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Sterility from Crosses between Sub-Species of the Tsetse Fly *Glossina morsitans*

C. F. CURTIS

Introduction

It is well known that closely related allopatric populations often show sterility when brought together and mated. The sterility may effect the cross-mating or the hybrids produced and may be due to incompatibility of factors in the cytoplasm or the nucleus [for example, LAVEN (1967a), review by EHRMAN (1962), DAVIDSON, PATERSON, COLUZZI, MASON & MICKS (1967)]. VANDERPLANK (1947) crossed the closely related tsetse species *G. morsitans* and *G. swynnertoni* and found reduced fertility in the crosses and the male and female hybrids. This work was extended to numerous other populations of tsetses of both the *morsitans* and *palpalis* groups (VANDERPLANK, 1948), but many of the results were based on few matings and the fertility of the control, intra-population, matings was low, with many individuals totally sterile. Methods of laboratory rearing of tsetse have greatly improved in recent years (e.g. NASH, JORDAN & BOYLE, 1968) so that it is now possible to quantify the proportion of zygotes which are killed in matings involving genetical lethal factors (e.g. CURTIS, 1968b, 1969). It seemed worthwhile, therefore, to re-investigate crosses of *G. morsitans* sub-species, using modern rearing techniques, and to investigate, by means of backcrosses, the nature of the sterility produced.

One of the aims of VANDERPLANK's work was to determine whether releases of alien types could be used to control tsetse populations and the initial stages of such a release project were described (VANDERPLANK, 1947). Field experiments on the use of natural genetic incompatibility for insect pest control were described by LAVEN (1967b) and DAVIDSON, ODETOYINBO, COLUSSA & COZ (1970) and some theoretical aspects of such techniques were considered by ZASLAVSKII (1967) and CURTIS & HILL (1971). The potential advantages of this approach, compared with other genetic techniques, include the fact that it avoids the reduced viability or other abnormalities often associated with sterilising doses of radiation (e.g. CURTIS & LANGLEY, in press) or with translocation homozygosity (e.g. CURTIS, SOUTHERN, PELL & CRAIG CAMERON, in press). The use of genetic incompatibility will only be effective where behavioural barriers to cross mating are weak or absent, and a preliminary laboratory test was made of this point.

Multiple insemination of females has been demonstrated in *G. austeni* (CURTIS, 1968b) and in assessing the potential control efficiency of alien males or of sterile hybrids it is necessary to consider their effectiveness in deterring the target female population from re-mating and/or in producing sperm which can compete for fertilisation.

Fig. 1 shows the distribution of the subspecies of *G. morsitans*, as mapped by FORD (1963) and using the revised nomenclature of MACHADO (1970). The geographical factors which keep the ranges of *G. m. centralis* separate from those of both *G. m. submorsitans ugandensis* and *G. m. morsitans* have been discussed by FORD (1970).

Materials and methods

In Fig. 1 the sites of origin of the *G. morsitans* populations used in the experiments are indicated by letters by which they will be distinguished in this paper. Further details are as follows:

U – *G. m. submorsitans ugandensis*, collected at Karuma Falls, Acholi, Uganda ($2^{\circ} 15' \text{ N}$, $32^{\circ} 16' \text{ E}$), received from Dr. T. Kangwagye.

S – *G. m. centralis* (subspecies referred to as *G. m. morsitans* by VANDERPLANK 1948 & 1949), collected at Singida, Tanzania ($4^{\circ} 50' \text{ S}$, $34^{\circ} 45' \text{ E}$), received from Dr. C. S. Tarimo.

M – *G. m. morsitans* (subspecies named *G. m. orientalis* by VANDERPLANK 1948 & 1949), collected at Morogoro, Tanzania ($6^{\circ} 49' \text{ S}$, $37^{\circ} 40' \text{ E}$), received from Mr. D. Robertson.

K – *G. m. morsitans*, collected at Kariba, Rhodesia ($16^{\circ} 35' \text{ S}$, $29^{\circ} 0' \text{ E}$), received from Dr. D. Dame in 1967 and subsequently colonised in this laboratory by NASH, JORDAN & TREWERN (1971).

Except where otherwise stated, all results quoted are from females bred in the laboratory, since there was inferior performance from individuals which had travelled by air from Africa at the pupal stage (JORDAN, NASH & TREWERN, 1970). In the U stock inferior performance continued into the first laboratory-bred generation and a steady high performance was not achieved until the following generation, and the results quoted are from that time onward.

The flies were fed on rabbits and most were kept in groups following the technique of JORDAN, NASH & BOYLE (1967), but the males and a few females were kept in individual cages (NASH, JORDAN & BOYLE, 1967). Virgin females were obtained by sexing within 24 hours of eclosion, they were fed and, at 2–3 days of age, were mated individually in tubes for 24 hours with males of

