Genet. Res., Camb. (1969), **13**, *pp.* 289–301
With 2 text-figures
Printed in Great Britain

The production of partially sterile mutants in *Glossina austeni*

By C. F. CURTIS

Tsetse Research Laboratory, University of Bristol, School of Veterinary Science, Langford, Bristol, England

(*Received* 19 *December* 1968)

1. INTRODUCTION

Tsetse flies (*Glossina* spp.) are the vectors of African trypanosomiasis and are therefore of great medical and veterinary importance; they feed exclusively on blood and have larviparous reproduction (Buxton, 1955). Very little is known about their genetics, mainly because of the difficulty, which used to be experienced, in rearing them in captivity. This difficulty has now been largely overcome (see, for example, Jordan, Nash & Boyle, (1967) and Nash, Jordan & Boyle, (1968)).

Heterozygotes for chromosome translocations have often been found to be 'semi-sterile' because they produce a proportion of duplication-deficiency gametes (Burnham, 1962). Translocation homozygotes, if they are viable, are generally fully fertile. Serebrovsky (1940) suggested that the rearing of a population of a pest species homozygous for a translocation and release of these into the wild might provide an effective control method for the species because heterozygotes are expected to be produced, with a consequent reduction in population fertility, for a number of generations following the release. Theoretical comparisons of this procedure with the release of sterile males showed that, on certain assumptions, the translocation method might be the more efficient one for tsetse control (Curtis, 1968*b*; Curtis & Hill, 1968). It has also been suggested that a translocation might be used to manipulate the frequency of a desirable gene in a pest population (Curtis, 1968*a*).

This paper describes an attempt to produce translocations in *G. austeni*. No marker genes are available and cytogenetic studies on *Glossina* are only in their early stages (Itard, 1966). Therefore the method used was to test the progeny of irradiated individuals for the occurrence of inherited partial sterility. This is essentially the same as the method first used by Snell (1935) to identify translocation heterozygotes in mice.

2. MATERIALS AND METHODS

Origin of stock and maintenance and radiation methods

The insects used originated from the large random-bred colony of *G. austeni* maintained in this laboratory. The colony was founded by importing many thousands of pupae from Zanzibar. All cases in which wild-type controls and

matings to wild type are mentioned refer to individuals taken from this laboratory colony.

The flies were maintained at about 24·5 °C and 70–80 % relative humidity and were offered blood meals every day except Sundays. The types of cage used were as follows:

'Geigy 10' (640 c.c.): could accommodate up to ten flies which were offered food by strapping the cage to a rabbit's ear for 15 min. (Jordan *et al.* 1967).

'Geigy 1' (74 c.c.): could accommodate one fly which was coaxed to feed by applying the surface on which the fly was resting to a rabbit's ear (Nash, Jordan & Boyle, 1967).

After deposition the larvae dropped through the netting of the cage and pupated in the container below. Pupae were collected daily and buried in pots of sand; the adults emerged about 34 days later.

Virgin females were obtained by sexing the newly emerged adults daily. The females were mated on their third day of adult life and the sperm received was used throughout the reproductive life. Males were mated on or after their tenth day of adult life. For mating, one male and one female were confined together for 24 h.

Doses of ^{60}Co γ-radiation from a radiotherapy source were given to males on their ninth day of adult life. The dose rate was about 100 rads/min and during irradiation the flies were in conditions of unrestricted aeration. Each irradiated male was mated to a single untreated virgin female on the day following treatment. With this procedure the spermatozoa transferred to the female were mature at the time of irradiation.

Methods for testing fertility

The methods for testing fertility depend on the facts that, at the temperature used, the female fly ovulates one egg 7–14 days after eclosion and, provided the fly is well fed and no genetic lethal factors intervene, the fertilized egg develops into a larva which is deposited after about 9·4 days. The next ovulation occurs immediately after deposition so that after the first one, pupae are expected at regular intervals. The interval between one ovulation and the next will be referred to as a cycle. The early death of an embryo in the uterus reduces the length of the cycle to about 7·5 days (Saunders, 1960; Curtis, 1968*c*). Where a proportion of genetically lethal zygotes are produced, repeated failures to produce pupae at the normal intervals are expected.

For some of the tests the females were kept individually in Geigy 1 cages. For comparison 42 controls (wild-type females mated to wild-type males) were kept under the same conditions; one failed to produce any pupae and died young, but the remainder were kept for an aggregate of 248 cycles and pupae were produced at every cycle (Curtis, 1968*c*). Thus, among experimental flies, the occurrence of an unproductive cycle can almost certainly be attributed to genetic abnormality in the female or its mate. In such cases the female was retained and the existence of an abnormality was then generally confirmed by repeated failures to produce pupae at the normal intervals. When a female produced pupae at its first four cycles it was classified as fully fertile and discarded.

