diagrammatically how this translocation originated after irradiation. Further investigations showed the presence of translocation trisomics (TN + X) which posses one additional X-chromosome compared to translocation heterozygotes (TN) (van Heemert 1974b). Figure 1b shows how numerical non-disjunction in TN ($33^{-}X^{3}X$) females can give rise to two unbalanced gametes. One carries an additional X-chromosome $3^{-}X^{3}X$, the other only has one normal chromosome 3 and no sex-chromosome. Fusion of a 3X gamete from the father with a $3^{-}X^{3}X$ gamete will give a ($33^{-}X^{3}XX$) = TN + X zygote (Fig. 1c). In females numerical non-disjunction can be observed regularly (19 %), while in males it seldom occurs (2 %) (van Heemert 1974a).

The flies were reared at 23°C, appr. 70 % r.h. and 16 hours of light per day. As the onion fly hardly mates in single pairs (appr. 10 % success) we mass-mated the flies and separated individual females in small cages (females only mate once) when oviposition had started. Eggs were incubated for three days at 29°C and nearly 100 % r.h. in an oven. To measure sterility, eggs were classified as white (unfertilized), empty (hatched larvae) and brown (late embryonic lethals), using a stereomicroscope (12 ×). The sterility was defined as the percentage of brown eggs after the exclusion of white eggs from the total. As shown by van Heemert (1975) white eggs are unfertilized and their frequency is very low (3 %) in crosses involving translocated and non-translocated flies. The percentage of brown eggs in the control is approximately 3 %. Both testcrossed TN and TN + X males and females had an average sterility of about 25-35 %. Cytological observations were made on eggs (8-16 hours), larvae (5-10 days), young males (1 day) and sometimes females (1 day). No special fixation of the tissues was needed after dissecting these tissues in Levy's saline solution. Larval brains were put in lactic acetic orcein directly after dissection. Testes and ovaries were first put in a hypotonic solution for 5 or 10 minutes, respectively, to obtain a swollen tissue before staining. Squashing was carried out after half an hour in 45 % acetic acid. Cytological analysis was usually carried out immediately. Photographs (Figs. 2A-L) were made from temporary preparations with a Zeiss photomicroscope using a high contrast Agfa-Gevaert ortho negative film (12 DIN) or an 11 ford pan film (18 DIN).

