Sex ratio distortion and semi-sterility in the progeny of irradiated *Glossina morsitans*

BY C. F. CURTIS AND P. A. LANGLEY

Tsetse Research Laboratory, Langford, Bristol, England

A. R. MEWS AND E. D. OFFORI

Seibersdorf Laboratory, International Atomic Energy Agency, Vienna, Austria

D. I. SOUTHERN AND P. E. BELL

Department of Zoology, University of Manchester, Manchester, England

SUMMARY

Male *Glossina morsitans* were subjected to various doses of gamma radiation in air or nitrogen and mated to untreated females. The sex ratio of the F_1 progeny was biased towards males, and this may be explained by the extra vulnerability to dominant lethal induction that the presence of the large X chromosome gives to female determining sperms. The mean fertility of the F_1 progeny was subnormal. This was due to the induction of 50 % sterility in a large proportion of F_1 individuals. Cytogenetic examination of the progeny of outcrosses of F_1 individuals indicated that in most of the semi-steriles there were translocations involving the large autosomes or the Y chromosomes. Almost all the normally fertile F_1 flies gave only cytogenetically normal progeny. The F_1 sex-ratio distortion and semi-sterility would provide a 'bonus' in the application of the sterile male technique, which would amount to a 15–50 % saving in the releases required to achieve a population control target compared with the requirement if the F_1 was normal.

1. INTRODUCTION

Muller (1954a) pointed out that, in the application of the sterile male technique, induced translocations and other abnormalities in the F_1 progeny of irradiated insects would contribute to the population suppressing effect, if a radiation dose below that required for 100 % dominant lethality were used.

In Lepidoptera, high radiation doses are required to achieve 100 % sterility of the irradiated insects and at doses below this the F_1 males and females may show a higher degree of sterility than the irradiated insects (e.g. Proverbs, 1962; Bauer, 1967; North & Holt, 1968a). Many translocations were found in the progeny of irradiated Lepidoptera by Astaurov & Frovlova (1935), Bauer (1967) and North & Holt (1968b) and a correspondence was proved in the Homopteran *Oncopeltus* between individuals with translocations and those with heritable partial sterility (LaChance, Degrugillier & Leverich, 1970). Knipling (1970) considered the potential

advantages, for control purposes, of this F_1 sterility. It is probable that the exceptional resistance to dominant lethal induction and the high rate of recovery of translocations in Lepidoptera can be explained by their holokinetic chromosomes (LaChance *et al.* 1967).

Even in insects with localized centromeres, work directed to the production of translocation-carrying strains has shown that, at suitable radiation doses, these are by no means rare mutations, e.g. in *Culex fatigans* (Laven & Jost, 1971) and in the tsetse fly *Glossina austeni* (Curtis, 1969). Another abnormality observed in the progeny of irradiated male insects is distortion of the sex-ratio in favour of males, e.g. in *Drosophila* (Catcheside & Lea, 1945) and in Lepidoptera (Proverbs, 1962).

In *Glossina austeni* the pupae fathered by irradiated males had subnormal weight; this appears not to be due to a genetic effect, but to a physiological effect of infrequent pregnancy in the female (Curtis, 1970). Itard (1973) found a similar depression of pupal weight in the progeny of irradiated *G. tachinoides*. He also found indications of sex-ratio distortion and there was a considerable proportion of semi-sterile individuals among the progeny. *G. morsitans* differs from *G. tachinoides* in its sensitivity to dominant lethal induction by radiation (Itard, 1970*a*). Studies have been in progress at Langford and Seibersdorf on radiation induction of dominant lethality in *G. morsitans*. Following Itard's observations, the pupae produced from irradiated parents were retained and this paper describes studies on the progeny which emerged. In *G. austeni*, cases of inherited semi-sterility have been shown to be due to translocations which can be observed at meiosis in the pupal testis (Curtis, Southern, Pell & Craig Cameron, 1972). These studies have now been extended to F_2 pupae from irradiated *G. morsitans*.

2. MATERIALS AND METHODS

The stocks used were *G. morsitans morsitans* Westwood (= *G. m. orientalis* Vanderplank) from the laboratory colonies maintained at Langford and Seibersdorf. The experimental flies were kept at 24·5–25·5 °C and 60–70 % rh and fed 6 days per week on rabbits' ears (Nash, Jordan & Boyle, 1966). Virgin females were obtained by daily sexing and separation of emergent adults. After feeding, the females were mated to males aged at least 6 days.

The flies studied were the surviving progeny of normal females mated to males which had been irradiated with ^{60}Co gamma rays. At Langford the dose rate was 150 rad/sec, treatment was at the late pupal stage and in some cases an atmosphere of nitrogen was used during treatment (Curtis & Langley, 1972). At Seibersdorf the dose rate was 100 rad/sec and treatment was at the early adult stage. With either irradiation procedure spermatogenesis was already complete in the irradiated males (Itard, 1970*b*) and the relationship of % dominant lethals to dose obtained at the two laboratories shows fair agreement.