The fertility of most of the males was tested by mating each to four wild-type virgin females and confining these four mates together in a Geigy 10 cage. The males were mated on days 10, 12, 19 and 21 of adult life and in this way the cycles of their mates were approximately synchronized. The tests were generally completed within 60 days of the first mating and over this period of time the difference in the length of productive and unproductive cycles may be ignored and it may be

Table 1. *Fertility tests in Geigy* 10 *cages: results of controls and criteria used to distinguish fully fertile from partially sterile individuals*

Aggregate no. of cycles completed	Observed no. of pupae from mates of 26 control males	Nos. expected to be $\leqslant$ production by mates of 95 % of fully fertile males	Nos. expected to be $\geqslant$ production by mates of 95 % of semi-sterile males
6	4, 6, 6	4	5
7	—	5	5
8	6	6	6
9	—	6	6
10	—	7	7
11	—	8	8
12	10	9	8
13	12, 13, 13	10	9
14	13, 13	11	9
15	11, 13, 14, 14	11	10
16	14, 16	12	10
17	12	13	11
18	13, 16, 17, 17, 17, 17, 17, 17, 18	14	12

Total: 339 from 376 cycles
= 0·90 pupae/cycle

assumed that successive cycles have been completed in successive 9-day periods once the females had reached maturity. Thus 30 days after the first mating two of the females in a cage have completed two cycles each and two have completed one each, i.e. an aggregate of six. After further 9-day intervals the aggregate has risen to 10, 14 and 18 and the fertility of the females can be expressed in the form x pupae produced from y cycles. If a female died, the aggregate number of cycles had to be adjusted appropriately.

Table 1 shows the pupal production from the mates of twenty-six wild-type control males against the number of cycles completed by the time that the flies were discarded. Over-all, pupae were produced at 90 % of cycles and this value agrees roughly with a large amount of other data for wild types maintained under the same conditions except that there were ten flies per cage (Jordan & Curtis, 1968). The inferior performance of flies in Geigy 10 cages compared to those in Geigy 1 cages is presumably due to the inferior feeding conditions in the former. Assuming that the probability of any female failing to produce a pupa at any cycle is a constant during the test period, the number produced, after a given number of cycles, from a series of cages of the mates of males with normal full fertility, would be expected to form a binomial distribution with $p = 0{\cdot}90$ and $q = 0{\cdot}10$.

https://doi.org/10.1017/S0016672300002986 Published online by Cambridge University Press

Similarly, for the mates of males with half the normal fertility the values of p and q would be 0·45 and 0·55. Table 1 (column 3) shows, for various numbers of cycles, the pupal production which is expected, on the above assumption, to be equalled or exceeded by 95 % of the mates of fully fertile males. Ninety-five per cent of the mates of semi-sterile males are expected to produce numbers equal or less than those shown in column 4 of Table 1. The figures in columns 3 and 4 were used as the criteria for distinguishing fully fertile and partially sterile males. Thus, after 10 cycles, if eight or more pupae had been produced the male was classified as fully fertile and its mates and progeny were discarded, if six or less had been produced it was classified as partially sterile and, if seven had been produced, testing was continued until an unequivocal answer was obtained. In practice the mates of males classified as partially sterile were generally retained for a long period and in almost all cases the original diagnosis was confirmed. As shown in Table 1, twenty-four out of twenty-six of the control results fell within the 95 % limits predicted on the assumption of a binomial distribution for fully fertile males and two fell just below these limits, but above the limits for semi-sterile males. Thus the binomial assumption seems to be at least a reasonable approximation.

When the mates of apparently partially-sterile males died or were killed during the 60-day test period, their spermathecae were examined at 600 × magnification for the presence of spermatozoa. In the very few cases when uninseminated females were found their contribution to the aggregate number of cycles for the cage of females was removed and this sometimes changed the classification of the male concerned.

3. RESULTS

(i) *The production of partially sterile mutants*

Radiation doses of 5, 6 or 7 kr were given to a total of about 100 males and this induced dominant lethals in 70–90 % of their sperm, but the remaining zygotes produced F_1 progeny which showed normal survival to maturity (Curtis, 1968*c*). A total of sixty F_1 males were obtained and tested for fertility by the method described. The results are shown in columns 1–3 of Table 2. The individuals with full fertility and their progeny and the sterile individuals were discarded.