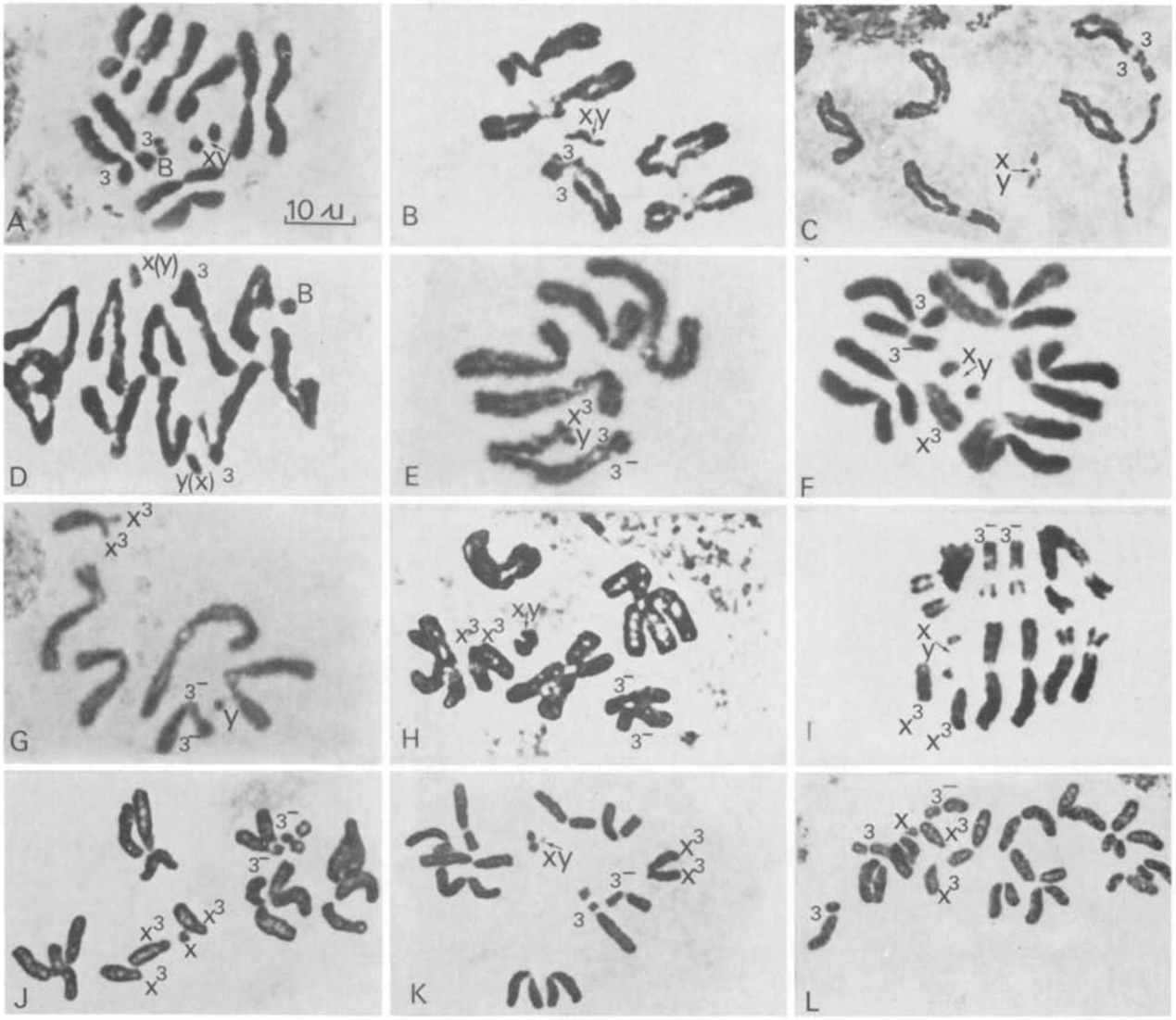

Fig. 2. A-D show normal (33XY) karyotypes of the onion fly (2n=12). A- spermatogonial metaphase; B - Diakinesis/prometaphase in the male; C - Mitotic prophase (late) in larval braincell 33XY (or 33XX); D - AI in the male; E - TN ($33^{-}X^{3}Y$) male diakinesis/prometaphase; F - TN + X ($33^{-}X^{3}XY$) male spermatogonial metaphase; G - TT + Y ($3^{-}3^{-}X^{3}X^{3}Y$) male, diakinesis/prometaphase; H— TT + 2X(X + Y), mitotic metaphase in larval braincell; I - TT + X + Y male, spermatogonial metaphase; J - TT + X^{3} + X(Y) larva, mitotic metaphase; K - TN + X^{3} + X(Y) larva, mitotic metaphase; L - Karyotype of triploid larva ($333^{-}X^{3}X^{3}X$). Note somatic pairing in all mitotic divisions. N.B. X(Y) indicates X and Y can not be discriminated cytologically

Results

In Table 1 the results of testcrosses of TN and TN + X females are compared. As shown in Tab. 1a, from 4 different meiotic orientation types in TN females, 8 different embryonic karyotypes can be produced and the frequencies have been published earlier (van Heemert 1974a). Only six of these appeared to be viable as larvae and adults. The $33^{-}XX$ and $33^{-}X$ types die at a late embryonic stage (brown eggs) because they lack a large piece of chromosome 3. These inviable brown eggs lead to the sterility which we measured by scoring the egg hatch. Table 1b shows the segregation in TN + X females. The presence of a primary trisomic NN + X (33XXX) type in the progeny indicates a TN + X parent, while a monosomic 33X karyotype can only be scored in the progenies of TN females

