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Chromosome translocations in *Glossina austeni*

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SUMMARY

An autosomal translocation in the tsetse fly *Glossina austeni* was studied genetically, cytogenetically and for its effects on viability. Flies homozygous for the structural change could be identified by outcrossing to wild-type and demonstrating semi-sterility in all the progeny.

A cytogenetical analysis of male meioses in samples of pupae which were sibs of the semi-sterile progeny showed them to be structurally heterozygous for the translocation. Matings of the translocation heterozygotes and homozygotes gave the expected progeny ratios, with the exception of a deficit of females classified as translocation homozygotes. This was due to their sterility or inviability. Those female homozygotes which did breed showed a subnormal lifetime pupal production. These deleterious recessive effects were probably due to the translocation itself although the influence of linked loci could not be ruled out. These effects would prevent the mass rearing of this particular translocation for a tesetse control project.

Two other stocks which showed semi-sterility were found to carry autosomal translocations and two which currently showed holandric inheritance have *Y*-autosome translocations. One stock with holandric inheritance of extreme sterility carries a double translocation involving two autosomes and the *Y* chromosome.

1. INTRODUCTION

Tsetse flies (*Glossina* spp.) are the vectors of African trypanosomiasis and both sexes feed only on the blood of vertebrates. Reproduction is larviparous; after insemination early in adult life the female can give birth every 9 days to a larva which pupates soon after deposition. Pupal life lasts about 1 month and male meiosis occurs only between days 7 and 10 of this period (Itard, 1970; Haring & Fraser, 1968). This unusual reproductive physiology determines the kind of genetic and cytogenetic observations that are practicable and useful.

Until relatively recently very little information was available on tsetse-fly chromosomes but interest in this subject is now increasing (e.g. Itard, 1971) and for *G. morsitans morsitans* and *G. austeni* details of nuclear phenotype and chromosome behaviour, during meiosis and mitosis, are available (Southern, Craig-

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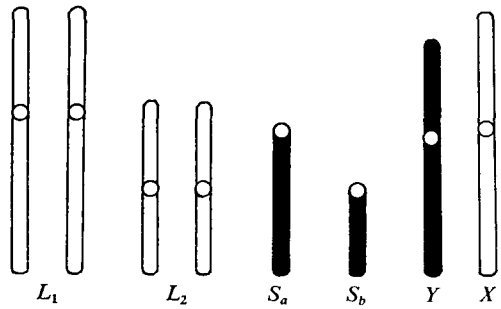

Fig. 1. Male karyotype of *G. austeni*.

Cameron & Pell, 1972*a*, *b*). To summarize: the chromosome number of wild-type *G. austeni* from the Langford colony is rather variable with a diploid count of $2n = 14 \pm 2$, XX/XY. The autosomes include a large pair of submetacentrics (L_1), a pair of shorter metacentrics (L_2) and a variable number of small telocentrics which we have grouped into two size categories: S_a and S_b (Fig. 1). Females of the species are the homogametic sex and carry two X chromosomes whereas in males the X and Y constitute a heteromorphic pair. Meiosis in the male appears to be achiasmate and there is no recognisable diplotene or diakinesis. Only L_1 and L_2 autosomes form bivalents (Plate 1). The S group remain as univalents and the X and Y chromosomes always associate, but the region of contact between them is restricted to a small area adjacent to their centromeres. All of the S chromosomes together with the Y exhibit allocyclic behaviour during both mitosis and meiosis.

The use of chromosome translocations for the control of insect pests was first proposed by Serebrovskii (1940) and the strategy of their use against tsetse was considered by Curtis (1968*a*, *b*), Curtis & Hill (1968, 1971) and Curtis & Robinson (1971).

Partially sterile mutants were induced with gamma radiation in *Glossina austeni* and they were inherited as unitary dominant factors (Curtis, 1969). Most showed an autosomal pattern of inheritance but a few showed holandric (father-to-son) transmission. Inbreeding of the autosomal stocks yielded some males which, when outcrossed to wild-type, were fully fertile but gave semi-sterile progeny (Curtis, 1971); these are the properties expected of translocation homozygotes. Preliminary evidence was presented of subnormal viability of these individuals. Two other mutant stocks were described (Curtis, 1971) which showed the autosomal type of inheritance for several generations, but subsequently 'switched over' to the holandric type.

It seemed probable that the partial sterility in these stocks was due to translocation heterozygosity, but it was important to confirm this by cytogenetical methods and to determine whether translocation homozygotes (T/T), heterozygotes (T/+) and wild-types (+/+) could be reliably identified cytogenetically. Following the production of moderate numbers of mutant homozygotes it was possible to study more thoroughly the breeding and fitness properties of the mutant.

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2. METHODS AND MATERIALS

The *G. austeni* wild-type stock, which originated from Zanzibar, has been maintained as a closed laboratory population of several thousand flies since 1966. The production of the partially sterile mutants and the numbering of the stocks was described by Curtis (1969). They were maintained in heterozygous form by outcrossing to wild-type and selecting the partially sterile segregants as parents for the next generation. After six generations of this the autosomal stocks were inbred and, in stock 68, after the production of individuals with the properties of translocation homozygotes, these were selected as parents of subsequent generations. The male for mating to a particular female was selected so as to minimize inbreeding.