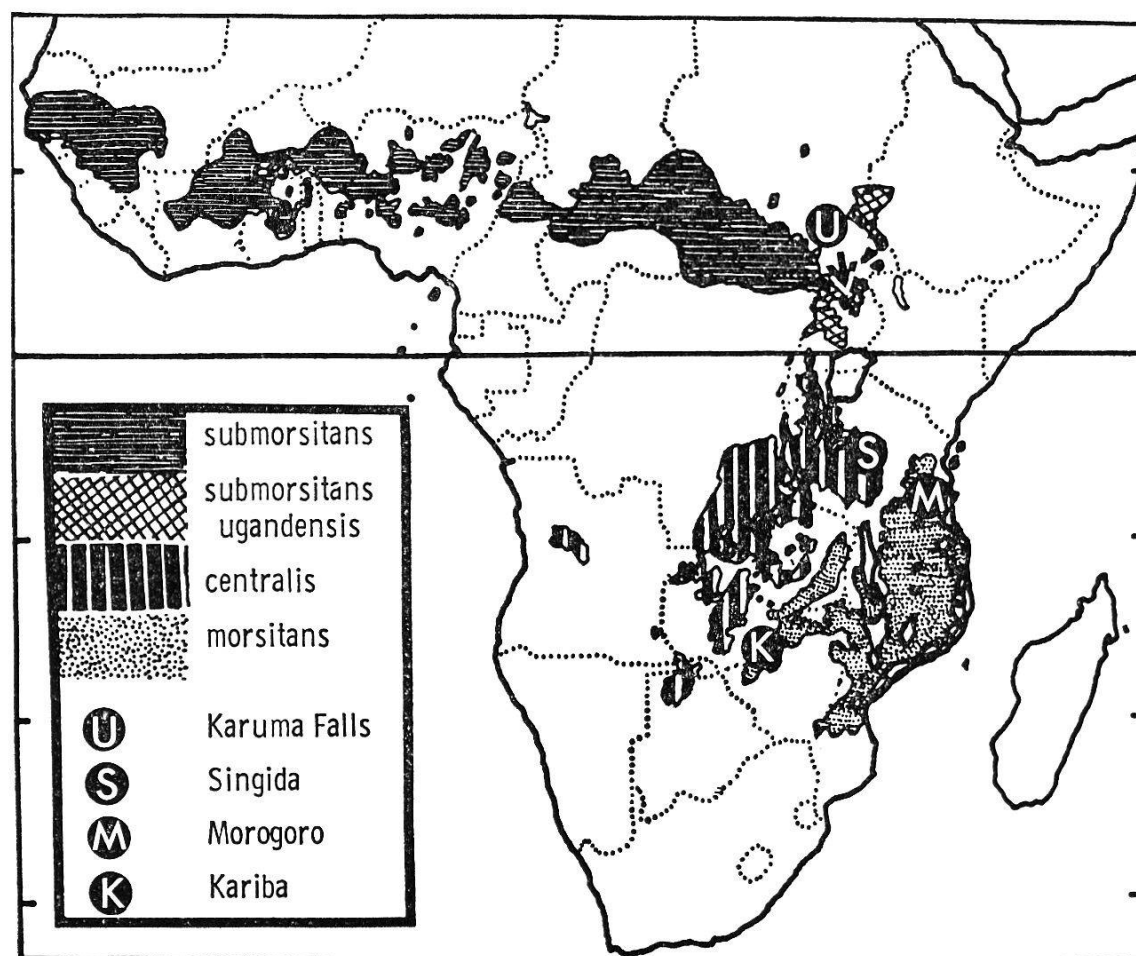


Fig. 1. The distribution of the subspecies of *Glossina morsitans* and the places of origin of the four populations used in the experiments.

10 or more days of age. Fertility of females kept in group cages was recorded in terms of pupae \div mature female days, i.e. the aggregate of the numbers of females, aged more than 15 days, alive on each day. Females were omitted if they were subsequently shown to be uninseminated by examination of the spermathecae at 600 \times magnification. Fertility recording continued for the period of maximum fertility, i.e. up to the 65th day of age. The number of mature female days (m) was converted to the number of ovulation cycles (c) with the formula:

$$c = \frac{m - 2p}{6.5}$$

where p is the number of pupae produced. This is based on the method of CURTIS (1968b) and the fact that under the conditions used (25.5 °C and 60–70% rh) a gestation period lasted about 8.5 days and the interval between ovulations when the embryo died young lasted about 2 days less. For females maintained individually observation of the intervals between pupal production readily gave data on pupae \div ovulation cycles; observation continued up to the 10th ovulation which, under these conditions, appeared to be the duration of maximum fertility.

Mating competition experiments were carried out with K and U males. A male of each type, from groups of the same average age and distinguished by the position of a paint mark, were put in a cage (15 \times 8.5 \times 5 cm), a virgin female was added and the male which succeeded in copulating was recorded. The flies were removed and the test replicated with a new trio.

Results

The crosses and the female hybrids

All matings involving pure and hybrid populations occurred readily and, with the exception of certain hybrids to be described below, insemination was normal with motile sperm visible in the spermathecae. The sex ratio was 1 : 1 from all matings.

Table 1 shows the data collected from all the matings made. Matings are listed with the female first and the products of successive backcrosses are indicated with their parentage enclosed by sets of parentheses. Lines 1–4 show the intra-population control matings: the K stock showed the best performance, perhaps because it has been colonised for longest and there has been more chance for selection of a laboratory adapted genotype.

Lines 5–11 of Table 1 show that crosses between the two *G. m. morsitans* populations (K and M) and backcrosses of both male and female hybrids were all very fertile. The average (weighted for sample size) of all the crosses was 0.886 pupae/ovulation, which is close to the mean of 0.872 for the intra-population controls (K \times K and M \times M). Thus, there is no evidence for incompatibility between these populations. Crosses of the M and K populations to the U and S ones produced sterility of various kinds and, in every respect, the results for M were homogeneous with those for K (Table 1) and in the following account and in Figs. 2–3 the results for M and K have been combined.