(ii) *Inheritance of the partial sterility*

Some of the progeny of each of the twenty-two F_1 males, which had been classified as partially sterile, were tested for fertility but many had to be discarded because of limited resources for feeding them.

Figure 1 shows representative parts of the pedigrees of the stocks descended from three of the partially sterile males. Each generation was derived from matings between partially sterile individuals and individuals from the laboratory colony; i.e. there was a process of repeated outcrossing and selection for the partially sterile type at each generation. Table 2 (columns 5–12) gives a summary of the data from similar pedigrees of the stocks descended from each partially sterile

F_1 male; each stock is distinguished by a serial number. The sterile individuals will be considered in the next section.

In stocks 57, 62, 612 and 625 there were no partially sterile offspring and the males are classified as non-transmitters of partial sterility (symbolized by N). All the other eighteen stocks showed inheritance of the character by at least a proportion of the male progeny and, excluding the sterile F_1 individuals, the over-all proportion of the F_1 males which showed heritable partial sterility was 34%.

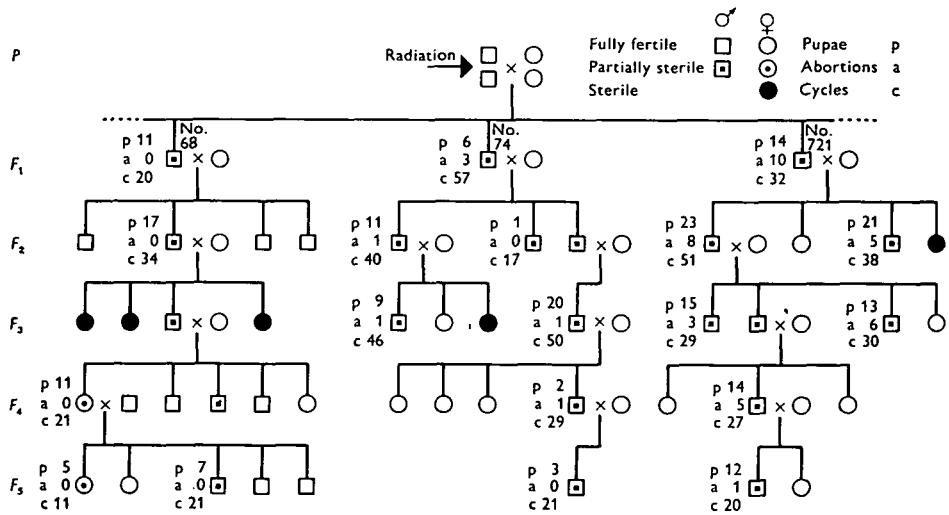

Fig. 1. Representative parts of the pedigrees of three of the stocks showing partial sterility. The fertility of partially sterile females in Geigy 1 cages and of those mates of partially sterile males which were kept in Geigy 10 cages is indicated in terms of the number of pupae and abortions produced in the aggregate number of cycles during the test period. Symbols not connected by lines to the tree represent individuals taken from the unirradiated laboratory colony.

In stocks 74 and 721 (Fig. 1 and Table 2) there was strictly patrilineal transmission of partial sterility. Three sons of fully fertile females of stock 721 and one of stock 74 were tested and all showed full fertility. This indicates that the absence of partially sterile females in these stocks was not due to sex-limited expression of the partial sterility factors but to non-transmission of the factor to females and it is therefore concluded that the factors involve the *Y* chromosomes of stocks 74 and 721 and this interpretation is symbolized by Y in Table 2.

Stock 79 showed the same pattern of inheritance as 74 and 721 with the exception of the occurrence of two fully fertile males. This stock has been tentatively classified as of the Y type. One possible explanation of the two anomalous males is that they result from numerical non-disjunction so that the mutant *Y* chromosome was not transmitted to them. If the partial sterility is due to translocation heterozygosity it is not unexpected that such numerical non-disjunction should occur (Burnham, 1962), but the fact that the presumed *XO* males were viable and fertile may argue against this suggested explanation.

Most of the stocks showed a segregation of partially sterile and fully fertile types among the males and the partial sterility factors are interpreted as involving autosomes only (symbolized by A). Most of these stocks also produced a proportion of partially sterile females and, as shown for stock 68 in Fig. 1, there was a segregation among their progeny just as in those of the males.