A full description of the fly maintenance methods was given by Nash, Jordan & Boyle (1967) and Jordan, Nash & Boyle (1967) and the method of distinguishing partially sterile from fully fertile individuals was described by Curtis (1969). In summary: the flies were offered food daily on rabbits' ears, virgin females were mated individually and the subsequent pupal production of the inseminated females was recorded and related to the number of ovulation cycles that they had completed. The partial sterility of the mutants was found to be mainly due to embryonic deaths and was detectable only as a reduced rate of pupal production, but in some cases deaths were at a later stage and dead larvae were expelled from the uterus. Females of the mutant stocks were maintained individually in small ('Geigy-1') cages and coaxed to feed; in the same conditions control matings ($+/+ \times +/+$) produced pupae from 100% of ovulation cycles (data from 48 flies and a total of 367 cycles). For testing males, they were mated successively to four $+/+$ females which were kept together in 'Geigy-10' cages; controls in these conditions produced pupae from 90% of cycles. The statistical criteria used for distinguishing partial sterility from normal fertility were as specified by Curtis (1969).

Measurement of the fertility of the various matings was made over the period of maximum productivity, i.e. unless otherwise stated, up to day 120 in 'Geigy-10' cages and up to the 20th ovulation cycle in 'Geigy-1' cages. Females shown, by observation of their spermathecae, to be uninseminated were omitted in calculating fertilities. The pupae for cytogenetical studies were bred at Langford, packed individually in labelled tubes and mailed to Manchester in polystyrene boxes (Kernaghan & Nash, 1964). Apart from their time in transit they were maintained at 25 °C and a humidity of 70% until the time of dissection.

The wild-type karyotype of this species (Text-fig. 1) is based upon chromosome morphology at mitotic metaphase in dividing cells of the central nervous system from pupae up to 5 days after larviposition. Meiosis was examined in the testes of male pupae between 9 and 10 days after deposition. A detailed account of the techniques employed for making these chromosome preparations has been given elsewhere (Southern *et al.* 1972*a*, *b*) and we will restrict ourselves to a résumé of the process.

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Testes were removed from male pupae, cleared of clinging fat by distilled water and then gently tapped out in 5% acetic orcein. After allowing the cells to take up the stain for 1 h the slides were gently warmed and finally flattened by finger pressure. A similar procedure was adopted for making mitotic preparations.

3. RESULTS

(i) *Correlation of fertility and cytogenetic observations in stock* 68

Among the members of stock 68 translocation complexes were repeatedly found during male meiosis. The translocation will be referred to as translocation no. 68. A careful analysis of first prophase and in particular second metaphase in these structural heterozygotes showed that the interchange involved a small terminal segment from one of the L_2 autosomes and a large terminal segment from an L_1 (Plate 2*a*, *b*). This results in four partially homologous chromosomes associating together and producing the type of pairing cross illustrated in Plate 2(*c*, *d*) at zygotene/pachytene.

At metaphase I the multiple can orientate in a variety of ways. The type illustrated in Plate 2(*e*, *f*) will produce gametes which carry deficiencies and duplications for various chromosome segments and, if involved in fertilization, inevitably lead to the formation of inviable zygotes. If the multiple orientates in an alternate manner, i.e. L_1, L_2 to one pole and L_1^2, L_2^1, to the other, then all gametes will be genetically balanced and should produce viable zygotes.

It seemed likely that heterozygosity for this translocation was the sole cause of partial sterility in this stock. In some species such a hypothesis can be tested by observations on fertility and karyotype in the same individual (LaChance, Degrugillier & Leverich, 1970), but such a test is not possible in tsetse because male meiosis is restricted to the pupal stage. The next best approach was to observe the fertility and karyotype of sibs produced by inbred individuals which had been shown to be fully fertile and which, on the above hypothesis, were either translocation or wild-type homozygotes. Fig. 2 shows the results of some of these tests; the results of fertility tests to known wild type are shown in terms of pupae/ovulations. Individuals A and B both showed high fertility and in each case there was no segregation among their progeny – all those from A were semi-sterile or T/+, and those from B were fully fertile or +/+. It is therefore reasonable to identify all semi-steriles as T/+, A as a T/T and B as a +/+.

The test mating of D showed it to be T/+, but C and E could not be identified because the fertility of a homozygote × T/+ mating cannot be distinguished with statistical confidence from that expected from a T/+ × T/+, with the limited number of zygotes that a tsetse female can produce.