Table 1. Segregating pattern in testcrosses of translocation heterozygous (TN) females shown in Tab. 1a and translocation trisomic (TN+X) females shown in Table 1b. 1c. Comparison of the numbers of the different larval karyotypes observed in the progenies of both types of testcrosses

a)	TN ♀ ($3\bar{3}X^3X$) / NN ♂	alternate (40,65 %) $3\bar{3}X^3$	3X	adjacent I (40,65 %) $3\bar{3}X$	$3X^3$	nondisjunction I (9,35 %) $3\bar{3}X^3X$	3	nondisjunction II (9,35 %) $3\bar{3}$	$3X^3X$	(4 orientation types and percentages)
	3X	$33\bar{3}X^3X$ TN	33XX NN	$33\bar{3}XX$ †	$33X^3X$ $33X^3X$	$33\bar{3}X^3XX$ TN+X	33X 33X	$33\bar{3}X$ †	$33X^3XX$ $NN+X^3$	
b)	TN+X ♀ ($3\bar{3}X^3XX$) / NN ♂	1 (2,8 %) $3\bar{3}X^3$	3XX	2 (47,2 %) $3\bar{3}X^3X$	3X	3 (2,8 %) $3\bar{3}XX$	$3X^3$	4 (47,2 %) $3\bar{3}X$	$3X^3X$	(4 orientation types and percentages)
	3X	$33\bar{3}X^3X$ TN	33XXX NN+X	$33\bar{3}X^3XX$ TN+X	33XX NN	$33\bar{3}XXX$ +	$33X^3X$ $33X^3X$	$33\bar{3}XX$ †	$33X^3XX$ $NN+X^3$	

c) ♀ parents	NN	TN	TN+X	$NN+X^3$	NN+X*	$33X^3X$	33X*	
TN ♀♀ (n=5)	29	26	9	2	0	17	6	N=89
TN+X ♀♀ (N=11)	21	1	28	12	1	1	0	N=64

† Karyotypes which cause death in the late embryonic stage and lead to brown eggs.

* NN+X, a primary trisomic for the sex-chromosome, can occur only in the progenies of TN+X mothers, while 33X (or 33Y), a monosomic karyotype only can occur in the progenies of TN mothers.

Note: Results are shown only with respect to female determining (3X) sperm; for each female zygote shown a corresponding male exists, e.g. $33\bar{3}X^3XY$(♂) corresponds to $33\bar{3}X^3XX$(♀).

and not in those of a TN + X female. Appearance of these two types can thus be used as a criterion for scoring the parents, which can not be done directly cytologically without sacrificing them before reproduction. In the progenies of TN females NN, TN and $33X^3X$ types predominate and there are only a few TN + X and NN + X^3. In the case of TN + X females NN, TN + X and NN + X^3 are predominant and there are only a few of the other types. Both have about the same frequency of NN karyotypes among their progenies, and therefore the frequencies of TN and $33X^3X$ or TN + X and NN + X^3 give a good classification. In Table 1c the distributions of the different larval karyotypes among the progenies of testcrossed TN and TN + X females are compared and it is evident that there is a great difference in the two distributions (van Heemert 1974a).

We decided to continue with the progenies of testcrossed TN + X females in order to produce a large proportion of TN + X in the following generation. Sibcrosses with flies of these progenies in fact will give an opportunity of finding translocation homozygotes containing two additional sex-chromosomes (Figs. 2, H and I) which would be rare after sibcrossing the progenies of TN females. This approach is an appropriate one to obtain a translocation stock homozygous in both sexes with two extra sex-chromosomes (Fig. Id). A "stock" which had no extra sex-chromosomes would contain only TT ($3\bar{3}\bar{3}X^3X^3$) individuals which are females, due to the absence of the Y-chromosome, and therefore the stock could not be self-propagating.

Table 2 shows the zygotes combining all 12 gametic types produced by TN + X fathers and all 8 types produced by TN + X mothers. The expected frequen-

Table 2

† Karyotype dies at a late embryonic stage
* Karyotypes as produced in a testcrossed TN+X ♀ (see Table 1b)
‡ Karyotypes as produced in a testcrossed TN+X ♂
0 Karyotypes (TT+X+Y and TT+2X) which were selected
†† Karyotypes duplicated for two X^3-chromosomes and/or deficient for one or two normal sex-chromosomes

Note: When no name or symbol has been indicated, no suitable name could be given e.g. $33\bar{3}X^3X^3$

Table 2. Gametic combinations following mating of TN+X($33^{-}X^{3}XY$) males and TN+X($33^{-}X^{3}XX$) females. The gamete frequencies were obtained after cytological analysis of eggs from testcrosses (see van Heemert 1974b)