Table 2. *Results of fertility tests on the sons of irradiated flies* (F_1♂♂) *and on the stocks descended from the partially sterile* F_1 *males up to the* F_6 *generation*

Dose (krads)	F_1 ♂♂	Serial nos. of stocks	Descendent stocks ♂♂ F	P.S.	S	D	♀♀ F	P.S.	S	D	Classification
5	12F 0S 3 P.S.	53	12	9	0	1	4	1	2	0	A
		57	5	0	0	0	0	0	0	0	N
		58	10	4	0	0	5	2	3	1	A
6	8F 4S 12 P.S.	62	5	0	0	0	0	0	0	0	N
		63	7	2	2	0	2	0	2	1	A
		68	10	5	0	3	3	2	5	1	A
		69	7	3	0	0	3	0	5	0	A
		612	1	0	4	0	0	0	1	0	N
		613	5	5	1	2	9	2	3	2	A
		615	5	6	0	2	6	2	4	2	A
		618	3	10	0	1	4	0	10	4	A
		620	5	6	0	1	8	0	5	4	A
		622	12	12	0	0	4	7	5	0	A
		625	5	0	0	0	0	0	3	0	N
		626	5	6	0	0	3	2	7	2	A
7	11F 3S 7 P.S.	73	8	16	0	3	5	3	6	4	A
		74	0	13	1	1	7	0	2	1	Y
		79	2	10	1	1	7	0	4	1	?Y
		719	8	12	0	1	4	2	4	1	A
		721	0	11	1	1	11	0	4	4	Y
		722	7	9	2	2	3	4	2	0	A
		723	14	5	0	2	9	3	3	2	A
Totals for F_1 ♂♂	31F 7S 22 P.S.	Totals for A stocks	118*	110*	5	18	72†	30†	66	24	

* $\chi^2_1 = 0{\cdot}21$, $0{\cdot}5 < P < 0{\cdot}7$, compared with expectation of 1:1 ratio.

† $\chi^2_1 = 16{\cdot}48$, $P < 0{\cdot}001$, compared with expectation of 1:1 ratio.

Abbreviations: F = fully fertile; P.S. = partially sterile; S = sterile; D = died too young to be tested; N = non-transmitter of partial sterility; A = factor involving autosomes only; Y = factor involving *Y* chromosome.

The over-all segregation ratio (fully fertile:partially sterile) in the A stocks was close to 1:1 for male progeny but among female progeny there was a very significant deficit of the partially sterile type. There appears to have been heterogeneity between the A stocks for this ratio: 622, 73 and 722 produced several partially sterile females, whereas 620, despite the testing of many females, produced none.

(iii) *The sterile progeny*

Some sterile females were found in each of the stocks tested (Table 2). Those classified as sterile only include ones which had been inseminated and which survived for at least one cycle without reproduction, but about 65 % of these died before the end of the four-cycle test period, whereas only 4 % of those that had produced at least one pupa did so. Thus there is a very strong correlation of sterility with low viability. Very few sterile males were found (apart from a concentration in stock 612) and the percentage of males and females which died young, before their fertility could be tested, was not greatly different. The sex ratio of all the progeny which hatched from partially sterile parents was 727 males: 666 females compared with a ratio in controls of 1 : 1. Thus there is no evidence of a category of defective males corresponding to the category of sterile and inviable females.

It seems possible that the deficit of partially sterile females, relative to the number of fully fertile females, in the A stocks (§ (ii)) is connected with the presence of the large proportion of sterile females, i.e. in some cases sterility is produced by a combination of the partial sterility factor with other radiation-induced modifying factors. However, these modifiers must have an important role and female sterility must sometimes occur in the absence of a partial sterility factor for the following reasons: (*a*) the numbers of partially sterile females plus totally sterile ones was considerably greater than the number of fully fertile ones; (*b*) the percentage of sterile females declined somewhat as the outcrossing proceeded; (*c*) the Y stocks produced some sterile females.

(iv) *The level of fertility of the partially sterile individuals*

The level of fertility of the partially sterile males whose wild-type mates were maintained in Geigy 10 cages was calculated from the pupal production up to day 120 after their first mating. Up to this time productivity was at a steady maximum. In the short test periods used for distinguishing fully fertile from partially sterile individuals it was adequate to assume that cycles occupy 9 days. Over the 120-day period, however, the fact that unproductive cycles were shorter than productive ones was significant and the procedure used, therefore, was first to calculate the productivity in the form of x pupae from y female days (excluding the immature period before the first ovulation) and then to convert this to x pupae from z cycles by the method given by Curtis (1968*c*). The results for the individuals of the stocks whose pedigrees are shown in Fig. 1 are indicated on the figure. For all stocks the sum of pupae produced and cycles completed for all males classified as partially sterile is given in Table 3 (columns 2 and 3).