Apart from individuals A and B, ten other fully fertile products of inbreeding in stock 68 were progeny-tested both by the fertility and cytogenetical methods, and in every case the methods agreed and the parent could be unambiguously identified as either T/T or +/+. The cytological method has the important advantage that it can be applied 10 days after pupation, whereas for a fertility

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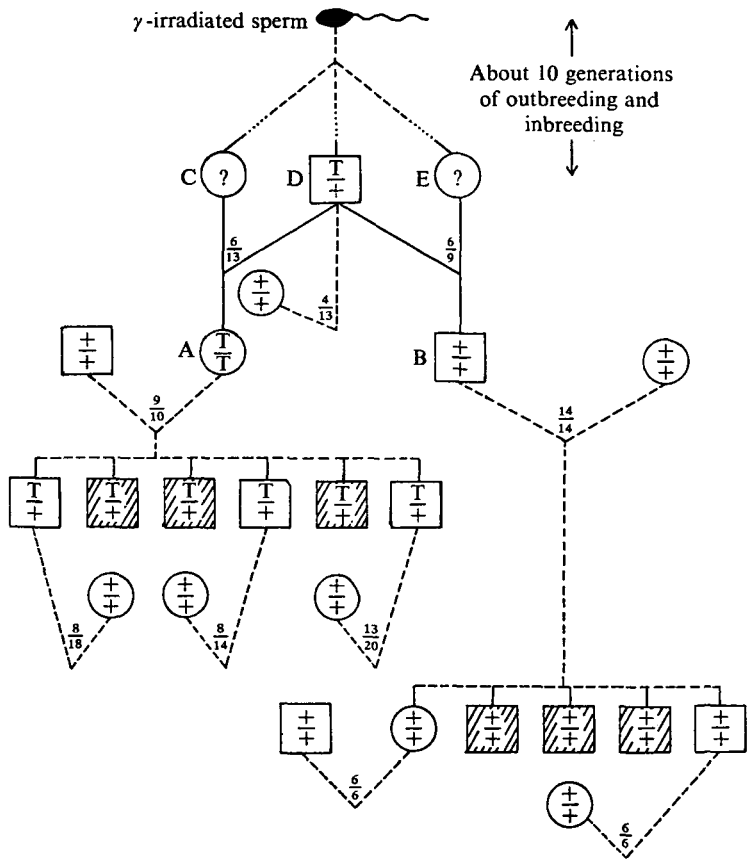

Fig. 2. Diagram of the pedigrees of two individuals, A and B, which were identified as T/T and +/+ respectively by both the cytogenetical and fertility test methods. □, Male; ○, female; —, Inbreeding; – – –, test mating to known wild-type; ▨, cytogenetically identified. Figures indicate (no. pupae)/(no. ovulations).

test the individual must be allowed to eclose, and it or its mates must be maintained for a further 60 days.

(ii) *The genetics of the no. 68 translocation*

Table 1 shows data on the fertility and progeny produced from nearly all the possible types of mating involving the no. 68 translocation. T/+ flies for these matings came from +/+ × T/T parentage and they therefore did not have to be selected by test mating. The fertility of T/+♀ × +/+♂ appeared to be higher than the reciprocal mating, but the difference was not statistically significant (total numbers of pupae and ovulations gave heterogeneity $\chi^2_1 = 1{\cdot}32$). The product of the estimates of fertility of the T/+ male and T/+ female (= 0·282) gave the expected proportion of fertilizations between genetically balanced gametes in T/+ × T/+ matings. The observed fertility (Table 1) was significantly greater

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Table 1. *The fertility and progeny produced from matings involving translocation no. 68*

(Classification of progeny was based partly on fertility tests and partly on karyotype observations. Males were classified as inviable if they died before mating on day 10 and females if they died before the end of the fourth ovulation cycle.)

Parents ♀	Parents ♂	No. of matings	Fertility (÷ +/+ × +/+ control)	♂ progeny +/+	♂ progeny T/+	♂ progeny T/T	♂ progeny Sterile and/or inviable	♀ progeny +/+	♀ progeny T/+	♀ progeny T/T	♀ progeny Sterile and/or inviable
T/+	+/+	52	0·575	9	7	0	—	—	—	—	—
+/+	T/+	36	0·492	21	22	0	—	—	—	—	—
T/+	T/+	51	0·351	10	18	1	—	—	—	—	—
+/+	T/T	35	0·961	0	68	0	—	0	32	0	4
T/T	+/+	3	0·857	0	7	0	—	0	4	0	0
T/+	T/T	10	0·547	1	17	15	3	0	9	2	9
T/T	T/T	3	0·857	0	0	7	2	0	0	2	11

than this ($\chi_1^2 = 4{\cdot}48$, $P < 0{\cdot}05$). This indicates that certain unbalanced gametes can complement each other's duplications and deficiencies and give viable zygotes; this has been shown to occur in *Drosophila* (Muller & Settles, 1927) and mice (Snell, 1946).

The data on the progeny of matings involving T/+ and +/+ came from cytogenetical observations and, as already explained, were made on male progeny only. Both reciprocal matings of T/+ and +/+ showed close agreement with the expected 1:1 ratio (Table 1).

A 1:1 ratio of +/+:T/T would be expected from the T/+ × T/+ matings but the observed numbers reveal a significant deficit of T/T ($\chi_1^2 = 5{\cdot}82$). As Table 1 shows, only one pupa was identified as T/T. At meiotic prophase in it the two L_1^2 chromosomes and the two L_2^1's formed their respective bivalents and there was very little difference between them in overall length. More critical evidence that it was T/T came from second metaphase nuclei, for at this stage the position of the centromeres can be accurately located and the ratio of the arms lying on either side of it can be compared. Every nucleus contained a submetacentric L_2^1 and an almost metacentric L_1^2.