The pupae produced were kept at 24–25·5 °C and they were sexed, usually at emergence, but in some cases by observation of the presence or absence of testes

on day 10 of pupal life. Fertility is best recorded in terms of proportion of viable zygotes. In living tsetse the zygotes cannot be seen, but the female has a regular reproductive cycle in which a single egg is ovulated and, if the fertilization is viable, a 3rd instar larva is subsequently deposited and it pupates immediately.

For the fertility tests at Langford, each F_1 male was mated to four normal females, following the schedule of Curtis (1969). The females were kept together in a cage and the pupal production per surviving mature female per day was recorded for 63 days after the first mating; this was converted to pupae per ovulation cycle by the method of Curtis (1972b). Each F_1 female was mated to a normal male and isolated in a cage where its pupal production was recorded for the first 10 cycles. For the fertility tests at Seibersdorf F_1 males or females were mated once only with a normal insect and the female was isolated in a cage. Matings were between 2-day-old females and 7- to 10-day-old males. Pupal production was recorded for 45 or 63 days from the eclosion of the female. Fertility appeared to be maximal only from days 28–45 (i.e. ovulation cycles 2 to 4) and the results quoted are for this period. Females, shown by dissection after the tests to be uninseminated, were eliminated from the fertility scores.

The cytogenetic studies were on pupae bred at Langford and mailed to Manchester. Male meiosis occurs on days 9 to 10 of pupal life at 24 °C and testes were dissected out at this stage and stained and squashed as described by Southern, Craig Cameron & Pell (1972a).

3. RESULTS AND DISCUSSION

(i) *Sex-ratio distortion*

Table 1 shows data for various radiation treatments on the fertility of the irradiated males (from Curtis & Langley, 1972) and on the sex-ratio of the progeny fathered by them. The sex-ratio was significantly biased in favour of males in the total over all treatments and in the individual treatments in which the sample sizes were largest (Table 1). It was surprising that at the dose which gave the highest dominant lethal rate (15 krad in air) there was no sign of sex-ratio bias. However, the numbers were too small to determine the significance of this.

Catcheside & Lea (1945) found a bias in favour of males in the sex-ratio of the progeny of irradiated male *Drosophila* mated to wild-type females and they showed that the bias was reversed if the irradiated males were mated to attached-X females. From such females, males derive from X-carrying sperms and females from Y-sperms and these results indicate that the sex-ratio bias was due to extra damage to X-sperms which was not sustained by Y-sperms. The sex determination system is similar in *Glossina* to that in *Drosophila* (Slizynski, quoted by Vanderplank, 1948; Southern, Craig Cameron & Pell, 1972a, b), and it is assumed that the sex-ratio bias has the same cause in the two genera. It could be postulated that the radiation damage in the X chromosome results either in induction of extra dominant lethals so that more XX zygotes die (Catcheside & Lea, 1945), or alternatively, that the damaged X chromosomes are lost at mitosis or function

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Table 1. *Data on fertility of irradiated male* Glossina morsitans *and the emergence rate and sex ratio of their progeny*

Treatment		Fertility of irrad. ♂ × untr. ♀ (pupae/cycle ÷ control)	F_1 sex ratio		
Dose (krad)	Gas		No. ♂	No. ♀	χ_1^2 (1:1 exp.)
6	Air	0·366	45	15	14·0***
7	Air	0·294	26	13	3·7
9	Air	0·128	16	3	17·0***
10	Air	0·117	5	2	—
12	Air	0·037	3	3	—
15	Air	0·012	3	4	—
7	N_2	0·381	32	20	2·3
10	N_2	0·218	11	7	0·5
15	N_2	0·059	25	5	12·0***
Total over all treatments			166	72	36·4***
Untreated control		1·0	26315†	26243†	

*** $P < 0.001$.
† Data from M. A. Trewern (personal communication).

Table 2. *Calculated dominant lethal rates in male and female zygotes of* Glossina morsitans *and metaphase chromosome lengths*

Treatment		Mean proportion of nonlethal zygotes ($= e^{-m}$)		Calculated no. dominant lethals per zygote (m)		
Dose (krad)	Gas	♂	♀	♂	♀	Ratio ♀/♂
6	Air	0·550	0·183	0·599	1·70	2·84
7	Air	0·392	0·196	0·939	1·63	1·74
9	Air	0·216	0·0403	1·54	3·22	2·09
10–15 (mean)	Air	0·070	0·057	2·66	2·81	1·06
7	N_2	0·471	0·294	0·753	1·23	1·63
10	N_2	0·268	0·171	1·32	1·77	1·34
15	N_2	0·0984	0·0197	2·32	3·94	1·70

Mean = 1·77

Metaphase chromosome lengths (μm):

L_1	L_2	X	Y	Short chromosomes	Ratio $\dfrac{L_1 + L_2 + X}{L_1 + L_2}$
7·60	4·92	7·15	4·44	4·29 (total for 2 chromosomes)	1·57

abnormally in sex determination, such that XO or XX progeny are produced with a male phenotype (L. E. LaChance, personal communication), or both of these factors might contribute.