To test the homogeneity between individuals within stocks, χ^2 was calculated from the numbers of pupae and cycles recorded for each individual and the mean pupae per cycle for that stock. The results are shown with their degrees of freedom and significance level in Table 3 (column 4) and in most cases there is no evidence for heterogeneity between the partially sterile members within each stock. The apparent heterogeneity within stocks 613, 626 and 73 may have been due to the

inclusion of aberrant sterile females among the flies taken from the unirradiated laboratory colony to mate to certain of the males, but this cannot explain the highly significant heterogeneity in stock 74, which was mainly due to one individual whose mates produced twenty pupae from 50 cycles, which was much above the very low fertility of the other members of this stock. As shown in the pedigree of this stock (Fig. 1), the relatively fertile individual had a son and grandson which reverted to the characteristic very low fertility of stock 74. The cause of the case

Table 3. *Productivity of the individuals classified as partially sterile in each stock*

	Males					Females		
Stock	Pupae	Cycles	χ^2 between individuals (+D.F.)	Abortions	Pupae hatched / Pupae tested	Pupae	Cycles	χ^2 between individuals (+D.F.)
53	85	260	12·50 (9)	2	8/11	5	8	—
57	20	43	—	0	—	—	—	—
58	89	157	2·00 (3)	1	63/74	21	25	0·20 (1)
62	17	40	—	2	—	—	—	—
63	47	103	2·46 (2)	6	—	—	—	—
68	35	75	2·35 (2)	0	28/30	16	32	0·14 (2)
69	56	143	1·53 (3)	13	39/49	—	—	—
612	18	35	—	2	—	—	—	—
613	75	140	7·81 (3)*	9	30/38	11	19	—
615	73	151	0·41 (3)	17	43/52	10	16	—
618	124	267	9·56 (8)	30	28/31	—	—	—
620	91	188	4·02 (4)	16	53/64	—	—	—
622	70	157	1·06 (3)	12	79/83	40	81	0·15 (2)
625	17	41	—	0	—	—	—	—
626	62	160	10·43 (3)*	4	57/68	13	28	3·98 (1)*
73	134	327	22·80 (10)*	33	61/71	25	48	3·07 (1)
74	69	381	34·23 (7)***	11	40/44	—	—	—
79	92	277	6·02 (7)	30	30/34	—	—	—
719	94	200	3·59 (6)	0	69/77	17	41	1·65 (1)
721	163	329	4·13 (10)	51	90/98	—	—	—
722	70	199	7·21 (5)	7	67/70	32	55	1·04 (3)
723	89	186	7·92 (4)	9	62/91	19	35	0·52 (1)
Totals	1590	3859		255	785/894†	209	388	
Controls	339	376		2	194/199	248	248	

Heterogeneity χ^2 between totals for each stock (+D.F.):

Males	Pupae/cycle (all stocks)	157·33 (21)***
	Pupae/cycle (excluding stocks 613, 626, 73 and 74)	55·14 (17)***
	Abortions/cycles not producing pupae	167·7 (16)***
	Pupal hatch (excluding stock 723)	17·96 (10)
	Pupal hatch of stock 723 compared with other stocks	24·88 (1)***
Females	Pupae/cycle	14·24 (9)

Symbols: † = total excludes data from stock 723.
* = $0{\cdot}01 < P < 0{\cdot}05$; *** = $P < 0{\cdot}001$.

Individuals have been omitted from the calculations of χ^2 where the expectation for them on the null hypothesis < 5·0.

https://doi.org/10.1017/S0016672300002986 Published online by Cambridge University Press

of relatively high fertility cannot therefore have been a change in the nature of the partial sterility factor (such as the dissociation by crossing-over of a multiple translocation complex) and it seems probable that an unlinked gene, modifying the effect of the factor, was responsible.

The homogeneity between stocks was tested by calculating χ^2 from the total pupae and cycles for each stock and the overall mean pupae per cycle. As shown there was highly significant heterogeneity between stocks. The greatest contribution to this was by the very infertile stock 74, but this stock showed internal heterogeneity, which may complicate the interpretation. However, as shown in Table 3, even if stock 74 and the three other stocks showing significant internal heterogeneity are excluded the re-calculated χ^2 between stocks is still highly significant.