The results for the reciprocal T/+ × +/+ mating exclude an explanation of the shortage of T/T males from T/+ × T/+ matings in terms of segregation distortion and results from T/+♀ × T/T♂ (see below) indicate no selective mortality of T/T pupae. Even if all the dead pupae found at dissection from T/+ × T/+ matings were T/T, it would not entirely account for the deficit of T/T individuals and this remains unexplained. The fertility of the +/+♀ × T/T♂ matings was normal (Table 1), i.e. the T/T males, as expected, produced only genetically balanced sperms. The fertility of T/T females was subnormal. The data given in Table 1 only covers the first ten ovulations, as T/T females which survived to that age tended to become permanently sterile after that time. The reduced fertility of the T/T

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Table 2. *Fitness of heterozygotes for translocation no. 68 compared with controls from the laboratory colony*

(The flies were of outbred origin and maintained in groups in 'Geigy-10' cages.)

Sex	Genotype	Parental origin	Pupal eclosion (%)	Mean adult survival: No. tested	Mean adult survival: Days	Mean adult survival: % of control	Mating competition (no. copulations achieved)
♂	+/+	Colony	98·5*	39	77·9	100	22
♂	T/+	+/+ × T/T	93·7	39	64·4	83	26
♀	+/+	Colony	98·5*	157	140*	100	—
♀	T/+	+/+ × T/T	93·7	71	162	115	—

* Data from A. M. Jordan (personal communication).

Table 3. *Fitness of heterozygotes and homozygotes for translocation no. 68*

(The translocation flies were of inbred origin and they and the wild-type controls were maintained individually in 'Geigy-1' cages.)

Sex	Genotype	Parental origin	Pupal eclosion (%)	Mean adult survival: No. tested	Mean adult survival: Days	Mean adult survival: % of control	Failure to mate	Failure to inseminate
♂	+/+	Colony	98·5*	10	125	100	4·9%*	1·2%*
♂	T/+	T/+ × T/T	83·0	12	77·6	62		
♂	T/T	T/+ × T/T and T/T × T/T		31	60	48	10/93	10/82

Sex	Genotype	Parental origin	No. sterile and/or inviable	Survived and bred: No.	Survived and bred: Mean lifetime pupal production
♀	+/+	Colony	2	48	17·6
♀	T/T	T/+ × T/T and T/T × T/T	22†	7†	6·7

Mean inbreeding coefficient of sterile and fertile T/T ♀♀: Own 0·143 ← t_{27} = 1·12 → 0·113; Dam's 0·100 — 0·099

* Data from A. M. Jordan (personal communication).

† Includes some data excluded from Table 1 because the genotype of the mother could not be identified with certainty.

females was presumably not due to the production of genetically unbalanced gametes, but to a subnormal ability to maintain pregnancies; other evidence for the reduced fitness of T/T females is given below. The subnormal fertility of T/T females means that they can only be distinguished with certainty from T/+ females by the presence or absence of segregation among their male progeny. Therefore, matings and female progeny have only been included in the last three rows of Table 1 where they could be identified by their male progeny.

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All the testable progeny of the reciprocal T/T × +/+ mating were T/+: the identification of one of their parents as T/T depended on this fact. It is notable that only 4 out of the 36 female progeny from these matings were sterile or inviable, i.e. died before completion of the fourth ovulation cycle. In the generation immediately following induction of the translocations a large proportion of the females were sterile and/or died young, and this was interpreted as being mainly due to an interaction of translocation heterozygosity with other radiation induced genetic damage (Curtis, 1969). It appears that in the subsequent ten generations of breeding this damage was selectively eliminated.

The male progeny of the T/+♀ × T/T♂ mating showed the expected 1:1 ratio of the parental genotypes (Table 1). The one +/+ male (Curtis, 1971) was a rare anomaly of doubtful significance. The female progeny of this mating showed an apparent deficiency of the T/T type with a large proportion of sterile and/or inviable females, i.e. a reappearance of the 'syndrome' mentioned above. If all the latter were T/T the deficit would be accounted for. The suggestion that there is a tendency for T/T females to be sterile and/or inviable is supported by the large proportion of such females in the progeny of T/T × T/T matings (Table 1).

(iii) *The fitness of heterozygotes and homozygotes for translocation no. 68.*

Data collected on various components of fitness for translocation heterozygotes and homozygotes in comparison with wild-types from the laboratory colony are shown in Tables 2 and 3. Table 2 shows that outbred T/+ males did not survive as well as controls. However, in mating competitions between individual T/+ and +/+ males (of the same age and distinguished by paint marks) in a cage with one +/+ female, the two types performed equally well. The outbred T/+ females survived somewhat better than controls, but their pupal eclosion rate was slightly lower.

Table 3 refers to inbred T/+ and T/T individuals and it shows that their pupal eclosion rate was markedly lower than controls. The genotype of the dead pupae of course could not be ascertained. Compared with controls maintained in corresponding environments the inbred T/+ males had a mean survival rate of 62% (Table 3) while for the outbred T/+ males the rate of survival was 83% (Table 2). This difference may be due to inbreeding depression: the inbreeding coefficient of the inbred T/+ males ranged from almost zero to 0·22. The T/T males did not survive as well as the T/+ males from the same parental origin and this may well be an indication of recessive deleterious effects following the break up and rearrangement of linkage groups. As shown in Table 3, the T/T males tended to have inferior mating and inseminating ability, although when they did inseminate their mates, fertility was normal (Table 1). For reasons already stated, females which were derived from T/T fathers and which proved to be sterile and/or died young were interpreted as T/T. Table 3 indicates that only about one quarter of the T/T females born survived and delivered progeny.