On the hypothesis that only differential vulnerability to dominant lethals is involved, the data have been converted to the survival rate for male and female F_1 zygotes (Table 2). The results for the small samples at 10, 12 and 15 krad in air have been combined. Several dominant lethal events may be induced in one

sperm and since in *G. morsitans* there is, approximately, a 'one-hit' relationship of fertility to dose (Curtis & Langley, 1972) it is assumed that there would be a Poisson distribution of sperms with 0, 1, 2, ..., *n* dominant lethals. The observed proportions of surviving male and female zygotes therefore represent the term e^{-m}, where *m* is the mean number of dominant lethals per sperm. The calculated values of *m* are shown in Table 2, together with the ratio of female:male *m* values at each dose and the mean of these ratios for all doses.

Plate 1(*a*) shows the wild-type chromosomes at metaphase I in *G. morsitans*, and Table 2 includes measurements of their lengths at metaphase II. On the simple assumption that the vulnerability of a chromosome to induction of a dominant lethal is proportional to its metaphase length, the ratio of the *m* values of females and males would be proportional to the relative length of chromosomes in *X* and *Y* sperms. In other insects with a differentiated *Y* its presence is not essential to life and the short chromosomes vary in number in different viable *G. morsitans* individuals (Southern *et al.* 1972*a*). Therefore it may be that dominant lethals cannot be induced in these chromosomes because loss of fragments from them would be non-lethal. The vulnerable chromosomes on this view are only the two long metacentric autosomes, L_1 and L_2 in male-determining sperms, and L_1, L_2 and *X* in female-determining sperms. The ratio of the total lengths of these sets is in reasonable agreement with the mean ratio of the *m* values for males and females and this gives some support to the hypothesis of differential vulnerability. However, it may have been incorrect to assume that the *Y* and *S* chromosomes would be invulnerable to dominant lethal induction. Von Borstel (1960) concluded that dominant lethals are mainly due to chromosome bridges delaying mitosis and, if the same applies to *G. morsitans*, damage to the *Y* or *S* chromosomes would be able to yield dominant lethals, even if these chromosomes carry no vital genes.

The alternative theory that damage to *X*-sperms causes them to give rise to males has not been directly tested by cytogenetic studies on sexed F_1 pupae. However, following 15 krad in nitrogen treatments, where the F_1 sex-ratio was about five males:1 female, all the F_1 males were able to mate and inseminate successfully which is not the case with *XO Drosophila*. Their progeny were dissected at day 10 of pupal life; their sex could be determined by the presence or absence of testes and the sex-ratio of these pupae was 65 male:50 female, which is not significantly different from 1:1 ($\chi^2_1 = 1.7$). No adequately tested F_1 male showed itself unable to produce male progeny. If the postulated sex determination anomaly persisted to the F_2 generation, some of the F_2 pupae with testes would have had *XO* or *XX* karyotype, but all were found to be *XY*. Thus there is no evidence that abnormalities of sex determination contribute to the sex-ratio bias in the F_1.

In *G. austeni*, only a slight and statistically insignificant bias towards males was found in the progeny of males which had received 5–7 krad in air (Curtis, 1968). Calculated as already described, the data give a ratio of values of *m* for the two sexes of 1·09. The relative length of the large metacentric autosomes and the *X* are about the same as in *G. morsitans* (Southern *et al.* 1972*b*). It may be that in *G. austeni* the larger number of short chromosomes provide a significant

Table 3. *Fertility of the progeny of irradiated male* Glossina morsitans

Treatment given to parents			F_1 individuals tested				Pupae/ cycle	Fertility corrected for control
Dose (krad)	Gas	Laboratory	Sex	No.	Pupae	Cycles		
6	Air	S	♂	37	89	111	0·802	0·887
6	Air	S	♀	12	24	35	0·685	0·768
9	Air	S	♂	15	35	45	0·778	0·852
10	Air	L	♂	3	38	39·6	0·960	0·991
12	Air	S	♂	3	4	9	0·445	0·493
15	Air	L	♂	2	28	39·8	0·704	0·727
15	Air	L	♀	3	15	29	0·517	0·535
10	N_2	L	♂	7	112	136·4	0·822	0·848
10	N_2	L	♀	3	11	23	0·478	0·492
15	N_2	L	♂	17	231	383·9	0·602	0·620
15	N_2	L	♀	3	12	16	0·750	0·770
Untreated control		S	—	42	115	126	0·913	1·0
Untreated control		L	—	*	292	300·6	0·971	1·0

* Mates of six males mated four times and 30 males mated once.
S = Seibersdorf; L = Langford.

source of dominant lethals, so that the X chromosome is relatively less important than in *G. morsitans*.

The explanation advanced for sex-ratio distortion in tsetse flies is different from that proposed for the superficially similar distortion phenomenon in Lepidoptera (e.g. Proshold & Bartell, 1970). In Lepidoptera the males are the homogametic sex and dominant lethals induced in the X chromosomes of spermatozoa would not therefore affect the sex ratio. Induced recessive lethals in the spermatozoa would, however, kill F_1 females and not males, thus altering the sex-ratio in favour of males.

(ii) *Reduced fertility of the* F_1 *generation*

Table 3 shows the data, collected at Langford and Seibersdorf, on the overall fertility of the F_1 progeny of irradiated males. The control values differed between the two laboratories and the appropriate value has been used to correct each set of F_1 data. At all doses, in both sexes, and at both laboratories, the F_1 fertility was considerably below the control values. The numbers tested were too small to determine the dose/response relationship, or whether the extent of the effect was the same in nitrogen and air or in F_1 males and females.