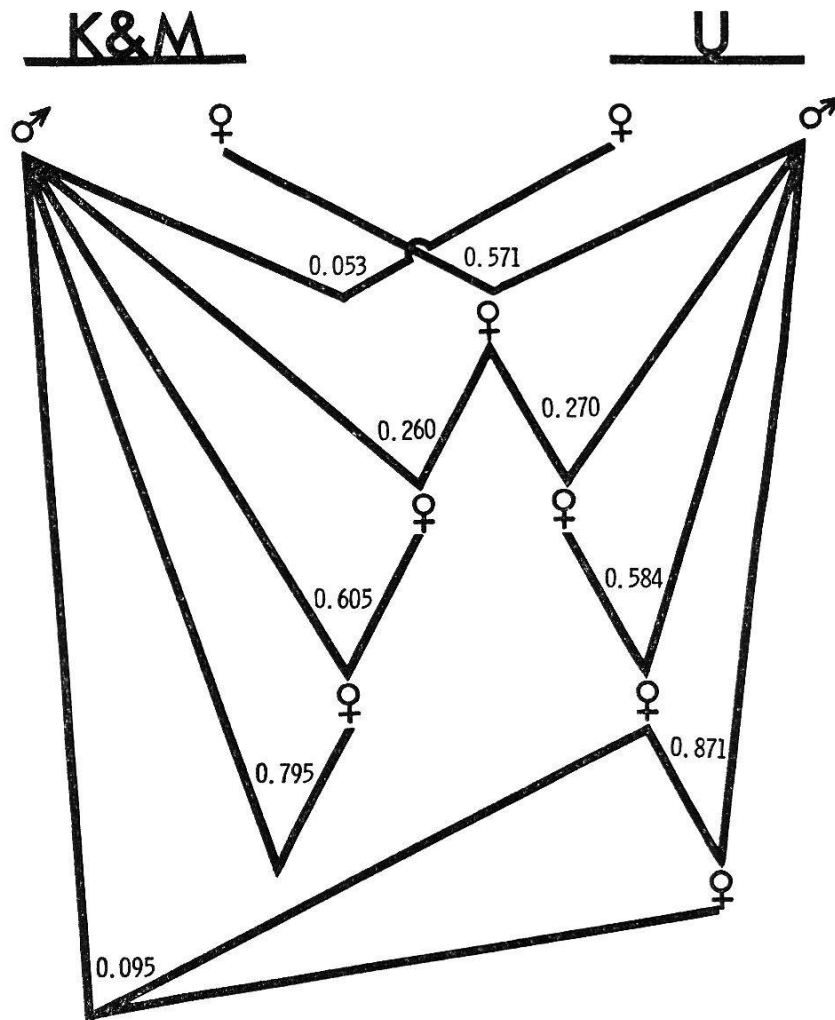


Fig. 2. The fertility of crosses of the K and M stocks with U and of the backcrosses of female hybrids, expressed as proportion of intra-population control values.

Fig. 2 shows the fertilities from crosses of M and K to U. Fertilities, in terms of pupae/ovulation, were divided by the fertility of the intra-population mating of the same type of female. After correction in this way an average, weighted for the relative sample sizes, was taken of the results for M and K. The control value used for correcting the results of mating hybrid females was the average of the parent stocks weighted for their genetic contribution to that hybrid.

Fig. 2 indicates a marked difference between the reciprocal crosses: where U was the mother there was very low pupal production and only 6 out of 11 hatched (K control = 97%), one of the progeny had a normal lifespan but was sterile and the remaining five had a mean adult lifespan of 2.5 days (K control = 100 days). Thus, the $U_{\text{♀}} \times K_{\text{♂}}$ cross was effectively sterile. There were only two aborted larvae, so that most of the deaths occurred early in development. By contrast, when U was the father, pupal production was more than 50% of control level (Fig. 2), pupal eclosion was 93% and the adult female lifespan was 90 days. Mating of the male hybrids will be described below.

Table 1. Results of the crosses

Parents			Fertility data				Male progeny	
♀	♂	No. of females	Pupae	Mature female days	Ovulation cycles	Pupae/cycle	Inseminator	Non inseminator
1	K	30	166	1,446	171	0.968	All	0
2	M	18	55	570	70.7	0.777	All	0
3	U	22	97	990	122.5	0.798	All	0
4	S	10	31	306	37.5	0.827	All	0
5	K	9	38	359	43.5	0.872	All	0
6	K × M	4	16	136	16	1.0	—	—
7	K × M	4	13	138	17.2	0.754	—	—
8	K	22	98	909	109.6	0.893	—	—
9	M	1 *	9	—	9	1.0	All	—
10	M × K	3	12	128	16	0.75	—	—
11	K	6	31	282	33.8	0.917	—	—
12	U	11	4	516	78.1	0.051	—	—
13	U	2	0	106	16.3	0	—	—
14	K	36	106	1,444	189.5	0.559	0	18
15	M	4	16	212	37.8	0.423	0	4
16	K × U	10	16	464	66.5	0.241	2	2
17	M × U	4 *	4	—	23	0.174	0	1
18	K × U	7	13	380	54.5	0.239	4	2
19	(K × U) × K	5 *	23	—	40	0.575	2	2
20	(M × U) × M	2 *	4	—	12	0.333	1	0
21	(K × U) × U	4 *	17	—	33	0.515	3	1