It seemed possible that inbreeding depression was the precipitating factor in

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making some T/T females sterile and/or inviable, and the inbreeding coefficient of each was calculated from its pedigree (Falconer, 1960). The mean inbreeding coefficient of the sterile females was higher than the fertile ones, but not significantly so (Table 3). There is a precedent for a maternal effect causing sterility in tsetse (Saunders, 1971), but there was no difference in the mean inbreeding coefficient of the dams of the sterile and fertile females (Table 3). Seven full sib matings (which give an inbreeding coefficient of 0·25) were made for comparison in non-mutant stock. There was 10% embryonic mortality, but of the five female progeny tested none were sterile or died young.

The seven fertile T/T females showed abnormally low mean lifetime production of pupae after mating to homozygotes. This was because of short mean life-span as well as their early cessation of pupal production.

(iv) *Stock nos. 53, 622, 626, 73 and 74*

Five other stocks carrying partially sterile mutants were studied cytologically and a translocation was found in each case. In stock 53 the interchange had occurred between an L_1 and an L_2 autosome. From the appearance of the pairing cross at meiotic prophase it was concluded that this translocation closely resembled the one already described for stock 68 (Plate 2*a–f*). Some difficulty was experienced in finding the translocation in stock 622, which was not surprising for only the minutest of L_1 and L_2 terminal segments had been interchanged. In consequence, multiples were rarely observed during early meiosis and in the vast majority of cells the chromosomes formed bivalents. Attempts to obtain males which were structurally homozygous for these two translocation types met with little success, and for each stock only one such male was produced.

Stocks 626 and 73 showed a 'switch-over' from apparently autosomal to holandric inheritance of semi-sterility (Curtis 1971) and three further generations of breeding have continued to show holandric inheritance.

Cytogenetical studies during the last three generations have revealed Y-autosome translocations in all 13 males examined from stock 626 and in a male from stock 73. In both stocks a small terminal segment of an L_1 autosome had interchanged with a large terminal segment from the long arm of the Y chromosome (Plate 3*a*, *b*). Plate 3(*c*, *d*) illustrates the appearance of the pairing complex at pachytene. The heterochromatic portion of the Y on the ${L_1}^Y$ remains embedded in the heterochromatic mass of the S chromosomes, while a small segment close to the centromere on the shorter arm of the X associates with a region adjacent to the centromere on the translocated arm of the Y^{L_1}.

The multiple is sufficiently flexible to enable it to orientate in several ways at metaphase 1; the alternate type allows the L_1 and X to move to one pole, giving a female-determining sperm, while the Y^{L_1} will travel to the same pole as the ${L_1}^Y$, giving a male-determining sperm (Plate 3*e*). Thus any zygotes which result following fertilization should be viable, one a +/+ female, the other a male heterozygous for the translocation. Adjacent orientation leads to gametes carrying

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duplications and deficiences (Plate 3*f*) but, rather surprisingly, not all the products of fertilization are inviable. Such a case was found in one individual where all the mitotic cells examined contained the Y^{L_1}, an X and two normal L_1's. This was, of course, an unbalanced combination as a complete segment of the Y was missing, being replaced by a third region of an L_1. Pupal development had been normal up to the time of dissection, but, although a major part of the Y was present, no testes (or ovaries) were found.

Stock 74 has shown holandric inheritance of extreme sterility from when it was first isolated (Curtis 1969) through 13 generations. One anomalous fully fertile male was found out of 24 tested. The mean fertility of the partially sterile matings was 0·232 pupae per ovulation, i.e. 0·257 of the control level.

When testicular cells were examined the L_1 and L_2 autosomes together with the X and Y were found to be involved in a complex pairing association at first prophase and metaphase. This we interpreted as being due to a double translocation. The cytogenetical evidence suggests that a large terminal segment from the longer arm of the Y had interchanged with a much smaller end segment from one arm of an L_2, while the second interchange involved a large segment from the other arm of the same L_2 autosome and a small terminal region of an L_1 chromosome. This scheme explains the holandric inheritance and such a double heterozygote could be expected to give 25% orthoploid gametes (Curtis & Robinson, 1971), which agrees with the observed fertility level.

4. DISCUSSION

The level of fertility in translocation heterozygotes 53, 68, 622, 626 and 73 was about 50% of that of the controls (Curtis, 1969). This must in large measure reflect an approximately equal ratio of adjacent to alternate orientation of the interchange multiple at metaphase I. Clearly the size of the segments exchanged does not materially influence this ratio in tsetse flies. In progeny-testing for the identification of T/T individuals the cytogenetic method saves 80 days compared with the use of fertility testing and this could be of the greatest value when screening numerous translocations in search of suitable T/T properties for a tsetse control project. In the case of the no. 68 translocation the cytogenetic method was shown to be completely reliable, but for translocations, such as 622, where very short segments had been exchanged and multiple formation was only an occasional event, the task of identifying T/+ proved far more difficult.