TN+X^3 ♀ / TN+X^3 ♂	0.014	0.014	0.236	0.236	0.014	0.014	0.236	0.236	
	-3		-3		-	3	-	3	
	3X	3XX	3XX	3X	3XX	3X	3X	3XX	
-3	--33	-3	--33	-3	--3	-33	--3	-33	
3X	33XX	33XXX	33XXX	33XX	33XXX	33XX	33XX	33XXX	
								3	
0.083	TT	TN+X	TT+X	TN	†		†	TN+X	
	-3		-3		-	3	-	3	
3XY	33XXY	33XXXY	33XXXY	33XXY	33XXXY	33XXY	33XXY	33XXY	
						3		3	
0.083	TN+X	NN+2X	TN+2X	NN+X	†	NN+X	†	NN+X+X	
-3	--33	-3	--33	-3	--3	-33	--3	-33	
3XY	33XXY	33XXXY	33XXXY	33XXY	33XXXY	33XXY	33XXY	33XXXY	
						3		3	
0.083	TT+Y	TN+2X	(TT+X+Y)	TN+X	†	TN+X	†	TN+X+X	
	-3		-3		-	3	-	3	
3X	33XX	33XXX	33XXX	33XX	33XXX	33XX	33XX	33XXX	*
								3	
0.083	TN	NN+X	TN+X	NN	†		†	NN+X	
-3	--33	-3	--33	-3	--3	-33	--3	-33	
3XX	33XXX	33XXXX	33XXXX	33XXX	33XXXX	33XXX	33XXX	33XXXX	
						3		3	
0.083	TT+X	TN+2X	(TT+2X)	TN+X	†	TN+X	†	TN+X+X	
	-3		-3		-	3	-	3	
3Y	33XY	33XXY	33XXY	33XY	33XXY	33XY	33XY	33XXY	*
								3	
0.083	TN	NN+X	TN+X	NN	†		†	NN+X	
-	--3	-	--3	-	--	-3	--	-3	
3XY	33XXY	33XXXY	33XXXY	33XXY	33XXXY	33XXY	33XXY	33XXXY	
0.083	†	†	†	†	†	TN+X	†	TN+2X	
3	-33	3	-33	3	-3	33	-3	33	
3X	33XX	33XXX	33XXX	33XX	33XXX	33XX	33XX	33XXX	
		3	3						
0.083		NN+X	TN+X		TN+X	††	TN	††	
-	--3	-	--3	-	--	-3	--	-3	
3Y	33XY	33XXY	33XXY	33XY	33XXY	33XY	33XY	33XXY	
0.083	†	†	†	†	†	TN	†	TN+X	
3	-33	3	-33	3	-3	33	-3	33	
3XX	33XXX	33XXXX	33XXXX	33XXX	33XXXX	33XXX	33XXX	33XXXX	
	3	3	3	3					
0.083	TN+X	NN+X+X	TN+X+X	NN+X	TN+2X	††	TN+X	††	
-	--3	-	--3	-	--	-3	--	-3	
3X	33XX	33XXX	33XXX	33XX	33XXX	33XX	33XX	33XXX	
0.083	†	†	†	†	†	TN	†	TN+X	
3	-33	3	-33	3	-3	33	-3	33	
3XY	33XXY	33XXXY	33XXXY	33XXY	33XXXY	33XXY	33XXY	33XXXY	
	3	3	3	3					
0.083	TN+X	NN+X+X	TN+X+X	NN+X	TN+2X	††	TN+X	††	

‡

Table 3. Distribution of larval karyotypes (N=59) as observed cytologically among progenies (8) of (TN+X)×(TN+X) crosses and the comparison with the distributions as expected from Table 2)

	TT	TT+X	TT+2X	TN	TN+X	TN+2X	TN+X^3	TN+X^3+X	
no. obs. (%)	0 (0)	6 (10.2)	6 (10.2)	4 (6.8)	18 (30.5)	4 (6.8)	2 (3.4)	4 (6.8)	
% exp. *	0.1	3.5	6.4	7.1	26.4	7.1	7.1	12.8	
+	0.1	7.0	12.8	6.3	23.5	6.3	6.3	11.4	
‡	0.1	3.2	5.8	6.4	23.7	6.4	6.4	11.6	
	$33^-X^3X^3$	NN	NN+X	NN+2X	NN+X^3	NN+X^3+X	$33X^3X$	††	% ster.
no. obs. (%)	1 (1.7)	6 (10.2)	2 (3.4)	0 (0)	6 (10.2)	0 (0)	0 (0)	0 (0)	37.3
% exp. *	0.3	6.4	3.5	0.2	13.2	3.5	3.5	0	37.8
+	0.3	5.7	3.1	0.2	11.7	3.1	3.1	0	37.8
‡	0.3	5.8	3.2	0.1	11.8	3.2	3.2	9.1	31.5

†† Three different karyotypes with large duplications pooled in one group (see †† Table 2)
* Expected distribution if the †† types die before egg hatching
\+ Proband correction for selection on TT+X and TT+2X
‡ Expected distribution if the †† types are viable as larvae.