22	$[(K \times U) \times K] \times K$	K	6	22	234	29.2	0.753	4	1
23	$[(K \times U) \times U] \times U$	U	2	12	133	16.8	0.714	0	1
24	$[(K \times U) \times U] \times U$	K	3	0	129	20	0	-	-
25	$[(K \times U) \times U] \times U$	K	5	5	299	44.5	0.112	0	1
26	$[(K \times U) \times U] \times U$	$(K \times U) \times U$	3	14	144	17.8	0.786	1	4
27	K	S	26	97	956	117	0.827	0	13
28	K	S	7	28	291	36.1	0.774	0	8
29	M	K	9 **	4	147	21.4	0.187	0	1
30	S	M	8	1	268	38.2	0.026	0	1
31	S	K	11	32	513	69	0.464	9	14
32	$K \times S$	K	7	9	142	19.1	0.472	0	1
33	$M \times S$	M	7	27	344	44.6	0.605	3	7
34	$K \times S$	S	2	10	119	15.2	0.657	1	1
35	$M \times S$	S	12	31	436	57.5	0.539	4	0
36	$(K \times S) \times K$	K	4	10	182	24.9	0.402	0	1
37	$(K \times S) \times S$	K	4	19	182	22.5	0.860	3	0
38	K	$(K \times S) \times K$	4	20	200	24.6	0.812	0	2
39	K	$(K \times S) \times S$	6	22	301	39.5	0.557	0	5
40	U	S	3 *	4	-	7	0.571	0	1
	S	U							

* Maintained individually: K \times K controls gave 0.985 pupae/cycle; U \times U controls gave 0.847 pupae/cycle.

** Wild collected individuals: S \times S controls gave 0.619 pupae/cycle.

The female hybrids were successively backcrossed with males of each subspecies as the recurrent parents (Fig. 2). The first backcrosses showed fertility markedly below that of the cross from which the hybrids came, but in successive backcrosses the fertility rose towards the normal level (Fig. 2). The female products of the first backcross were maintained individually and each one showed partial sterility. Females from the second and third backcross to U were mated with K males and the pupal production was very low (Fig. 2) and of the three offspring tested one survived normally, but the other two both died on the second day of life.

The results in Fig. 2 give information on the causation of the sterility. The simplest hypothesis for the high sterility of the $U \text{♀} \times K \text{♂}$ cross would be inviability of zygotes or larvae heterozygous for genes from these two parents. This is ruled out, as the major factor, by the fact that the reciprocal cross, which would give zygotes with the same combination of autosomal genes, was quite fertile. The same argument applies to genes on the sex chromosomes, since only males receive a different combination in the reciprocal crosses, but the production of both sexes was equally drastically curtailed from the $U \text{♀} \times K \text{♂}$ cross.

The difference between the reciprocal crosses suggests an analogy with *Culex pipiens* geographical races, where maternally inherited factors in the cytoplasm of the egg and sperm interact to prevent fertilisation in certain crosses but not in the reciprocals (LAVEN, 1967a). Alternatively, one might postulate lethal inter-actions between maternally inherited factors in the egg cytoplasm and products of alien genes. On either of these hypotheses the surviving hybrids and all the products of backcrossing would have inherited the cytoplasmic factors down the female line from the K or M stocks. On the cytoplasm-cytoplasm interaction hypothesis all matings of these females to K or M males would have been as fertile as controls. On the gene-cytoplasm inter-action hypothesis these fertilities would have been intermediate between that of the $K \text{ or } M \text{♀} \times U \text{♂}$ mating and control. In fact, however, the first backcross to K or M males was about 25% fertile and the mating to K males of the products of the second and third backcross to U were almost as sterile as the $U \text{♀} \times K \text{♂}$ cross. The former females would have had one half of U genes and the latter ones $7/8 - 15/16$ of U genes. The results point very strongly to an inter-action of gene products of the mother with alien factors in the sperm, zygote or larva as the cause of the sterility. The higher fertility of $K \text{ or } M \text{♀} \times U \text{♂}$ crosses can be explained by postulating higher "tolerance" in K or M mothers and one would then predict that the fertility of the F_1 hybrids, when backcrossed, would be intermediate between the two reciprocal crosses of the parents and this was found to be the case. (Fig. 2). However, on a simple model of additive gene action one would have expected considerably higher

fertility in the first backcross to U (where the progeny would have been $\frac{3}{4}$ U) than in the other backcross (where the progeny would have been $\frac{1}{4}$ U). Only a very small difference of this kind was found, but larger samples would be required before it would be profitable to try to fit the data to more complex models of gene action.

The fact that each individual product of the first generation of backcrossing showed partial sterility indicates that several gene loci were concerned with the incompatibility – if a single locus had been involved there would have been a 1:1 segregation of fully fertile homozygotes and semi-sterile heterozygotes. The steady rise in fertility in both lines of backcrossing was to be expected as the proportion of incompatible genes between mother and progeny declined. In addition to this “dilution”, natural selection would be expected to speed the process, since individual females, which were more compatible with the genes of the recurrent male parent, would have made a dis-proportionate contribution to the next generation.

The genes of the mother might be thought to have their effect in causing this sterility by provoking a hostile reaction to alien sperm or influencing the laying down of the egg cytoplasm so that the egg could not be fertilised by alien sperms. However, eggs removed from the uterus after this cross showed sperms accumulated at the micropile as with normal matings (R. ALLINGHAM, personal communication). No attempt has yet been made to observe whether normal syngamy between the sperm and egg nuclei occurs in the cross. The fact that the $U \text{♀} \times K \text{♂}$ mating produced a few pupae, but these tended to die young, suggests that the uterus of a U female provides a continuing hostile environment for an embryo or larva containing K genes and this usually kills it early in development, but, if not, it is born defective.