The technique of chromosome analysis would be useful in the stage of a control programme at which field trials were carried out. It would be necessary to monitor the frequency of stucturally mutant individuals that have been established in a natural population, following the release of a translocation stock. This will probably best be done by capturing males and mating them to +/+ females. A cytogenetical examination of the pupae at the appropriate date after deposition should enable one to establish their chromosome constitution. By using +/+ females as one parent the progeny will be either +/+ or T/+. This would avoid the problem of

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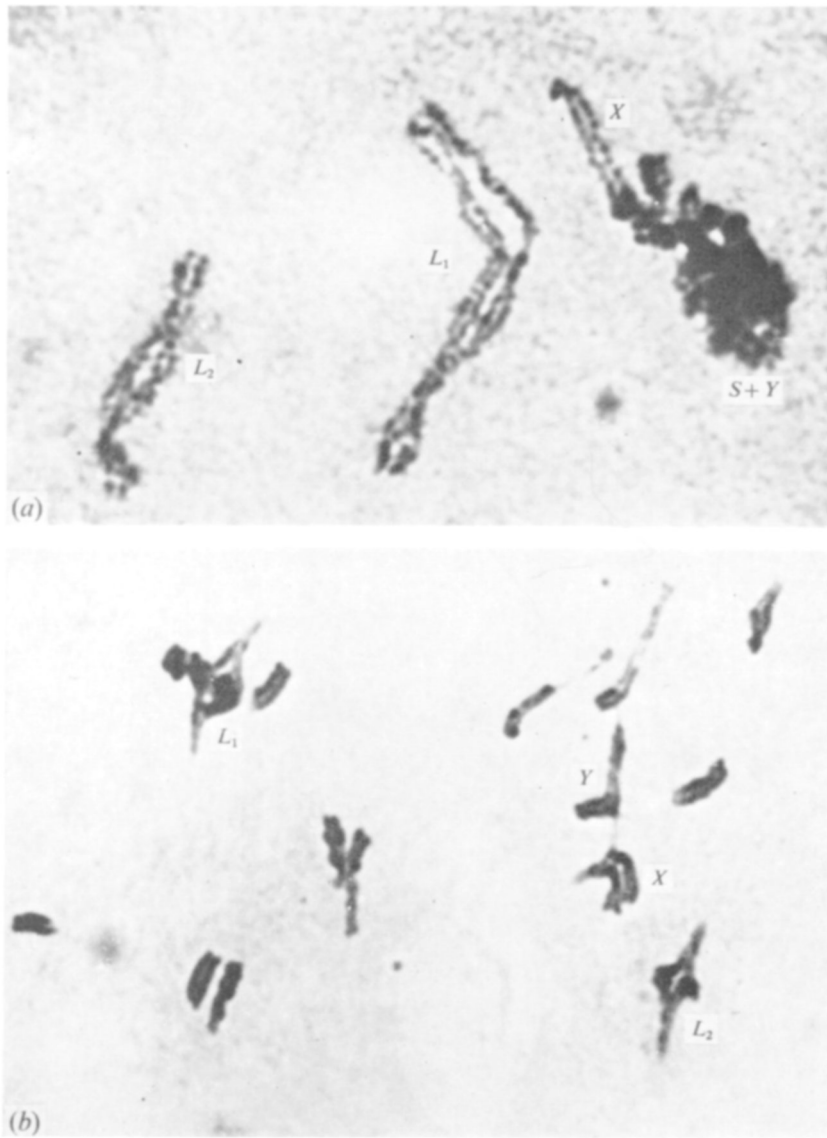

(*a*) Prophase I during + / + male meiosis, showing the *X* chromosome and L_1 and L_2 bivalents. The heterochromatic *Y* and *S* chromosomes remain associated throughout this stage and do not separate one from another until they move to the poles at Anaphase I.

(*b*) Late metaphase I/early anaphase I during + / + male meiosis. The *S* chromosomes are moving towards the poles, the *X* and *Y* have started to separate but the L_1 and L_2 chromosomes remain as bivalents.