Note: X and Y cannot be discriminated and therefore TT+X can also be TT+Y

cies are derived from segregation data (egg stage) as estimated from earlier testcrossing data (van Heemert 1974a and b). Twenty seven different karyotypes (X- and Y-chromosome not discriminated) can exist in early embryonic stages. Nine of these (†), carrying deficiencies, will die at a late embryonic stage (brown eggs). Of the remaining 18 karyotypes most will reach the larval stage, but, only 11 different larval karyotypes were observed (Table 3). This is probably due to the relatively small number of larvae (59) observed. Also, three of these 18 different larval karyotypes (††: $33X^3X^3$, $33X^3X^3X(Y)$ and $33X^3X^3XX(Y)$) possess large duplications and probably consequently will have a low larval viability, or cause lethality in the embryonic stage before hatching thus contributing to the brown egg class. In Table 3 the result of the cytological analysis of the larvae from eight crosses between two TN + X parents is presented. The presence of TT and one or two extra sex-chromosomes conclusively identifies such matings and distinguishes them from e.g. (TN + X) × NN crosses from which no homozygotes can be produced. Only five of these eight progenies were used for sibcrossing in the next generation (Table 4).

Figure 2 shows several photographs of karyotypes such as NN, TN, TN + X(Y), TT + 2X(X + Y), TT + X^3 + X(Y) and the triploid $333^-X^3X^3X(Y)$. The percentages of the different larval karyotypes observed are compared with the expected percentages as calculated on the basis of Table 2. The expected percentages were calculated in three ways after having excluded the deficient types (marker † in Table 2), e.g. TN + X^3 zygotes occur from six combinations (Table 2). By including the deficient zygotes, 4.4% ($2 \times 0.083 \times 0.236 + 4 \times 0.083 \times 0.014$) of all zygotes will be TN + X^3. By excluding the deficient zygotes (37.8%), which die in the embryonic stage, the expected distribution in the larval stage becomes 7.1%. In the first case (*) no correction was made. In the second case (+) a proband correction was calculated to improve the statistical comparison, because of the preferential selection of only those progenies in which TT + (1 or 2 sex chromosomes) types out of a sample of 6 larvae were found. In the third case (‡), without the proband correction, we assumed that the three different karyotypes which carry two large duplications for about half the length of the long arm of chromosome 3 (††: $33X^3X^3$, $33X^3X^3X(Y)$ and $33X^3X^3XX(Y)$) remain alive as larvae until the stage in which the larvae were examined cytologically.

The mean observed sterility in the eight TN + X × TN + X crosses was 37.3%. The sterility from the expected distributions was estimated as 37.8% and 31.5% for the * and ‡ cases respectively.

The observed distribution shows some resemblance to the uncorrected distribution (* Table 3), but statistically no significance could be established ($0.02 < p < 0.05$). However in the case of TT + X,

Table 4. Course of the selection procedure to obtain a translocation homozygous line with two additional sex-chromosomes

Generation	Type of cross	No. of cages with fecund female	Mean perc. sterility	Selection** for TT+	Selection** against NN+	No. of progenies* tested cytologically	No. of progenies selected	Mean % sterility of selected crosses	Mean % TT+ larvae among progeny
G_7	Test ♀♀	11	± 30	-	-	11 (6)	11	31.52	0
G_8	Sib 1	63	27.60	yes	no	29 (6)	‡ 5 (8)	‡ 34.19 (37.29)	‡ 19.44 (20.3)
G_9	Sib 2	52	35.98	yes	yes	34 (6)	5	35.07	41.38
G_{10}	Sib 3	39	38.77	yes	yes	33 (2)	15	36.49	46.67
G_{11}	Sib 4	59	33.40	yes	yes	29 (2)	20 ↓ 1	29.19 33.33	68.79 100.0
G_{12}	Sib 5	47					29	30.84	100.0
G_{13}	Sib 6	masscage	23.33						100.0