The data from each individual fly at Seibersdorf only covers three ovulation cycles and the controls showed 10 % of zygotic deaths; therefore it is impossible to identify which F_1 individuals had subnormal fertility. In the data from Langford, however, the sperm from each F_1 male was tested in a total of more than 20 fertilizations in its four mates and the controls showed very few zygotic deaths. Text-fig. 1 shows the distribution of the observed fertility from the individual F_1

Text-fig. 1. Lower part: distribution of fertility scores for the male progeny of irradiated males. The karyotype of each male, deduced from cytogenetic examination of its progeny, is indicated, as described in the text.

Upper part: the distribution of fertility scores expected for normally fertile ($P = 0.971$) and semi-sterile males ($P = 0.478$), and the observed distribution (shaded) of scores for six control males.

males deriving from all the radiation treatments at Langford. Text-fig. 1 also shows the data from six controls mated according to the same schedule as the experimentals.

None of the F_1 males were sterile, many of their fertility scores fell within the control range and the remainder spanned a zone about 50 % of the control. On the hypothesis that the F_1 consisted of two types only, 50 % sterile and normally fertile, a considerable scatter of fertility scores would be expected, because only about 20 zygotes were sampled from each male. The upper part of Text-fig. 1 shows the binomial distributions for a sample size of 20 and probabilities of pupal production/cycle, $P = 0.971$ (the control mean) and $P = 0.478$ (the mean for all those scoring below 0.75). Most of the F_1 scores fell well within these two distributions, but the occurrence of four individuals in the range 0.7–0.85 is hardly consistent with expectation and it seems that, in addition to the majority of the F_1 which were either normally fertile or semi-sterile, a few with intermediate fertility also occurred.

Each of the F_1 female individuals at Langford could be classified as normally

fertile or partially sterile, but, because their fertility scores could only be based on 10 cycles, their distribution cannot be meaningfully compared with that for the F_1 males. The mean fertility of the partially sterile females was 0·448, i.e. not far from the male mean. These means for both sexes were close to those found for radiation-induced partially sterile mutants in *G. austeni* (Curtis, 1969).

(iii) *Cytogenetic studies*

The chromosome complement and male meiotic sequence in $+/+$ *G. m. morsitans* have been described by Southern *et al.* (1972a). Plate 1a shows a metaphase I nucleus in such a normal male, and plate 1b is a polar view of early anaphase I. The diploid chromosome number in these males was $2n = 8 \pm 2$ autosomes plus an XY pair. There are two pairs of large autosomes, L_1 and L_2, which can be readily distinguished from one another on the basis of their length. The other autosomes, which can vary in number from individual to individual, are much smaller, almost certainly telocentric, and there is little to distinguish one of these S chromosomes from another during either mitosis or meiosis. The sex chromosomes constitute a heteromorphic pair, for the X, which is comparable to the L_1 in length and centric location (Plate 1b), is appreciably larger than the markedly submetacentric Y chromosome. The Y and S chromosomes show allocyclic behaviour during both mitosis and meiosis.

Male meiosis is normally achiasmate and only the L_1 and L_2 chromosome pair along their entire length to form bivalents. The region of association between the X and Y is limited to a small segment on each chromosome (Plate 1a) and the S autosomes remain as univalents.

Among the progeny of the sons of irradiated males several kinds of translocation were found. The commonest type had a large segment from the long arm of an L_1 interchanged with a small segment from the longer arm of an L_2 autosome. This translocation could be easily identified. The four partially homologous chromosomes always associated to form a characteristic pairing cross at zygotene/pachytene (Plate 1c), and at anaphase I (Plate 1d) or metaphase II the relative length of the arms lying on either side of the centromeres could be measured and compared with normal chromosomes. At metaphase I the multiple orientated in one of two ways, adjacent non-homologous (never adjacent homologous), or alternate. Only the latter type of orientation would lead to genetically balanced gametes.

A more complex L_1–L_2 interchange was detected in one male individual. From the appearance of the prophase pairing multiple (Plate 1e) and relative length of the chromosome arms at anaphase I/metaphase II (Plate 1f) a possible scheme for its derivation was constructed and this is illustrated in Text-fig. 2.