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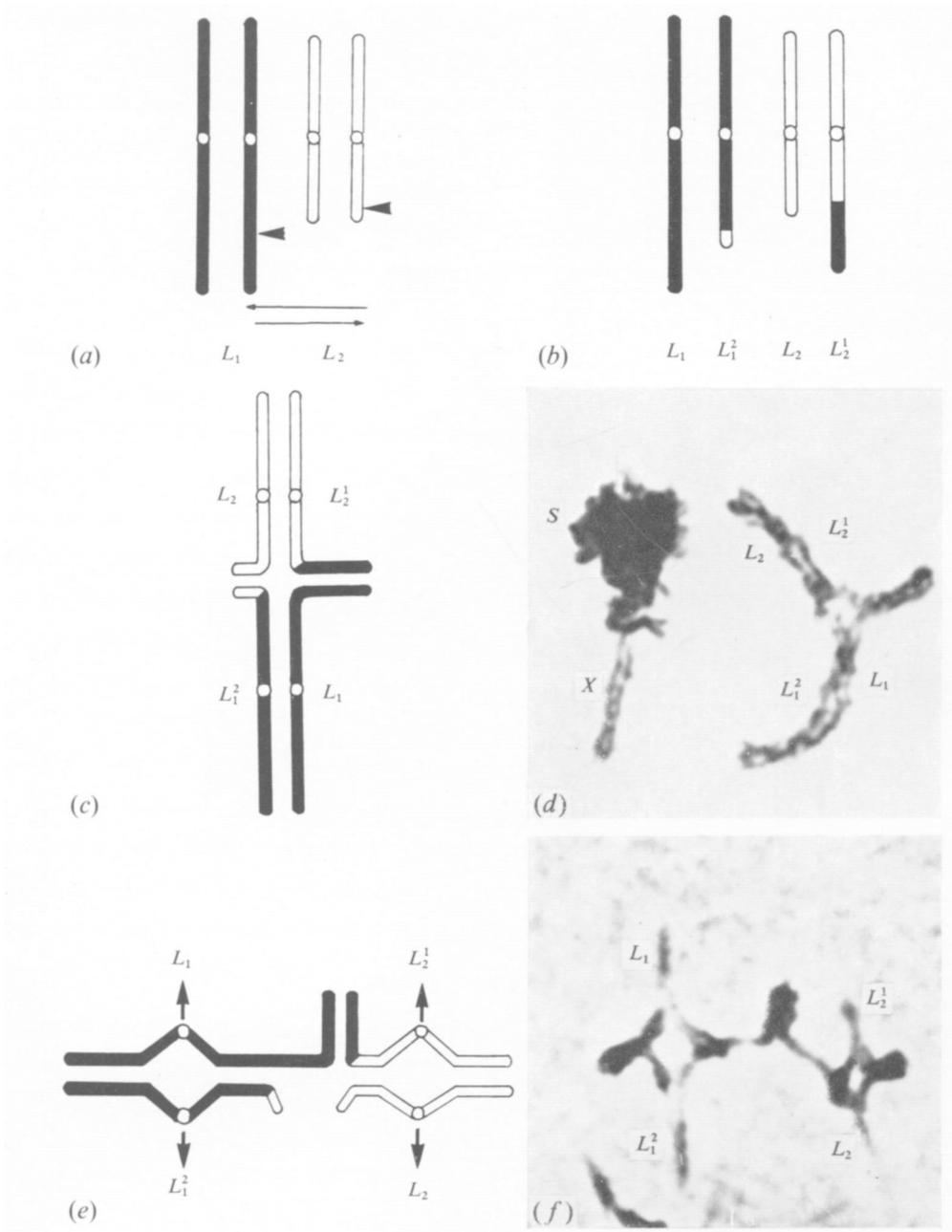

(*a*, *b*). Scheme for the production of the L_1–L_2 interchange in stock 68 and stock 53.

(*c*, *d*) The L_1–L_2 pairing cross at pachytene in the structural heterozygote.

(*e*, *f*) Adjacent non-homologous orientation of the L_1–L_2 multiple at metaphase I.

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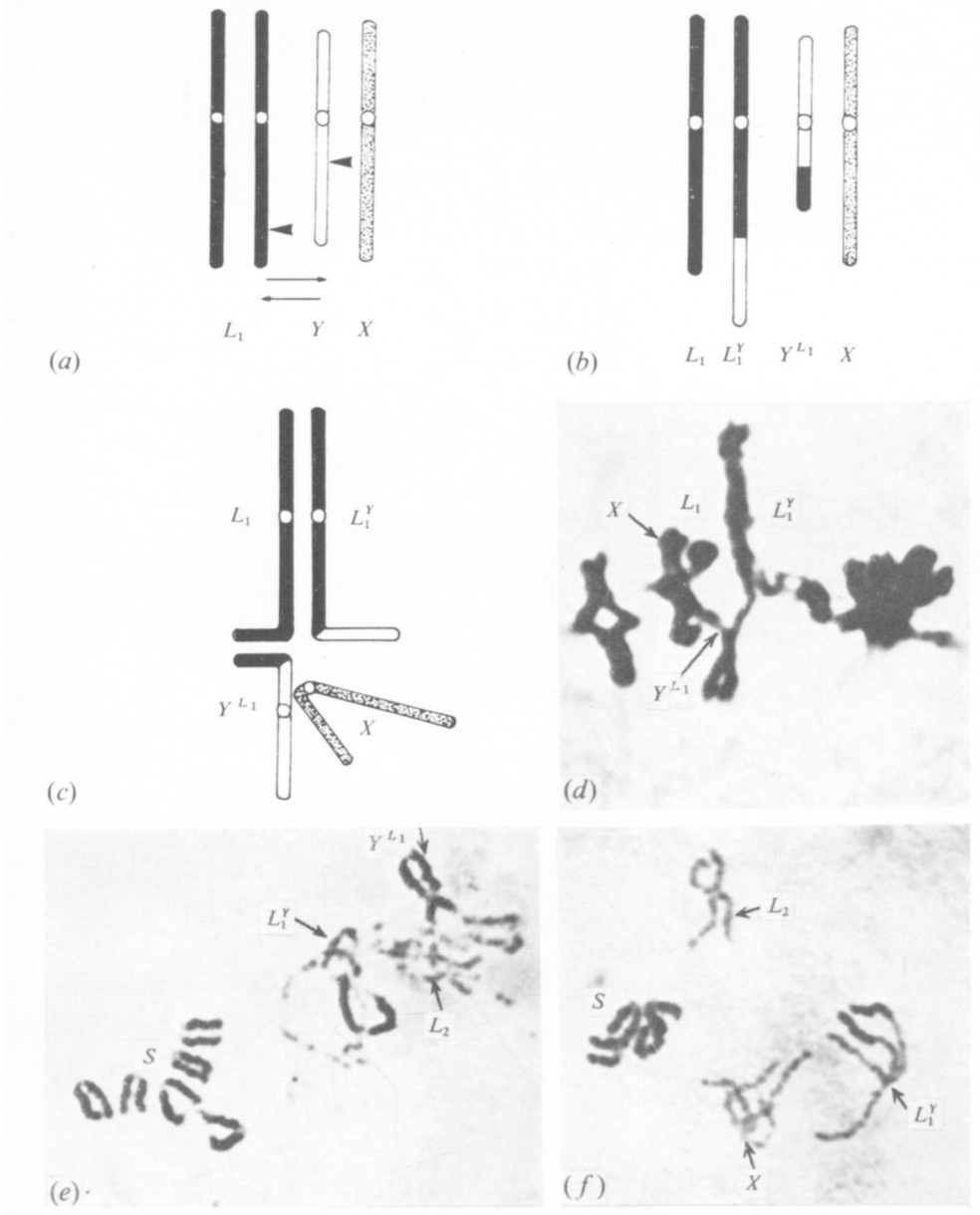