Fig. 3 shows the data for crosses of K and M to S expressed in the same way as in Fig. 2. The fertility of the $S \text{♀} \times K$ or $M \text{♂}$ mating was very low and the survival of the few progeny was sub-normal. The reciprocal cross was quite different: fertility was only slightly below the control level. Backcrosses of the female hybrids gave results intermediate between the reciprocal matings of the parents, with the backcross to S being the more fertile. There was fairly low fertility when the female products of the first backcross to S were crossed to K. These results fit the same type of hypothesis as above, i.e. mother-larva incompatibility with the S mothers being less “tolerant” than the K or M; however, the incompatibility was rather less severe than in the cross to the U stock. The second backcross to K, surprisingly, showed little improvement in fertility on the first backcross. The data shown was for flies kept in bulk. Data for this mating from flies kept individually was too sparse to confirm that, as with the corresponding cross with U, each individual showed partial sterility.

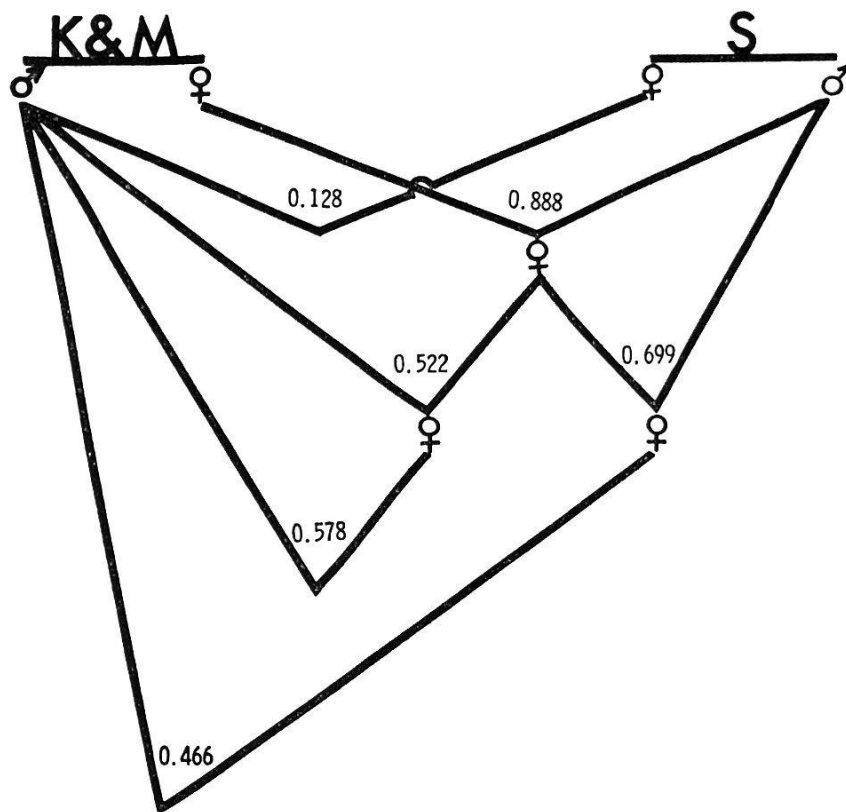


Fig. 3. The fertility of crosses of the K and M stocks with S and of the backcrosses of female hybrids, expressed as proportion of intra-population control values.

The above results suggested that the U and S stocks might be genetically similar and limited data from the reciprocal crosses between them (lines 39 and 40 of Table 1) show that both had moderate fertility.

Male hybrids

Male hybrids, derived from mating K or M ♀ × S or U ♂, were found to be totally sterile when mated to K or U females. On examination of the spermathecae of their mates all were found to be uninseminated. Sperm was present in the testes of the hybrid males but subjective assessment of the ruptured testes, in saline without a coverslip, indicated sub-normal motility of the sperms in most cases. Motility varied between individuals of the same parental origin, however, and some could not be distinguished from normals.

Copulation of the hybrid males lasted more than one hour but there was an indication that the average duration was sub-normal. A spermatophore (POLLOCK, 1970) was usually deposited in the uterus of the mates of the hybrid males and, in some cases, sperm was introduced into it (W. A. FOSTER, personal communication). Thus it can be concluded that there is a defect in the sperm of hybrid males which prevents its migration from the spermatophore to the spermatheca of the female.

Mates of hybrid males at 30 days of age showed the pattern of ovulation and oogenesis normal for a mated fly of that age (W. A. FOSTER, personal communication). This is to be expected since, though copulation must have taken place for normal ovulation to occur (MELLANBY, 1937), sperm transfer is not necessary (SAUNDERS & DODD, 1972).

Tests on the male progeny of backcrossing female F_1 hybrids to either of their parents showed that some individuals from each mating could inseminate and others could not (lines 16, 17, 18, 31, 32, 33 and 34 of Table 1) and repeated tests on the same individuals gave the same result, i.e. there was a segregation of males into two categories. This contrasts with the females of the same generation, each of which had intermediate fertility. The sperm in the testes of males that could inseminate showed normal motility. From all the first backcross males derived from K or $M \text{♀} \times U$ or $S \text{♂}$ matings, the ratio of inseminators : non-inseminators was 19:28, which is not significantly different from 1:1 ($\chi^2 = 1.36$). This suggests that this character is controlled by a single locus, with the parent sub-species homozygous for different alleles and heterozygosity causing the sperm defect. The limited data from males of later generations of backcrossing (lines 19–25 and 35–36 of Table 1) are consistent with the expectation from this hypothesis (e.g. the second backcross gave a 10:3 ratio of inseminators : non-inseminators, which is close to the expected 3:1 ratio).