The difference in cyclic behaviour of the long autosome and Y chromatin during early meiotic prophase facilitated the detection of Y–L_1 and Y–L_2 translocations. Plate 2a illustrates a case where a small end segment from the long arm of the Y chromosome and a larger terminal segment from the longer arm of an L_1 had interchanged. These chromosomes, together with the normal L_1 and X pro-
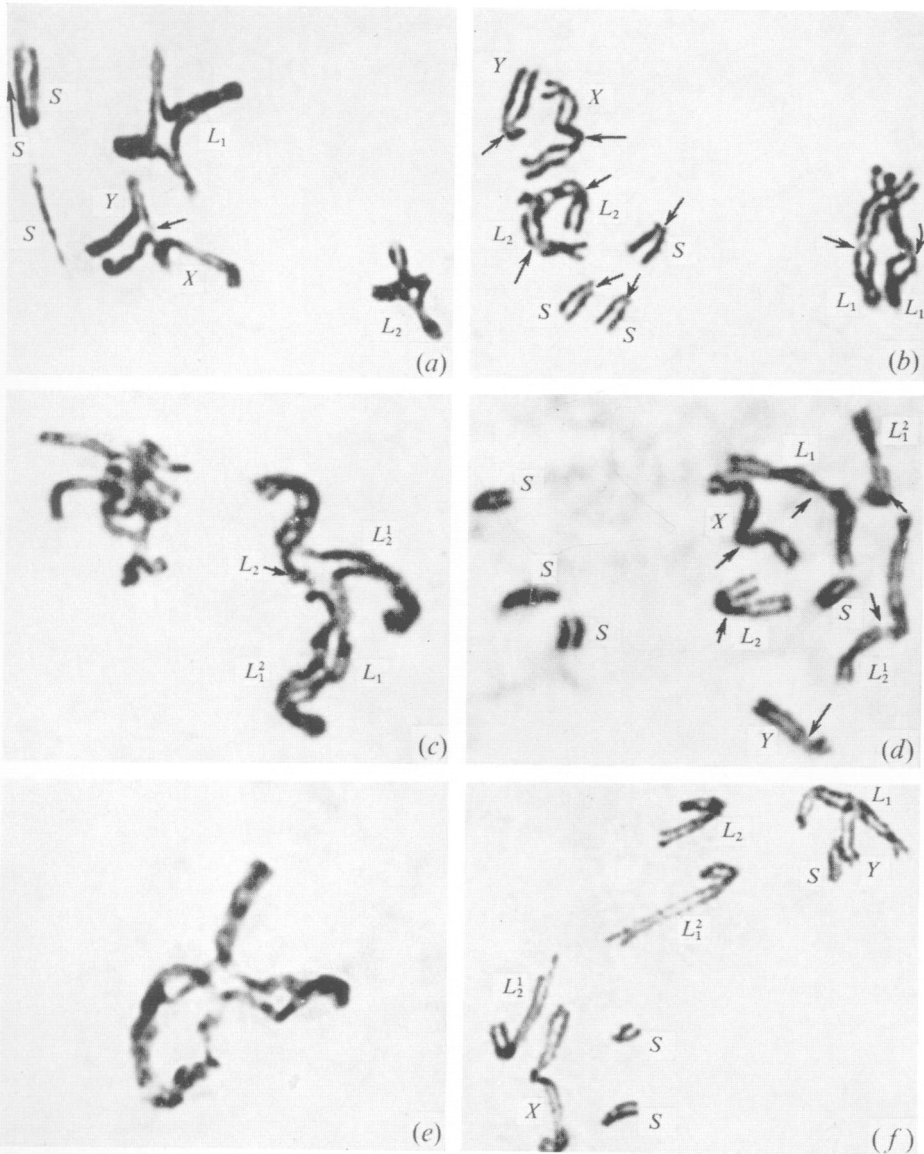
(a) Metaphase I in a wild-type *G. m. morsitans* male. Arrow indicates region of association between X and Y chromosomes.

(b) Early anaphase I in a wild-type *G. m. morsitans* male showing chromosome morphology. Arrows indicate position of centromeres.

(c) L_1–L_2 pachytene pairing cross.

(d) Anaphase I showing relative arm lengths of normal and translocated L_1 and L_2 chromosomes. Arrows indicate position of centromeres.

(e) L_1–L_2 multiple at prophase I. Compare with Fig. 2.

(f) Anaphase I in the L_1–L_2 interchange heterozygote.

