

## X-CHROMOSOME INVOLVEMENT IN MALE HYBRID STERILITY FROM *GLOSSINA MORSITANS* SUB-SPECIES CROSSES

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### SUMMARY

Crosses between sub-species of the tsetse fly *Glossina morsitans* yield males which can mate but are unable to inseminate. To test the hypothesis of X-chromosome involvement in this form of male sterility, *G. m. morsitans* carrying the X-chromosome marker *ocra* were mated to wild type *G. m. centralis*, and the F<sub>1</sub> females backcrossed to each parental population. The *ocra* gene was used to distinguish those male progeny which had inherited their X-chromosomes from each sub-species and these categories of male from each backcross differed significantly in the proportion which could inseminate. It is concluded that inability to inseminate is caused by an interaction of the X-chromosome from one sub-species with the Y-chromosome and/or autosomal factors from the other. The results are discussed in relation to similar observations from crosses within two *Anopheles* species complexes and to the genetic control of tsetse flies.

### 1. INTRODUCTION

*GLOSSINA MORSITANS* tsetse flies are the main vectors of nagana (trypanosomiasis of domestic animals) in African savannas. Several sub-species of *G. morsitans* were recognised on morphological grounds by Vanderplank (1949) and were re-named by Machado (1970). Crosses between different sub-species and between these and *G. swynnertoni* were shown to be partly sterile and to produce sterile male hybrids (Potts, 1944; Vanderplank, 1944; Vanderplank, 1948). Mating competitiveness was tested in the field (Jackson, 1945) and the first-ever field trial of a genetic control system was carried out between 1944 and 1946 using the sterility from species crosses (Vanderplank, 1947 and personal communication).

The genetics of the sterility produced by crossing *G. morsitans* sub-species was studied with more reliable laboratory rearing techniques (Curtis, 1972). It was concluded that the partial sterility in the crosses was probably due to incompatibility between the genes of pregnant females and their hybrid larvae *in utero*. The male hybrid sterility from all possible reciprocal crosses between three sub-species appeared to be due to a sperm defect which prevented normal migration of sperm from the spermatophore to the spermathecae in the mates of hybrid males. The ability or inability to inseminate was characteristic of an individual in repeated tests and this character segregated in the progenies of backcrosses of female hybrids to either parent. It was suggested that the sterility might be due to heterozygosity at an autosomal locus. In an analogous case in the *Anopheles maculipennis* complex an explanation in terms of incompatibility of alien X- and Y-chromosomes was preferred (Fraccaro *et al.*, 1977). Involvement of

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the X-chromosome in causation of the male hybrid sterility in the *An. gambiae* complex was proved using an X-chromosome marker (Curtis, 1978*a* and *b*).

An X-chromosome marker, *ocra*, has become available in *G. morsitans* (Bolland *et al.*, 1974). This paper describes a test for X-chromosome involvement in causing the male hybrid sterility in this group.

## 2. MATERIALS AND METHODS

The stocks used were:

*G. morsitans morsitans* originating from Kariba, Zimbabwe, and carrying the sex linked recessive gene *ocra*, causing yellowish body colour, selected from within a laboratory stock by Bolland *et al.* (1974). The mutant stock is now maintained at Langford.

*G. m. centralis* originating from Singida, Tanzania, were supplied by I.L.R.A.D. in 1979 and maintained at Langford. The stock has wild type (dark brown) body colour.

The stocks and crossed females were maintained by feeding through artificial membranes (Mews *et al.*, 1977). Crosses were made by isolating virgin females within 24 hours of emergence and mating them on day three to males aged at least seven days. The mated females were maintained for 90 days and the survival and number of pupae produced was recorded daily from day 16 of life so that fecundity could be calculated in terms of total pupae per aggregate number of mature female days. Each cross was made with a total of 120 females (15 in each of 8 cages). Each backcross was made with 75 females, 15 in each of 5 cages.

The ability of male progeny to inseminate was tested by confining them at the age of seven days with three day old *G. m. morsitans* virgin females and, after 24 hs, dissecting the females and examining the spermathecae at 280 $\times$  magnification for the presence of sperm.