(*a*, *b*) Scheme for the production of the L_1–Y interchange in stock 626.

(*c*, *d*) The pairing cross during prophase in the structural heterozygote.

(*e*) A second prophase nucleus containing both an $L_1{}^Y$ and Y^{L_1} chromosome in stock 626 following alternate orientation of the interchange multiple.

(*f*) An unbalanced second prophase nucleus containing an $L_1{}^Y$ and X chromosome following adjacent orientation in stock 626.

Note the differential staining of L_1 and Y segments in (*e*) and (*f*).

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identifying T/T individuals, which would be difficult if the translocations were symmetrical.

The Y–L_1 translocations satisfactorily explain the holandric inheritance of semi-sterility found in stocks 626 and 73. It was suggested (Curtis, 1971) that the change in pattern of inheritance in these stocks might have been due to selection for a new system of sex-determination not involving the Y-chromosome, but this hypothesis is disproved by the cytological observations. Occasional anomalous fully fertile males (such as occurred once in stock 74) might be explained by non-virginity of +/+ females used for mating, since W. A. Foster (personal communication) has shown that the technique employed of separating the sexes within 24 h of eclosion is not entirely reliable for ensuring virginity in *G. austeni*. However, this will not explain the several semi-sterile females which occurred in the early generations of stocks 626 and 73, and it seems that some type of non-disjunction must be postulated to explain them.

Apart from a slight reduction in pupal eclosion rate and male survival, the outbred T/+ of stock 68 displayed good viability (Table 2). The T/T males proved to be subnormal both as regards survival rate and mating success (Table 3), although neither factor was sufficient to inconvenience a rearing operation of these homozygotes. There was, however, a strong tendency to sterility in the T/T females (Table 3). Sterile T/T females did not have a significantly higher inbreeding coefficient than fertile ones, but this does not exclude the possibility that sterility was due to homozygosity for recessive deleterious factors linked to, but not identical with, the translocation breakpoint.

The 100 % survival from zygote to pupa found in control matings (see Methods section), where the females were maintained in individual cages, indicates that the genetic load in tsetse flies is extraordinarily light – perhaps because of their low natural fertility. On the other hand, sib-mating of non-mutant stock did yield some zygotic mortality, which indicates that some genes which are deleterious when homozygous are carried in the genome. Moreover, radiation damage other than chromosome rearrangements in the translocation stock may not have been entirely eliminated by backcrossing to wild-type. Therefore, although it seems probable that unfitness of the T/T females was due to gene damage or position effects, following the translocation, other linked loci may have made a contribution. In future attempts to produce viable T/T stocks, it would probably be wise to establish several separate lines of backcrossing of the same translocation to wild-types and to attempt to produce T/T individuals by crossing these lines. Because, as seems probable, there is no crossing-over during male meiosis, the suggested backcrossing should be carried out using T/+ as the female, since one's aim is to establish different alleles at loci linked to the translocation in the separate lines of backcrossing.

Reduced fitness of translocation homozygotes is common in insects (Lea & Catcheside, 1945; Laven *et al.* 1971; Robinson, 1971) unlike the situation in mice (Carter, Lyon & Philips, 1955) and maize (Gopinath & Burnham, 1956) so it would be very difficult to produce a translocation homozygote in tsetse which would

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survive and compete with the members of a wild population. However, if two different T/T populations were reared and crossed and the resulting double heterozygotes released, reduced fitness of the homozygotes in the wild would hardly effect the efficiency of population control (Curtis & Robinson, 1971). The only requirements are that any deleterious effects of the translocations are recessive, so that the double heterozygote is competitive in the wild, and the T/T flies are sufficiently fit so that they could be economically mass reared. The no. 68 translocation would not fulfil the latter condition, but we have little doubt that an intensive search, using the methods outlined, would produce the necessary translocation stocks.

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