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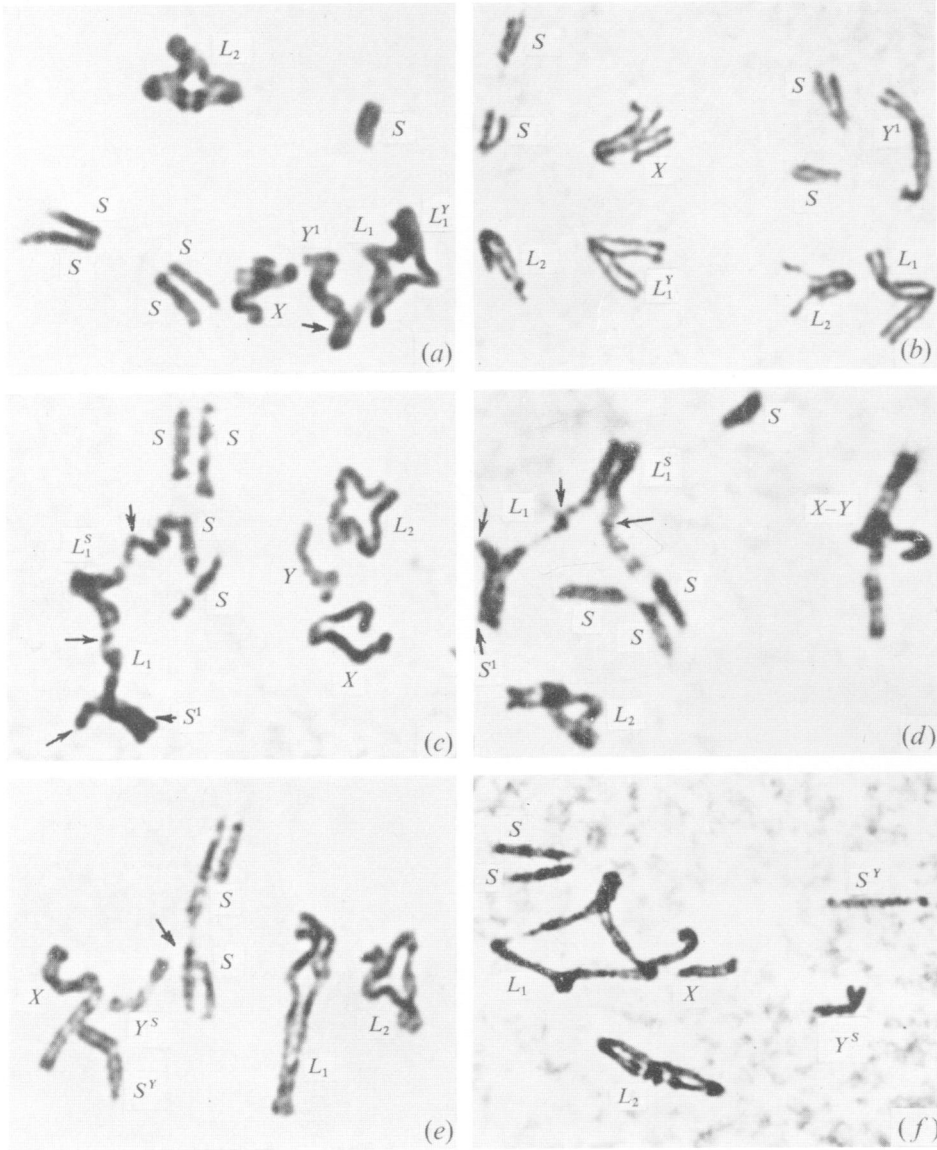
(a) L_1–Y interchange multiple at metaphase I. Arrow indicates region of association between L_1 and Y^1.

(b) Anaphase I in the L_1–Y interchange heterozygote. Compare enlarged Y^1 with normal Y in Plate 1b.

(c–d) Metaphase I in the L_1–S translocation heterozygote. Arrow indicates centromeres within the multiple. Note telomeric association of an S chromosome with the translocated S segment on L_1^S.

(e) Association of the Y^S with the X at early metaphase I in a Y–S translocation heterozygote. Arrow indicates telomeric attraction between S chromosomes and Y^S.

(f) Early anaphase I showing segregation of Y^S and S^Y from the X chromosome.

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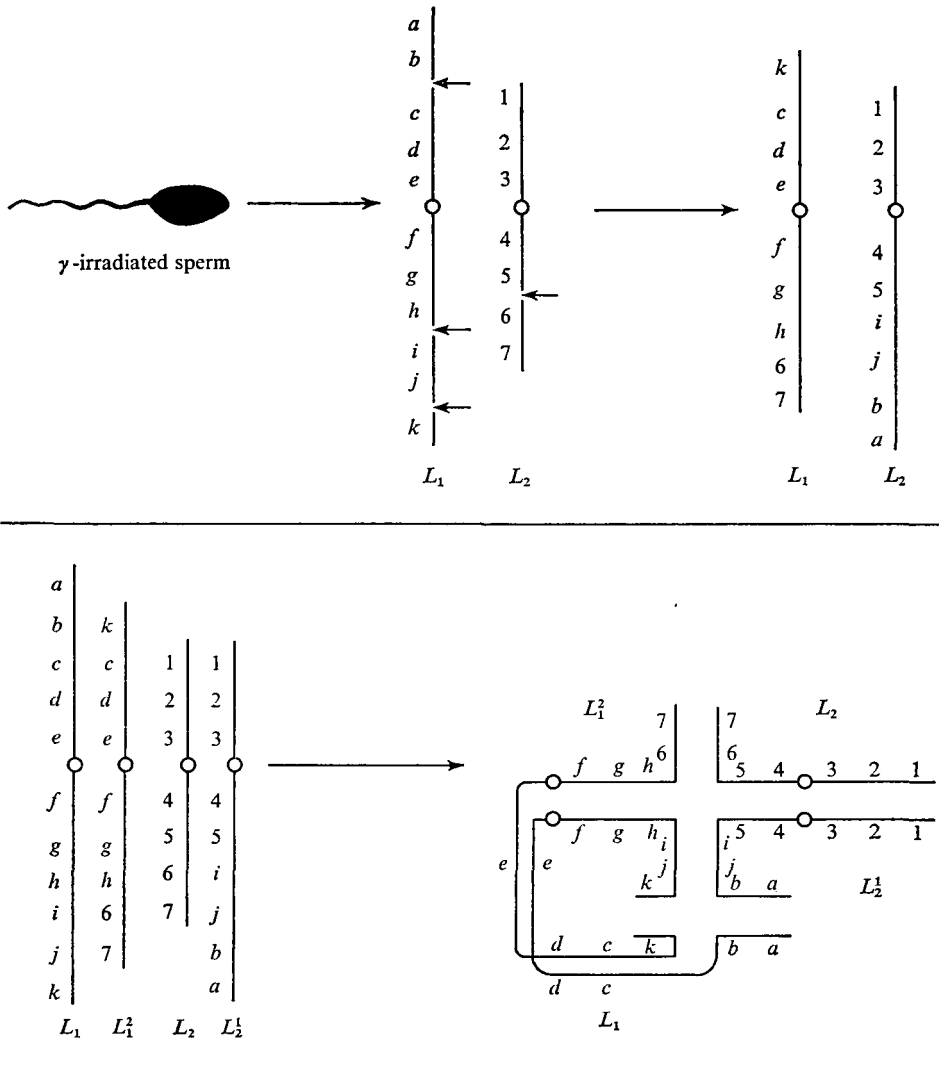
Text-fig. 2. Proposed scheme for the derivation of the L_1–L_2
multiple translocation illustrated in Plate 1e.