* The number of larvae per progeny analyzed cytologically is indicated in brackets
‡ The data from the total number of progenies which had karyotypes with TT+1 or 2 sex-chromosomes are in brackets (see text)
** TT+ means TT + 1 or 2 sex-chromosomes

TT + 2X, TN + X, NN and NN + X, more of each type was found than expected and fewer TN, TN + 2X, TN + X^3, TN + X^3 + X and NN + X^3 were found than expected. A few karyotypes which were expected were not observed, probably due to the insufficient total sample size (N = 59) of larvae examined for such a broad spectrum of different karyotypes. After the proband correction a significant resemblance was obtained ($0.5 < p < 0.9$). Assuming that the †† types are viable as larvae (‡) an equivalent fit to the uncorrected expectation (*) was obtained.

In Table 4 the course of the selection for translocation homozygosity is outlined. TN + X females (generation 7) were testcrossed and in the progenies (larvae) of the first sibcross generation (G_8) there was a selection for the presence of TT + 1 or 2 sex-chromosomes. G_8 is the 8th generation after induction of the translocation. In the second sibcross (G_9) between flies from the selected progenies of G_8 we selected for the presence of translocation homozygotes (TT)+1 or 2 sex-chromosomes and at the same time for the absence of normal karyotypes (NN) + 1 or 2 sex-chromosomes. In the next two generations (G_{10} and G_{11}) this procedure was repeated. Among twenty crosses in G_{11} one was between a TT + 2X(♀) and a TT + X + Y(♂), because all ten larvae analyzed appeared to be TT + 2 sex-chromosomes. From the progeny of this particular cross we have established a homozygous strain, with two extra sex-chromosomes. Unfortunately there is a small residual sterility of approximately 25%, the cause of which will be discussed below.

Discussion

Following the regime just described, a homozygous line for an X-linked translocation in the onion fly was obtained. In spite of the rather complex multiple combinations of gametes, as shown in Table 2, the results given in Tab. 3 show that the percentages of the observed larval karyotypes from (TN + X) × (TN + X) crosses are in rather good agreement with the expected percentages. In particular, the observed percentage (20.4) of individuals with the genotype TT + 1 or 2 sex-chromosomes, even from the relatively few larvae analysed, corresponds well with the expected percentage (19.8) after applying a proband correction. The percentage of TN + X karyotypes was higher than expected, perhaps due to some tangling with TT (+ 1 or 2 sex-chromosomes) types during the selection. No obvious explanation for the relative high percentage of NN karyotypes is available. The occurrence of a relatively small percentage of observed TN + X^3 and

TN + X^3 + X(Y) karyotypes is probably due to the long duplication which they possess causing a certain fraction of these types to die in the embryonic stage before hatching. This will also be the case for the types symbolized †† in Table 2 ($33X^3X^3$, $33X^3X^3X(Y)$ and $33X^3X^3XX(Y)$), which carry two large duplications for the long arm of chromosome 3. These types were indeed never observed in the larval stage. The expected sterility (37.8%) where the †† types do not hatch is somewhat higher than that observed (37.3%), but if the †† types hatch the expected sterility is consequently lower (31.5%). The percentages of the other karyotypes are in good agreement with the expectation, in spite of the relatively small number of larvae analyzed cytologically. The selection procedure took a few generations of sibcrossing before the tetrasomic TT stock could be isolated (Table 4). In the case of an autosomal translocation this relatively long route is probably not necessary, because only TT × TT crosses have to be isolated to obtain a disomic TT stock without extra chromosomes. Continued selection as carried out on the X-linked translocation line for TT + 1 or 2 sex-chromosome individuals and against NN is apparently effective. Although we were finally only able to select one particular progeny at the G_{11} generation, this method should have the advantage of yielding numerous crosses between TT + 1 or 2 sex-chromosome types to give a broader genetic base for further rearing. This suggests the possibility of producing several lines homozygous for the translocation, which could be crossed thus minimising inbreeding effects during further rearing.