The partially sterile females mated to wild-type males were maintained individually in Geigy 1 cages and the number of cycles they completed can readily be obtained from the intervals between pupal production. The rate of production seemed to be constant up to about the 25th cycle and results are given up to that time or to when they were killed, whichever was the earlier. The pooled data for all the partially sterile females of each stock are shown in Table 3 (columns 7 and 8). Where there were sufficient data, χ^2, between individuals within stocks, and between stocks, was calculated by the same methods described for the males. There is little evidence for heterogeneity within or between stocks, unlike the situation for the males. This difference may be because much less data are available for females and it is notable that the partially sterile males and females of stock 58 both appear to be more fertile than the males and females of all the other stocks.

To compare the average fertility of the males with the females it is necessary to correct the value for males for the fact that controls gave a lower fertility in the conditions used for testing the males (Geigy 10 cages) than in those used for testing females (Geigy 1 cages), and it also seems best to omit those stocks in which no partially sterile females occurred. After these corrections the average value for males is 0·484 pupae per cycle compared with the average for females of 0·539. The corrected data is not in a form in which this apparently greater fertility of females can be tested for statistical significance.

(v) *The stages at which the partial sterility factors cause death*

A crude indication of the stage at which death occurs in cycles when pupae were not produced could be obtained by recording the cases in which visible abortions occurred and those when there was none. The abortions ranged from early larval stages to superficially normal larvae of mature size which did not move after birth and failed to form a hard black puparium. At cycles at which neither a pupa nor an abortion was observed it is presumed that death occurred in the embryonic stage and the embryo was either resorbed or was too small to be noticed when extruded. The number of abortions from the mates of the partially sterile males of each stock are shown in Table 3 (column 5). The abortion rate among the controls agrees quite well with that from a much larger amount of data on wild-type flies

(A. M. Jordan, personal communication). The mean abortion rate per cycle of the partially sterile stocks was very much higher than in controls, i.e. a proportion of the deaths caused by the partial sterility factors occurred during larval development in the uterus. There was highly significant heterogeneity between stocks in the ratio of number of abortions to the total of cycles at which pupae were not produced. This ratio was highest in stock 721 and, as shown in its pedigree in Fig. 1, all the individual males of this stock showed this feature.

For some of the pupae deposited by the mates of partially sterile males records were kept of the proportion which hatched and the totals for each stock are shown in Table 3 (column 6). Excluding stock 723, there was insignificant heterogeneity between stocks and a mean emergence rate of 87·8 %, which was significantly less than the rate for wild-type controls ($\chi^2_1 = 15{\cdot}22$, $P < 0{\cdot}001$). This difference in

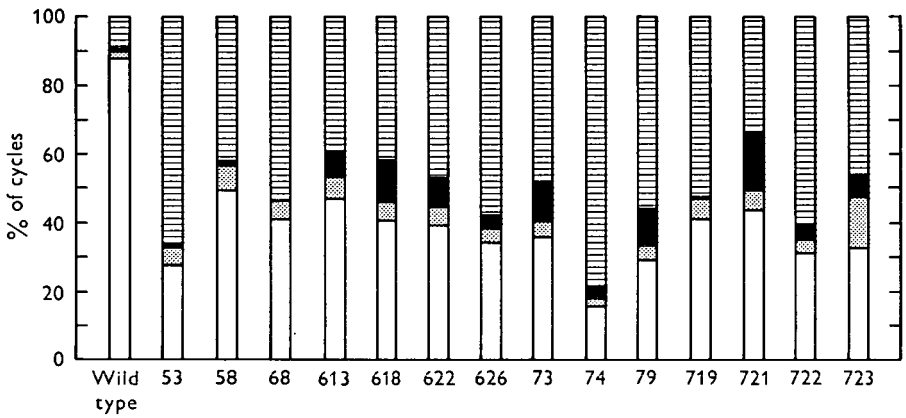

Fig. 2. The reproductive performance of the mates (in Geigy 10 cages) of the partially sterile males of the various stocks and of the wild-type control. The histograms show the percentage of cycles which led to embryonic death (horizontal shading), aborted larvae (black), pupal death (dotted) and survival to the adult stage (white). The rate of pupal survival is taken as 87·8 % for all stocks except wild type and 723 where the rates characteristic of these particular stocks are used.

emergence rate may be only indirectly caused by the partial sterility factors, via the possible adverse effects of infrequent pregnancy on the physiology of the female fly, which might have an effect on the quality of the pupae produced (Curtis, 1968*c*). However, this will not explain the fact that the partially sterile individuals of stock 723 gave pupae with the exceptionally low hatching rate of 68·1 % which is highly significantly less than that for the other stocks. Within stock 723 the results for the partially sterile individuals tested were homogeneous ($\chi^2_2 = 2{\cdot}65$; $0{\cdot}3 > P > 0{\cdot}2$). Pupae from several segregants from stock 723 with normal pupal production gave a hatching rate which was significantly higher than the pupae from partially sterile members of this stock ($\chi^2_1 = 13{\cdot}41$; $P < 0{\cdot}001$). Thus in this stock it appears that some of the deaths caused by the partial sterility factor itself (and not other unlinked factors) were delayed until the pupal stage.