duced a chain multiple which persisted throughout first prophase until the commencement of anaphase I. At this stage (Plate 2b), it was usually possible to measure the enlarged Y^1 and shorter L_1^Y and compare them with normal Y and L_1 chromosomes.

A variety of abnormalities involving the S chromosomes was found:

1. L_1-S reciprocal interchanges. These mutant heterozygotes and the position of the exchange points could be easily and positively identified. At early prophase I there was a difference in chromaticity between L_1 and S chromosome segments, and at metaphase I, the part of the S translocated on to the longer arm of the L_1

chromosome usually entered into a telomeric association with one or more S chromosomes (Plate 2c–d).

2. *Y-S reciprocal interchanges.* In these cases chromosome segments which share a similar condensation cycle had been exchanged, but measurement of the chromosomes left no doubt that a translocation had occurred. Throughout first prophase the Y^S chromosome associated normally via a region on its short arm with the X chromosome and not infrequently the end of its translocated long arm was attracted to the non-centric ends of the S chromosome (Plate 2e). In all anaphase I cells the Y^S was observed to segregate to one pole and the X to the other, while the S^Y chromosome moved independently of either chromosome (Plate 2f).

3. *S-S translocation.* It was concluded that centric fusion following fracture and exchange within the centromeres of two S chromosomes gave rise to the metacentric univalent illustrated in Plate 3a–b. The finding of normal fertility in these F_1 males would suggest that fusion has occurred between non-homologous telocentric chromosomes and that there has been regular alternate segregation at meiosis.

4. *S interstitial deletion.* In all cases only a small centric fragment remained of one S chromosome (Plate 3c) and it was not unusual to observe precocious chromatid separation of this element at anaphase I (Plate 3d).

(iv) *Correlation of cytogenetic and fertility data*

As expected, in those F_2 families in which one individual with a translocation involving the Y chromosome was found, all male members of the family were found to carry it (five families and a total of 26 progeny). Among the nine families in which an autosomal translocation was found, the overall ratio of wild type : translocation heterozygotes was 10:21. The deviation from the expected 1:1 ratio can probably be explained by the fact that the number examined in each family was small and the existence of a translocation in the parent was only ascertained if it was found in one of the progeny. Therefore translocation-carrying families in which all progeny examined were, by chance, wild type were omitted from the sample.

Where a translocation was detected in at least some of the progeny of an F_1 male, the chromosomes involved in the translocation are indicated in Text-fig. 1 in the square corresponding to the fertility score of the F_1 male ($1 = L_1$, $2 = L_2$, S = the short chromosomes). Where all the male's progeny examined had normal chromosomes the corresponding square is marked $+/+$. An asterisk indicates the existence of a centric fragment (Plate 3c and d). The blank square represents an individual from which no progeny were examined.