As outlined in the introduction, eight different translocation stocks of the onion fly had previously been subjected to a sibcrossing programme and, in five of these, translocation homozygotes were obtained as larvae (van Heemert 1975, Robinson and van Heemert 1975). No stocks homozygous for these translocations could be isolated, although TT × NN crosses (or the reciprocals) were definitely found in three out of these five. In such a case the fertility of the cross was about normal and all the progeny larvae appeared to be TN. In several other organisms difficulties have been encountered during the isolation of translocation homozygotes. Ross and Cochran (1975), indicated that one translocation homozygote in the German Cockroach is lethal before it can hatch. Curtis et al. (1972) could not establish a homozygous translocation stock in *Glossina austeni* because of the inviability of female TT pupae. LaChance et al. (1964) came to the conclusion that the homozygous karyotype for a translocation of *Cochliomyia hominivorax* does not emerge. In the housefly McDonald and Overland (1973a) could only isolate 4 TT stocks out of 18 translocations. Sobels (1972) and Ytterborn (1970) showed that 62.2% and 66%, respectively, of II-III translocations in *Drosophila* are lethal in the homozygous condition. From the reviews of Rai and McDonald (1972) and Robinson (1976) it can be concluded that the success rate in making translocations homozygous in pest insects is rather poor. Several authors (van Heemert and Wijnands-Stäb 1975, Robinson and van Heemert 1975, Ytterborn 1970) have suggested lowering the radiation dose for the induction of chromosomal rearrangements in order to obtain fewer genetic side effects such as recessive lethals linked to the breakpoints. A disadvantage of this procedure would be the increased amount of initial screening work required due to the inverse relationship between the number of induced chromosomal rearrangements and the dose of radiation applied. In mice (Carter et al. 1955) and plants (Burnham 1964) viable homozygotes are relatively easy to isolate and to maintain. Jaylet (1971), isolated a fertile translocation homozygote in the amphibian *Pleurodelis waltlii*. The fertility (egg-hatch) of most viable homozygous translocation stocks in general is somewhat less than the control fertility, the reasons being similar to those causing inviability of TT types during rearing (Lorimer et al. 1972, McDonald and Overland 1973a, Rai et al. 1974).

The reduced egg hatch of our homozygous translocation stock may be partially explained in another way. As shown in Figs. 1d, 2H and 2I, 4 sex-chromosome centromeres are present in the TT + 2 sex-chromosome karyotypes ($3^-3^-X^3X^3XX(Y)$). In the homozygote these 4 centromeres of the sex-chromosomes will behave independently of the two centromeres of the 3^- chromosomes. At the time of meiotic disjunction, in either the male or the female, X^3X can disjoin from X^3X (or X^3Y). This is a normal disjunction and the genetic material is equally distributed. If X^3X^3 disjoins from XX a different situation occurs. When these gametes fuse with a balanced X^3X gamete, $3^-3^-X^3X^3X^3X$ (Fig. 2J) or $3^-3^-X^3XXX$ zygotes are produced. Both

types are unbalanced. The first type has a large duplication and the other type a large deficiency and they cause post-embryonic lethality or egg sterility, respectively. Numerical nondisjunction occurs regularly in this translocation (van Heemert 1974a, 1974b). Unbalanced gametes carrying X^3X^3X, X, X^3XX or X^3 probably also occur and give duplicated or deficient karyotypes following fusion with an X^3X gamete from a TT + 2 ($3^-3^-X^3X^3XX$) sex-chromosome type.

McDonald and Rai (1971) suggested, on the basis of computer simulations, that the use of X-linked translocations offers a better opportunity for genetic control purposes than Y-linked translocations (Curtis 1975, Curtis and Hill 1971). The X-linked translocation described here has the great advantage over an ordinary X-linked translocation that it can be reared as a homozygous stock. It has the further advantage that, after release of homozygotes, a number of unbalanced karyotypes ($33^-X^3X^3$, $33X^3X$, $33X^3XX$, $33X$, $33^-X^3X^3X$ e.g.) occurs in the progeny of the F_1 generation which do not survive to the reproductive stage. However, a disadvantage would be the damage to the onion crop produced by these types because they are viable as larvae (damaging stage). Even triploid larvae can be observed cytologically (Fig.2L) which survive to the pupal stage. In the near future, population studies will be carried out to establish the competitiveness, longevity and other crucial biological parameters of this stock, to assess its suitability for the genetic control of the onion fly.

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