Lines 26, 37 and 38 of Table 1 show matings to K females of those first backcross males which could inseminate. As expected fertility was quite high, because these matings would have combined K mothers with progeny that had more than half K genes. The expectation on the above hypothesis is that males from the backcross to K (line 37, Table 1) would produce all inseminator sons and those from the backcrosses to U or S (lines 26 and 38, Table 1) would produce all non-inseminators. These expectations were fulfilled, with one exception. Because of the possibility of error the occurrence of such an exception requires to be repeated before rejecting the above hypothesis of the control of male sterility in favour of something more complex.

As would be expected, the only two males surviving to maturity, from $S \text{♀} \times K$ or $M \text{♂}$ matings, both failed to inseminate (lines 29 and 30 of Table 1).

Crosses of either K or M females to either S or U males gave male hybrids with similar sperm defects and it was therefore supposed that crosses within these pairs of populations would yield normal males. This was true for $K \times M$ (lines 5 and 9, Table 1) but, when S and U were crossed it was found that the male hybrids could not inseminate (lines 39 and 40, Table 1). The above hypothesis can be modified to cover this fact by postulating three alternative alleles at the same locus with any heterozygous combination having the sperm defect. It would

also be consistent with the available facts to postulate that U differs from K and M at one locus and S differs from K and M at another locus and that heterozygosity at either causes the same sperm defect.

Multiple mating of females

For these experiments only the K and U stocks were used. The matings were made between single pairs in tubes and the results are shown in Table 2. Males of the K stock mated in 100% of cases with fed virgin females aged between two and five days and, with one exception, this was true of U males also. When females were presented with a second male two days after their first mating, some individuals re-mated and others did not. This variation in response applied to all combinations of types of male tested, including those where the first mate was with hybrids which could not inseminate (table 2). Similar results were obtained in a limited experiment with three days between the matings. Failure of the second mating seemed to be due to increased resistance in mated females, but it is possible that it was due to reduced female attractiveness. Most of the apparent variation between the various combinations is not statistically significant, but there is an indication that U males were less successful than K ones in securing second matings with K females.

From K females which accepted matings first to U and then to K, some male progeny were shown to be able to inseminate and some could not, i.e. some derived from fertilisation by sperm from the second

Table 2. Results of multiple mating of females

Female	1st mate (day 3)	2nd mate (day 5)	No. of females offered matings	% accepting the matings	Fertility of females accepting the matings (pupae/cycle)
K	K	—	30	100	0.968
K	U	—	37	97	0.559
K	K	K	48	75	—
K	K	U	37	46	0.588
K	U	K	22	59	0.922
K	(K × U) hybrid	K	18	89	(64% inseminated)
K	(K or M × S) hybrid	K	28	79	(91% inseminated)
U	U	—	22	100	0.798
U	K	—	24	100	0.051
U	U	K	16	88	0.668
U	K	U	25	80	0.264

} av. = 0.763

} av. = 0.755

} av. = 0.424

} av. = 0.466

mating and some from the first. This point was confirmed by cytological examination of the pupae: the Y chromosomes of the K and U stocks can be distinguished (SOUTHERN, PELL & CRAIG CAMERON, in preparation) and, among these pupae, individuals with each type were found (P. E. PELL, personal communication). From U females mated in the same sequence, all 11 pupae scored were of the U type (P. E. PELL, personal communication): this is to be expected, since the great majority of hybrid zygotes die early in U mothers.

The fertility of females which accepted two matings varied with the order in which the two matings were made (Table 2). With U females the first insemination had a predominant effect, as in *G. austeni* (CURTIS, 1968b), but in K females it appears that the second insemination was predominant. This anomalous result with K females is based on a rather small number of females which accepted second matings and requires to be re-checked with a larger sample size. After a first mating to a hybrid male, some females were not inseminated despite accepting a second mating to a K male (Table 2).

For both U and K females the average of the fertility of females mated to U and K males in the two orders was similar to the average for the same female mated only to K or only to U (table 2). This indicates that, eliminating the effect of mating precedence, U and K sperm were equally competitive for fertilisation in the genital tract of each type of female.

Mating competition between K and U males

The results of mating competitions, conducted as described in the Methods section, are shown in Table 3. When competing for either U or K females, K males succeeded in copulating significantly more frequently than did U males. The data with the two types of female are homogeneous ($\chi^2 = 0.78$), so they have been pooled and the overall totals show a highly significant superiority of the K males.

Table 3. Results of mating competitions

Female	No. of males succeeding in copulating		χ^2_1	P
	K	U		
K	50	31	4.00	< 0.05
U	26	10	6.25	< 0.02
Total	76	41	24.4	< 0.001

Discussion

VANDERPLANK (1948) crossed populations originating not far from stocks M and S and, despite very uneven performance of his intra-population control matings, the reciprocal crosses showed similar relative fertilities to those shown in Fig. 3. There is also agreement between the present work and that of VANDERPLANK in the absolute sterility of F_1 male hybrids, the partial sterility of female hybrids and the fact that backcrossing the latter gave some fertile males. The only major point of disagreement, where a direct comparison is possible, is in VANDERPLANK's statement that the sterile hybrid males could inseminate. It may be that this was based on observation at low magnification of fluid in the spermathecae. The present work left no doubt that, at a magnification at which many individual spermatozoa could be seen in the mates of normal males, these were never visible in the mates of F_1 hybrid males.