For the partially sterile males of the stocks for which there are most data, the proportion of zygotes which survived each stage of development have been assembled in Fig. 2. This emphasizes that the partial sterility factors, which arose as independent radiation-induced events, have characteristic profiles of lethal activity at the embryonic, larval and pupal stages.

4. DISCUSSION

This work was initiated with the aim of producing translocations in *G. austeni*. By analogy with the results in other organisms (Burnham, 1962) it seems very probable that the partially sterile individuals in the A and Y stocks were translocation heterozygotes. The evidence for this is:

(*a*) The partial sterility was inherited as if due to unitary dominant factors.

(*b*) It was induced at high frequency (34%) in sperm by doses of 5–7 krads; this rate is comparable (allowing for the differences in the doses used) with data on the induction of translocations in sperm of *Drosophila virilis* (Alexander & Stone, 1955), but is very much higher than could be expected for mutations at specific gene loci.

(*c*) Linkage of the sterility to the *Y* chromosome in a small proportion of cases is to be expected if translocations were involved and two mutants apparently of this type were found. (Translocations involving the *X* chromosome would not have been detected because the fertility of F_1 females was not tested.) No information about the sex determination mechanism of tsetse has previously been obtained by breeding experiments, but the fact that the male had the constitution *X Y* had previously been suggested on cytological grounds by Dr B. M. Slysinski (quoted by Vanderplank (1948) and personal communication).

It is just possible that the results were due to inversion heterozygosity, but this seems unlikely as many other Cyclorrhaphous Diptera are known to be protected against the potentially deleterious effects of crossing-over in inverted sections of chromosome by the absence of crossing-over in male meiosis (Wagoner, 1967; La Chance, Dawkins & Hopkins, 1966) and, in the case of paracentric inversions, by the direction of the dicentric and acentric fragments to the polar bodies in female meiosis (Sturtevant & Beadle, 1936). Assuming that tsetse share these properties with their relatives, inversions would not explain the results.

The occurrence of differences between the fertility of the different stocks conforms with what is known for tranlocations in other organisms. The extreme sterility of the males of stock 74 suggests that in this case there may be a translocation complex formed by mutual exchange of segments between three chromosomes. The fact that the high sterility factor did not break down due to crossing-over might indicate that there is no 'differential segment' (John & Lewis, 1965) in this translocation complex. Since this factor was only inherited by males (Fig. 1), even if there was a differential segment, dissociation of the complex would still not occur if there is no crossing-over in the tsetse male. The lesser variation in fertility between the other stocks may be explained by differences in the lengths

of chromosome segments exchanged and in the length of the interstitial segments, causing variation in the proportion of alternate and adjacent segregations (Burnham, 1962).

Most of the deaths caused by the partial sterility factors occurred early in development but in several cases a proportion survived to the larval or pupal stages before dying (Fig. 2). This contrasts with the effects of dominant lethals induced in sperm of *G. austeni*, all or almost all of which cause death early in development (Curtis, 1968*c*). Differences in the time of death caused by dominant lethals induced in sperm and by duplication-deficiency zygotes from translocations were found by von Borstel & Rekemeyer (1959) in *Habrobracon* and *Drosophila*.

In view of all the analogies of the partially sterile mutants in *G. austeni* with translocation heterozygotes in other organisms it has been decided, even in the absence, so far, of cytological confirmation of the existence of translocations in these stocks, that it is worthwhile to inbreed the most suitable ones (i.e. those which produced the expected proportion of partially sterile females) to attempt to produce stocks with the properties of translocation homozygotes.

SUMMARY

An attempt is described to produce chromosome translocations in *Glossina austeni* which, it is hoped, might ultimately be used for tsetse fly control. The method consisted of selecting among the progeny of irradiated males for cases of inherited partial sterility. Thirty-four per cent of the testable sons of males which received doses of 5–7 krads showed these properties. In two cases the inheritance was patrilineal which suggests that the *Y* chromosome was involved. In most of the other cases a segregation among both male and female progeny of the partially sterile and normal types occurred and in these cases it appears that autosomes only were involved. Among males in these stocks the segregation ratio was close to 1:1, but in females there was a deficit of the partially sterile type. This may be partly associated with the fact that a large proportion of totally sterile and inviable females were produced by these stocks. The proportion of zygotes which died at the embryonic, larval and pupal stages as a result of the action of the partial sterility factors varied between factors of different mutational origin. In view of the pattern of inheritance of these factors and their high frequency of induction it is argued that in all probability they are translocations in the heterozygous state.