## 3. RESULTS

The results are summarized in table 1 and fig. 1. The two reciprocal crosses between *G. m. centralis* and *G. m. morsitans (ocra)* showed a marked difference in fecundity, the cross with *G. m. morsitans* as the female parent being much the more fecund. The *morsitans* females were also more fecund than the *centralis* females in the within-subspecies matings and it seemed best to use the *morsitans*  $\times$  *morsitans* mating as the control for the between sub-species cross where *morsitans* was the female parent and the *centralis*  $\times$  *centralis* mating as control for the reciprocal. On this basis fecundity was reduced to 89 per cent of control in the *morsitans*  $\text{f} \times$  *centralis*  $\text{m}$  cross and to 28 per cent of control in the reciprocal cross. The eclosion rates and weights of hybrid pupae were similar to those of controls (table 1). The  $F_1$  males from both the reciprocal crosses mated readily but of those tested none was able to inseminate, *i.e.*, to introduce sperm into the spermathecae of their mates. Only the cross with *G. m. morsitans* as the female parent yielded sufficient progeny for an adequate backcrossing test. Samples of  $F_1$  females from this cross were backcrossed to each of the parental stocks and the fecundity of both backcrosses is shown in table 1. The *ocra* and wild type male progeny were separated and the inseminating ability of each was tested

TABLE I  
Results of matings within and between the sub-species. Data from 75-120 pairings in each category

Parents		Fecundity (pupae/mature female day)	Adult emergence rate (%)	Pupal weight (mg)	No. of male progeny able to inseminate	
Female	Male	<i>centralis</i>	95.6	31.5	All	
		<i>morsitans (ocra)</i>	0.0738	91.0	31.2	All
		<i>centralis</i>	0.1007	93.4	31.2	0/30
		<i>morsitans (ocra)</i>	0.0205	97.0	32.1	0/18
		<i>morsitans (ocra)</i>	0.0898	91.8	32.5	35/84
		<i>F<sub>1</sub> [morsitans (ocra) ♀ × centralis ♂]</i>	0.0733	96.4	31.2	12/73
<i>F<sub>1</sub> [morsitans (ocra) ♀ × centralis ♂]</i>	0.0729					

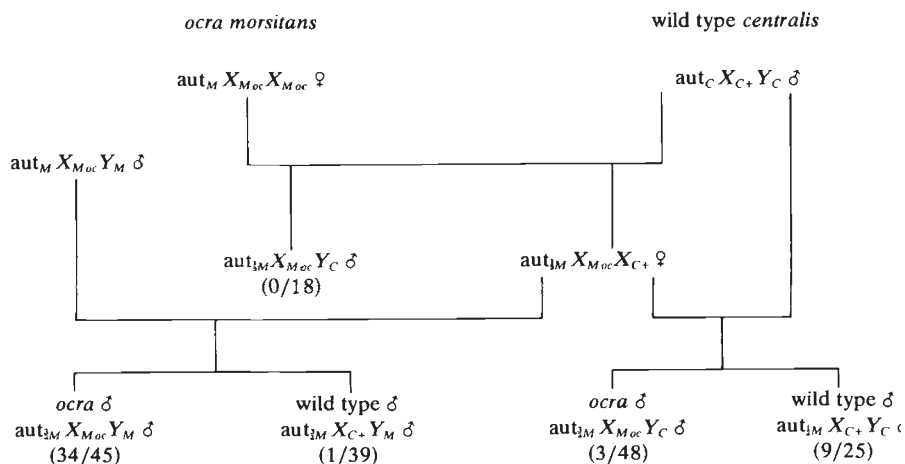


FIG. 1.—The *morsitans* (*ocrans*) ♀ × *centralis* ♂ cross and male progeny of both backcrosses of the F<sub>1</sub> females. The fractions in parentheses indicate the number of males which were able to inseminate/total number tested. The chromosomal compositions of each type are indicated (subscript *M* and *C* indicate *morsitans* or *centralis* origin of the chromosome(s), *oc* = *ocrans* gene and + = its wild type homologue, aut = autosomes whose average composition, with respect to sub-species origin, is indicated.

(fig. 1). From the backcross to *morsitans* (*ocrans*) males, the majority of the *ocrans* progeny could inseminate (76 per cent) but almost none (3 per cent) of the wild type males could do so. From the other backcross the difference between the inseminating ability of the males was less clear cut, but the proportion successful was 6 per cent in *ocrans* and 36 per cent in wild type. On the null hypothesis of no involvement of the X-chromosome in the causation of the male sterility, the proportion of inseminating males would not vary between the *ocrans* and wild type segregants from the backcrosses. This null hypothesis is decisively rejected for both the backcross to *morsitans* ( $\chi^2_1 = 43.2$ ,  $P < 0.001$ ) and for that for *centralis* ( $\chi^2_1 = 8.6$ ,  $P < 0.01$ ).