Of the four stocks tested, only K and M showed full fertility of the crosses and hybrids. These two stocks have been classified on morphological grounds as the same subspecies and small scale maps show continuity of the distribution of this subspecies over the 900 miles which separates their places of origin (Fig. 1). The only combination of populations which VANDERPLANK (1948) found to give full fertility of crosses and hybrids was two populations of *G. m. centralis* from 400 miles apart. Thus the morphological resemblances and differences recognised by taxonomists seem to correlate with reproductive compatibility. It is hoped to test the validity of this statement in crosses of *G. m. submorsitans* from Senegal and Nigeria with each other and with the U stock: these have been given the same tri-nomial by MACHADO (1970) but are separated by up to 3,500 miles, with apparent discontinuities of their range (Fig. 1). The present results led to the conclusion that the three different subspecies tested are mutually distinct at a locus (or loci) which controls male hybrid sterility. However, the relative fertility of the crosses and the female hybrids suggests that *G. m. centralis* is an evolutionary intermediate between the other two subspecies tested, but that is closer to *G. m. submorsitans ugandensis*.

The three populations have not yet diverged to the stage of full biological speciation, since gene exchange is still possible (at least in captivity) via the female hybrids of one of the reciprocal matings. *G. swynnertoni* was not tested in the present work, but the results of VANDERPLANK (1947, 1948) suggest that it is about as genetically compatible with *G. morsitans* subspecies as they are with each other.

VANDERPLANK (1948) showed that *G. m. centralis* eggs could be fertilised *in vitro* with *G. swynnertoni* sperm and that, as with intra-

population controls, development proceeded to the egg hatching stage, but no further. He concluded that the sterility of crosses in the *morsitans* group of tsetse was either due to lowered activity of sperms in an alien environment, or to mortality of larvae owing to "ineffective gene combinations" or to "an alien environment in the uterus". The first suggestion is made unlikely by the observation of motile spermatozoa in the spermathecae and sperms penetrating the eggs in sterile crosses and it seems to be ruled out by the data in Table 2 which show that, after correcting for mating precedence effects, alien sperm is fully competitive for fertilisation in multiply mated females. As already pointed out, the existence of high fertility in one of each of the pairs of reciprocal matings rules out "ineffective gene combinations" as the principal lethal factor. This leaves incompatibility between certain combinations of mothers and zygotes or larvae as the remaining possibility. Some of the progeny "escape" death from this cause until the pupa or adult stage, but most die early in uterine life and are re-absorbed or expelled when so small as not to be readily visible, as with deaths due to radiation induced dominant lethals (CURTIS, 1968b) and most, but not all, translocations (CURTIS, 1969).

The existence of differences between reciprocal matings due to mother-progeny incompatibility suggests an analogy with the human Rhesus blood group system but further work will be needed to determine whether the analogy is merely superficial. Rhesus incompatibility involves the immune system, but there is very little information about immunity in insects (STEPHENS, 1963). It is rather difficult to imagine how antigenic stimulus could move from progeny to mother with the system of adenotrophic viviparity in tsetse, but it can readily be imagined that either humoral or cellular immune attack could be transmitted in the uterine gland secretion.

Recently, in two cases of insect sterility in crosses or hybrids, cellular symbionts have been implicated in the mechanism (YEN & BARR, 1971; EHRLMAN & KERNAGHAN, 1971). There are "bacteroids" in tsetse midgut cells (BUXTON, 1955) which must be transmitted from mother to offspring via the egg or uterine gland secretion. Genetic incompatibility between alien bacteroids and larvae might be postulated, but this would lead to crossing results implicating a maternally transmitted factor, and the data (Figs. 2 and 3) contradict this.

The high sterility of the female hybrids is to be expected on the hypothesis of mother-progeny incompatibility, but the insemination defect of hybrid males seems to be a separate phenomenon. Sperm defects have frequently been found in hybrid insects (e.g. in *Anopheles gambiae*: DAVIDSON et al., 1967; *Drosophila* data reviewed by EHRLMAN, 1962). There are chromosome differences between the *G. morsitans* subspecies (SOUTHERN, PELL & CRAIG CAMERON, in preparation) but

these are not of a kind which would explain defective spermatogenesis and this seems to be genic in origin.

The results on multiple mating, showing normal competitiveness of alien sperm (Table 2), indicate that the occurrence of multiple mating in the wild and details of mating precedence may be ignored when considering the potential effectiveness of released males of an alien subspecies for population control. However, the sterile male hybrids would be completely ineffective in controlling fertility if their mates were inseminated by another male. The hybrids, like the normal males, had some ability to prevent their mates from re-mating and the hybrids sometimes prevent their mates from being inseminated if they do re-mate (Table 2). The mechanism obviously does not involve sperm transfer itself and, in view of the precedents in other Diptera (CRAIG, 1967; RIEMANN, MOEN & THORSON, 1967), one would suspect that male accessory gland secretions were involved. In the wild, where females are not forced into close contact with males as they were in these experiments, it may be that the deterrent to re-mating would be more effective and hence the sterile hybrids might have a considerable population suppressing effect. Unless this suggestion can be confirmed, attempts to suppress *G. m. morsitans* populations by release of males of either of the other two subspecies cannot be expected to be efficient, because neither the cross-matings nor the female hybrids would be sterile and the male hybrids would only sterilise females which mated with no other type.