I am grateful to Mrs E. Spincer and Mrs L. Rowney for technical assistance and to the staff of the Tsetse Research Laboratory for help of various kinds.

Radiation facilities were kindly provided by the staff of the Cobalt Unit, Bristol General Hospital.

I thank Dr A. M. Jordan and Dr T. A. M. Nash for criticizing the manuscript.

REFERENCES

ALEXANDER, M. L. & STONE, W. S. (1955). Radiation damage in the developing germ cells of *Drosophila virilis*. *Proc. natn. Acad. Sci. U.S.A.* **41**, 1046–1057.

BURNHAM, C. R. (1962). *Discussions in Cytogenetics*. Minneapolis, U.S.A.: Burgess.

Buxton, P. A. (1955). The natural history of tsetse flies. *Mem. Lond. Sch. Hyg. and Trop. Med.* no. 10. London: Lewis.

Curtis, C. F. (1968*a*). Possible use of translocation to fix desirable genes in insect pest populations. *Nature, Lond.* **218**, 368–369.

Curtis, C. F. (1968*b*). A possible genetic method for the control of insect pests, with special reference to tsetse flies (*Glossina* spp.). *Bull. ent. Res.* **57**, 509–523.

Curtis, C. F. (1968*c*). Radiation sterilization and the effect of multiple mating in *Glossina austeni*. *J. Insect Physiol.* **14**, 1365–1380.

Curtis, C. F. & Hill, W. G. (1968). Theoretical and practical studies on a possible genetic method for tsetse fly control. *Isotopes and Radiation in Entomology*, pp. 233–247. Vienna: I.A.E.A.

Itard, J. (1966). Chromosomes de glossines (Diptera-Muscidae). *C. r. hebd Séanc. Acad. Sci., Paris* **263**, 1395–1397.

John, B. & Lewis, K. R. (1965). *The Meiotic System*. Vienna: Springer-Verlag.

Jordan, A. M. & Curtis, C. F. (1968). Productivity of *Glossina austeni* maintained on lop-eared rabbits. *Bull. ent. Res.* **58**, 399–410.

Jordan, A. M., Nash, T. A. M. & Boyle, J. A. (1967). The rearing of *Glossina austeni* Newst. with lop-eared rabbits as hosts. I. Efficacy of the method. *Ann. trop. Med. Parasit.* **61**, 182–188.

La Chance, L. E., Dawkins, C. & Hopkins, D. E. (1966). Mutants and linkage groups of the screw worm fly. *J. econ. Ent.* **59**, 1493–1496.

Nash, T. A. M., Jordan, A. M. & Boyle, J. A. (1967). A method for maintaining *Glossina austeni* Newst. singly, and a study of the feeding habits of the female in relation to larviposition and pupal weight. *Bull. ent. Res.* **57**, 327–336.

Nash, T. A. M., Jordan, A. M. & Boyle, J. A. (1968). The large-scale rearing of *Glossina austeni* Newst. in the laboratory. IV. The final technique. *Ann. Trop. Med. Parasit.* **62**, 336–341.

Saunders, D. C. (1960). The ovulation cycle in *Glossina morsitans* and a possible method of age determination for female tsetse flies by the examination of their ovaries. *Trans. roy. ent. Soc. Lond.* **112**, 221–238.

Serebrovsky, A. S. (1940). On the possibility of a new method for the control of insect pests. *Zool. Zh.* **19**, 618–630. (Russian.)

Snell, G. B. (1935). The induction by X-rays of hereditary changes in mice. *Genetics* **20**, 545–567.

Sturtevant, A. H. & Beadle, G. W. (1936). The relation of inversions of the X chromosome of *Drosophila melanogaster* to crossing-over and disjunction. *Genetics*, **21**, 554–562.

Vanderplank, F. K. (1948). Experiments in cross breeding tsetse flies. *Ann. trop. Med. Parasit.* **42**, 131–154.

Von Borstel, R. C. & Rekemeyer, M. L. (1959). Radiation-induced and genetically contrived dominant lethality in *Habrobracon* and *Drosophila*. *Genetics* **44**, 1053–1062.

Wagoner, D. E. (1967). Linkage group-karyotype correlation in the house fly determined by cytological analysis of X-ray induced translocations. *Genetics* **57**, 729–739.