With a few exceptions, the semi-sterile individuals were shown, by their progeny, to carry translocations, and the normally fertile ones were not. Thus most of the reduced fertility shown in Table 3 can be explained by the induction of translocations giving 50 % genetically balanced gametes. The two males with fertilities of 0·5–0·55 are classified as '$+/+$' on the basis of two and one progeny examined. The F_1 males concerned probably carried autosomal translocations and
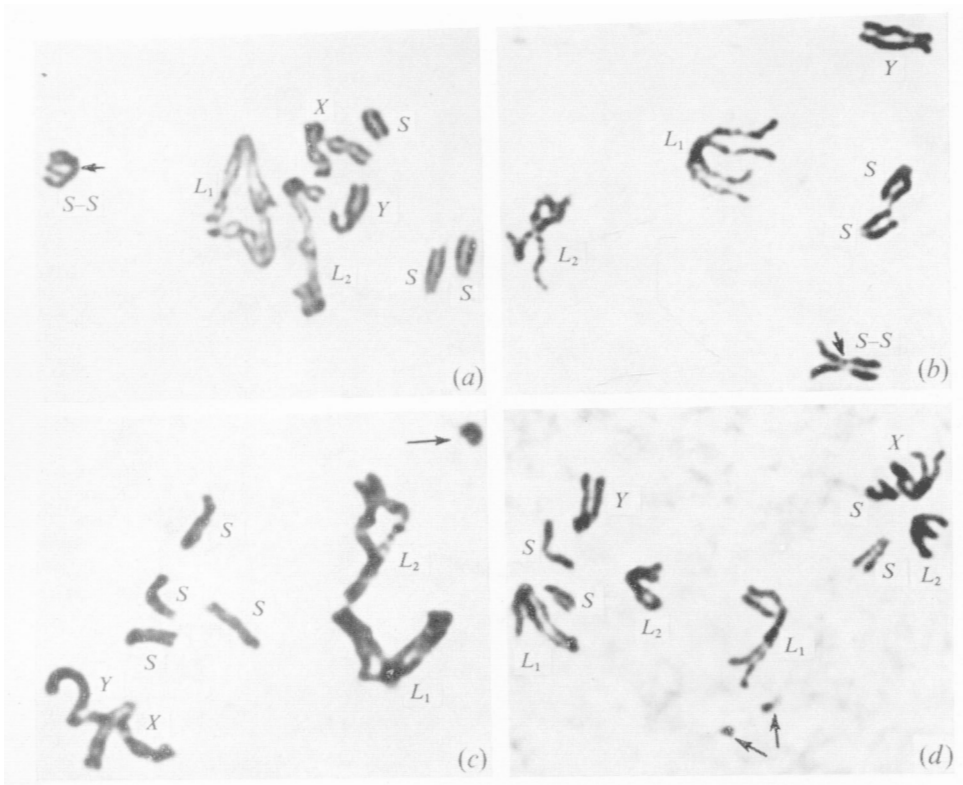
(a) Anaphase I showing univalent *S–S* metacentric. Arrow indicates centromere.
(b) *S–S* metacentric at prophase II. Arrow indicates centromere.
(c) Late prophase I showing *S* centric fragment (arrow).
(d) Precocious chromatid separation of the *S* fragment at anaphase I (arrow).

the pupae examined were probably from among the expected 50% wild-type segregants. However, the '$+/+$' male with a fertility of 0·7–0·75 was so classified on the basis of seven cytogenetically normal sons and it may be that in this case there was radiation damage, other than a translocation, with a limited sterilizing effect. The two individuals with translocations involving only the short chromosomes and the Y had fertilities which seemed to be intermediate between semi-sterility and the normal level. The number of short chromosomes varies between individuals and it may be that only in those zygotes which have the minimum viable number of short chromosomes is there lethality due to a deficiency for part of a short chromosome, following an unbalanced segregation of a translocation. However, in the three cases where only a centric fragment remained from one of the short chromosomes the fertility was in the normal range. Of the two examples of L_1–short chromosome exchanges, one was semi-sterile and one was normally fertile. Perhaps the latter case is explained by involvement of a non-vital section of the L_1 or by the occurrence of directed alternate segregation in this translocation.

The male which was shown to have a three-fold exchange involving the L_1 and L_2 (Plate 1e and Text-fig. 2) is indicated by 1–2 in Text-fig. 1: its fertility was within the range of the other semi-steriles. This is to be expected for a multiple exchange involving only two chromosomes in males which have no crossing-over (Curtis & Robinson, 1971).

The F_1 males showed examples of involvement of all possible chromosomes in translocations. It was anticipated that the six semi-sterile females might reveal an example of involvement of the large X chromosome, but they did not do so. From two of these females no translocation was found among a total of eight male progeny examined. It seems possible that the semi-sterility in these two females was due to cross-overs occurring in peri-centric inversions, though inversions were not detected cytologically.

(v) *Significance for the application of the sterile male technique*

Semi-sterility in the progeny of irradiated males would be a 'bonus' factor in attempts to control *G. morsitans* by the sterile male technique. The same is true of the sex-ratio bias against females, because virtually all adult female tsetses are inseminated in the wild (Glasgow, 1963), so that the reproductive potential of the population is limited by the numbers of breeding females and not the males.

For efficient use of the sterile male technique it will be necessary to choose the irradiation dose which gives the optimal combination of male survival, sexual competitiveness and sterilization, and the bonus from the F_1 sex-ratio distortion and semi-sterility should be taken into account in making the choice. A method has been developed for simulating the interaction of these various factors and for computing predictions of the population suppressing effect of releases of males which had received various doses (Curtis, 1972a). The method treats the sex-ratio distortion as differential rates of dominant lethality in male- and female-determining sperms, and hence assumes that the distortion would not be inherited. The

semi-sterility is assumed to be due to translocations which would be inherited like Mendelian dominants. As shown in this paper, these assumptions seem to be substantially valid. The simulation technique is based upon the assumption of a maximum two-fold population recovery potential per generation, an initial 90 % population suppression with insecticide, and sterile male releases at six successive generation intervals. Using male performance data for different radiation doses, interpolated from Tables 1 and 3 it was found that to achieve a target population reduction of $100 \times$ or $1000 \times$, the requirement for releases would be 15–50 % less than if the F_1 abnormalities did not exist. The saving due to the F_1 abnormalities would be at the upper end of this range where the target population reduction was large ($1000 \times$) and where a relatively low dose (e.g. 12 krad in nitrogen) was used.

To obtain more complete data on the dose/response relationship for the F_1 abnormalities, one could make use of the close correlation, which has been shown, of F_1 semi-sterility with translocations. Cytogenetic analysis and determination of the sex of the F_1 individuals early in pupal life would be considerably less time-consuming than the methods used in this work.

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