Releases of *G. m. morsitans* males to control either of the other two populations are much more hopeful because the cross-matings would be virtually sterile. The true potential of this method depends on the competitiveness of alien males for mating after release. In the experiments (Table 3) the K males showed superior competitiveness to U males. This might indicate a higher sex drive in *G. m. morsitans* males and, if so, it would be favourable for control attempts. However, the conditions of the competition experiments were extremely unnatural and the results may merely reflect superior laboratory fitness of the K strain compared with the U because of its longer laboratory colonisation: it is noteworthy that the K females showed superior control fertility (Table 1, lines 1 and 3). The superior mating competitiveness of K cannot be due to direct selection for this character during colonisation since all the matings were made between single pairs (NASH, JORDAN & TREWERN, 1970) and with *G. morsitans* there are no failures to pair (M. A. TREWERN, personal communication).

Though the laboratory mating competitions must be interpreted with extreme caution it was encouraging that the results with the two types of female were homogeneous, i.e. there was no evidence for discrimination by the female in favour of its own type of male. VANDERPLANK

(1948) reported a tendency, in the laboratory, towards assortative mating of *G. m. morsitans* and *G. m. centralis*, but quantitative data were not given. Owing to shortage of material, this combination has not been competed in the present work. However, these subspecies seemed quite willing to mate when single pairs were placed in tubes.

Sibling species of the *Anopheles gambiae* complex will readily mate in captivity (DAVIDSON et al., 1967), but, where they are sympatric in the wild, cross-mating is rare (e.g. WHITE, 1971). However, in tsetse there is some evidence that behavioural isolation is ineffective in the wild. The ranges of *G. m. centralis* and *G. swynnertoni* show small areas of overlap, and in one of these areas, VANDERPLANK (1947) recorded at least one hybrid among 51 individuals. This implies a considerable frequency of cross-matings because the fertility of these is very low. JACKSON (1945) released males and females of *G. m. centralis* and *G. swynnertoni* into the wild and caught copulating pairs and showed that the four combinations of male and female types occurred in the proportions expected with random mating. The question of whether alien males of a particular type will give sufficient cross-mating for control purposes can probably only be settled by making a release, capturing wild females and determining what proportion have received sterile inseminations.

If behavioural barriers to cross-matings are found, it might be possible to combine the alien sterility factor with the rest of the gene complex of the target population, including the genes controlling mating behaviour. This procedure would be quite easy where sterility was due to cytoplasmic factors (LAVEN, 1967b), but would be very difficult with the several or many genes implicated in this work in causing the sterility of the crosses and female hybrids. However, this procedure might be feasible with the single locus which seems to be responsible for the hybrid male sterility. As pointed out this factor would probably not be efficient for population suppression. However, it would lead to the selective disadvantage of heterozygotes in a mixed population and might therefore be used to cause selective increase in the frequency of a desirable gene (CURTIS, 1968a; WHITTEN, 1971) such as one which made the insect resistant to infection by trypanosomes.

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Zusammenfassung

Kreuzungen zwischen Populationen von *Glossina morsitans* aus verschiedenen Teilen Afrikas wurden gezüchtet. Zwei Populationen von *G. m. morsitans* erwiesen sich als genetisch gleichwertig; allerdings ergaben Kreuzungsexperimente von Männchen dieser zwei Populationen mit Weibchen von *G. m. centralis* und von *G. m. submorsitans ugandensis* Sterilität. Reziproke Kreuzungen waren teilweise fertil, aber die Bastard-Weibchen waren steriler und die Bastard-Männchen hatten die Fähigkeit verloren, Weibchen zu befruchten. Resultate aus Rückkreuzungen weisen darauf hin, daß die Sterilität der Kreuzungen und der Bastard-Weibchen wahrscheinlich auf eine Unverträglichkeit zwischen Mutter und Nachkommenschaft zurückzuführen ist, welche durch verschiedene Genorte kontrolliert wird, während die Sterilität der Bastard-Männchen möglicherweise durch einen einzigen Genort bestimmt wird.

Mehrfache Begattungen von Weibchen zeigten, daß das Fremdsperma in bezug auf Befruchtung konkurrenzfähig war; kompetitive Paarungsexperimente im Labor ergaben keinen Hinweis auf unterschiedliches Kopulieren der Unterarten.

Ausgesetzte Männchen von *G. m. morsitans* scheinen die andern zwei Subspecies kontrollieren zu können. Felduntersuchungen über die Konkurrenzfähigkeit ausgesetzter fremder Männchen sollten durchgeführt werden.

Résumé

Des croisements ont été réalisés avec des populations de *Glossina morsitans* provenant de différentes régions d'Afrique de l'Est. Une compatibilité génétique a été mise en évidence chez deux populations de *G. m. morsitans*, mais les mâles de ces dernières laissent stérile après accouplements les femelles de *G. m. centralis* et de *G. m. submorsitans ugandensis*. Les croisements réciproques étaient modérément fertiles, mais les femelles hybrides étaient beaucoup plus souvent stériles, et les mâles hybrides incapable d'inseminer leurs compagnes. Les résultats des croisements rétrograde indiquent que la stérilité des hybrides était probablement liée à une incompatibilité entre la mère et la progéniture contrôlée par plusieurs gènes loci chez les femelles hybrides alors que chez les mâles hybrides la stérilité était vraisemblablement liée à un seul locus.

De multiples accouplements ont montré que le sperme étranger était compétitif pour la fertilisation, et chez des femelles placées expérimentalement en position d'accouplements concurrentiels aucune tendance à des copulations préférentielles n'a été mise en évidence au niveau des sous-espèces. Des lâchers de *G. m. morsitans* mâles doivent permettre de contrôler les populations des deux autres sous-espèces, mais des expériences sur les accouplements concurrentiels de mâles étrangers libérés dans la nature sont maintenant nécessaires.