#### 4. DISCUSSION

The data reported involve a *centralis* stock from the same area as that used by Curtis (1972) but collected about eight years later. The *morsitans* stock was wild type in the earlier work but mutant in the present case. Despite these differences the present work agrees with the earlier work in all respects where comparisons can be made, i.e., (i) the fecundity was much lower in the *centralis* ♀ × *morsitans* ♂ cross than in the reciprocal; (ii) the fecundity of the backcrosses of the F<sub>1</sub> females was lower than that of the *morsitans* ♀ × *centralis* ♂ crosses from which they had been produced; (iii) all F<sub>1</sub> males from both reciprocal crosses were unable to inseminate; (iv) the overall proportion of inseminating males from all the first backcrosses involving *centralis* and *morsitans* did not differ significantly between the earlier and the present work ( $\chi^2 = 0.27$ ).

Use of the *ocrans* gene to follow the inheritance of the *morsitans* X-chromosome gave clear evidence that the X-chromosome was an important element in an interacting system which causes the male sterility. With

regard to the other component(s) of the system, the results from the backcross to *morsitans* suggest that the Y-chromosome is involved, *i.e.*, the combinations of the *morsitans* Y with X-chromosomes from the same or different sub-species yield a compatible or incompatible combination respectively. The exceptions (*i.e.*, the sterile *ocra* males and the single fertile wild type male) might possibly result from crossing-over between the X-chromosomes in the F<sub>1</sub> females so that the *ocra* gene does not always remain associated with the *morsitans* male fertility factor(s). However, it is not known whether crossing-over can occur between the X-chromosomes of different tsetse sub-species; it does not occur between the X-chromosomes of different *An. gambiae* sibling species (Curtis and Chalkley, 1979). Now that a second X-chromosome marker (*salmon eye*) has been found in *G. morsitans* (Gooding, 1979), it would be possible to investigate crossing-over in sub-species hybrids.

In the case of the backcross to *centralis*, there was again evidence for a significant effect of the X-chromosome but the fertility of the wild type segregants was much less than would be expected on a simple hypothesis of X-Y interaction being the cause of male hybrid sterility. We suggest that autosomal factors must be involved here, an average of 25 per cent *morsitans* autosomal genes being sufficient in many cases to produce an adverse interaction with the *centralis* X-chromosome.

The more important role of the X- than the Y-chromosome in causing the hybrid male sterility would not be expected from the cytogenetic studies of Southern and Pell (1973), which showed that the sub-species have visible differences between their Y-chromosomes, but not between their X-chromosomes.

The backcross results showing a strong influence of the X-chromosome, but also showing more sterility than would be expected on a simple X-Y interaction hypothesis, closely resemble those obtained in *An. gambiae* crosses (Curtis, 1978*a* and *b*; Curtis and Sawyer, in preparation). The results suggest that interaction of the hemizygous X-chromosome with alien Y and autosomal factors is the basis of Haldane's rule, *i.e.*, that when only one sex of hybrid is sterile it is the heterogametic sex.

Recent field trials involving genetic control of tsetse flies have used radiation sterilisation (Dame *et al.*, 1980; Cuisance *et al.*, 1978). This has several advantages but the capital cost of radiation sources is considerable and it is possible that sterile males with better competitiveness could be produced if the sterile hybridization concept for tsetse control were revived. If barriers to cross mating exist between the *G. morsitans* sub-species (which are almost completely allopatric), F<sub>1</sub> hybrid males might be ineffective in the field. It seems likely that males such as the wild type products of the backcross to *morsitans* (fig. 1) would have behaviour compatible with that of *morsitans* females in the field because their genetic composition is largely of *morsitans* origin, apart from the *centralis* X-chromosome which makes them sterile. It is unlikely that such males could be produced economically from a segregating system such as that shown in fig. 1. It may be feasible, however, by crossing appropriate products of the two backcrosses to produce a fertile strain with its X- and Y-chromosome of the same origin but its autosomes of mixed origin. Crossing this strain to one of the pure sub-species would be expected to generate sterile males with their genetic composition largely that of one of the pure sub-species. Non-inseminating males can partially

deter their mates from re-mating in confined conditions in the laboratory (Curtis, 1972); it remains to be seen whether they would do so more effectively under natural conditions. This ability would be essential if hybrid males are to have any prospects as a tsetse control agent.

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