AGENCY FOR INTERNATIONAL DEVELOPMENT
WASHINGTON, D. C. 20523

BIBLIOGRAPHIC INPUT SHEET

FOR AID USE ONLY

1. SUBJECT CLASSIFICATION

A. PRIMARY
Agriculture — AL72-0000-0000

B. SECONDARY
Pests of animals

2. TITLE AND SUBTITLE
Observations on sex attraction and mating behavior of the Tsetse fly,Glossina morsitans orientalis Vanderplank

3. AUTHOR(S)
Dean,G.J.W.; Clements,S.A.; Paget,J.

4. DOCUMENT DATE	5. NUMBER OF PAGES	6. ARC NUMBER
1968	11p.	ARC

7. REFERENCE ORGANIZATION NAME AND ADDRESS
USDA/ARS

8. SUPPLEMENTARY NOTES *(Sponsoring Organization, Publishers, Availability)*
(In Bul.of entomological research,v.59,p.355-365)

9. ABSTRACT

10. CONTROL NUMBER
PN-RAA-557

11. PRICE OF DOCUMENT

12. DESCRIPTORS
Sex behavior
Tsetse flies

13. PROJECT NUMBER

14. CONTRACT NUMBER
PASA RA-1-00 Res.

15. TYPE OF DOCUMENT

AID 590-1 (4-74)

Bull. ent. Res. (1968) 59, 355–365
Published 1969

Observations on sex attraction and mating behaviour of the tsetse fly *Glossina morsitans orientalis* Vanderplank

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Introduction

Tsetse fly populations can exist at densities as low as 100 individuals/sq. mile (Glasgow, 1963), and Teesdale (1940) found 98% insemination of non-teneral female *Glossina fuscipes* Newst. caught from a very sparse population. In wild populations it is uncommon to find a single non-teneral female that has not mated, and it seems likely that a wild female fly may mate only once (Glasgow, 1963). However, Dame & Ford (1968) with chemically induced sterile sperm demonstrated that multiple mating of female *G. morsitans* Westw. can occur under laboratory conditions, while males can copulate several times before depleting their sperm. This paper presents results from some studies into sexual attraction and mating in *G. morsitans orientalis* Vanderplank.

Materials and methods

Tsetse flies used in laboratory tests were obtained from puparia found in natural sites at Kariba, Rhodesia. Emerging flies were collected daily, and the sexes were held separately to ensure their virginity in standard 8×8×11-in. cages (Dame & Ford, 1966) at 25±2°C and about 70% r.h. The flies were fed on a guinea-pig placed for one hour each day within a mesh insert in the floor of the cage. Male and female flies were sexually mature 7 and 2–3 days, respectively, after eclosion (Dame & Dean, unpublished).

The possibility that a pheromone was produced to attract the male fly was investigated by collecting volatile and soluble compounds from mature virgin female tsetse. Batches of 750 virgin females were exposed for 45–60 min to a flow of either pure nitrogen or air at 35°C, and any volatile compounds that may have originated from the flies were condensed with dry ice. Soluble compounds were extracted by crushing similar numbers of virgin females and placing them into a 3:1 solution of analytical grade chloroform and methanol for three days. The condensate from the gas flow was stored in a deep freeze and the solvent extract was kept at 2°C. For test purposes one-inch squares of filter paper were soaked in the collection samples and air-dried, and similar-sized pieces of filter paper were soaked in the chloroform—methanol solvent, air-dried and used as controls.

Attraction of male flies to these test samples was investigated under diffused overhead lighting in both a 7×10×14-in. glass tank and a 3·5×48-in. glass tube. The floor of the tank was divided into one-inch squares (designated A–G, 1–13), and sector preferences, in still air, in relation to the test samples shown by 10 mature males were recorded every half hour. The length of the tube was divided into twelve 4-in. sectors and the extract sample was placed in sector 5. Air was passed slowly along

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the tube from sector 1 to 12 where 25 mature males were released, and the number seen in each sector was noted every half hour.

In a separate test, eight mature virgin females in a 3×1-in. vial, capped at both ends with mosquito mesh, were placed at one end of the glass tube, and an air flow was passed across them towards male flies released at the other end. The distribution of the males was recorded every half hour for four hours before the females were released into the tube to confirm their ability to mate.

The possibility of olfactory sensillae on the antenna or arista being used to determine the presence of the opposite sex was investigated by removing, with fine scissors, these appendages at their basal segment from flies inactivated at 2°C. All treated flies were allowed to recover for four hours before the start of a test. Groups of 25 of the treated sex were paired in a standard cage with equal numbers of the opposite, untreated sex for 24 or 48 h. The success of mating was determined by dissection of the female flies and microscopic examination of the spermathecae.

The possible existence of a pheromone was investigated further by a trial in a stand of *Colophospermum mopane* in the Zambezi valley. Two oxen were tethered 100 yd apart, and a cage containing 50 three-day-old virgin females was suspended just above one of the oxen. Each ox was specified for a particular site and was baited with the caged females on alternate days. Comparative catches of wild flies were made off both animals between 06.00 and 18.00 h on nine successive days.

The effect on mating due to visual attraction between the sexes was studied in three ways. First, an 18×12×4-in. wooden-sided box with a transparent plastic top was divided into two equal volumes (9×12×4 in.) by a cotton mesh septum across the centre. Ten virgin female flies were placed in one section and 10 mature males were released into the other. The individual flies were relatively static and easily visible to the observer through the cotton mesh. The numbers of the two sexes seen on the dividing septum and the two end walls were recorded every half hour. Secondly, virgin flies were released singly into a standard cage containing 20 flies of the opposite sex and mating behaviour was observed continuously. Thirdly, the compound eyes and ocelli of either sex were covered with black enamel paint while the flies were inactivated at 2°C. Groups of 25 of the treated sex were paired with 25 normal flies of the opposite sex in standard cages for 24 or 48 h. Insemination of the female flies was checked by described procedures.

The influence on mating of sounds made by haltere or wing vibration, and the position of the female's wings during mating attempts by male flies, were investigated by removing or glueing these appendages and observing the mating behaviour of pairs in 3×1-in. glass vials. In tests to study multiple mating behaviour, copulation was stopped after 1, 5, 15, 30 and 45 min by immobilising the flies with CO_2 and gently separating the individuals, and the females were re-mated with new males either immediately or 24 h later. Behaviour was recorded as the time between liberating the two sexes into the vial (pairing) and the start of copulation (mating), the duration of mating, and percentage of pairs copulating for longer than 60 min, the minimum period considered necessary to ensure insemination (Dame & Dean, unpublished). Statistical analysis of the data was not attempted since effects due to the different treatments were either large, or if small could probably be attributed to experimental conditions.

Results and conclusions

Pheromone

Responses by male tsetse to condensates from the nitrogen and air flows, and the extract with chloroform—methanol, gave similar results. Typical horizontal sector preferences in the glass tank shown by male flies in still air to condensates from nitrogen are given in Table I. No male flies were seen in the sector containing the test extract (X), and the majority were observed around the perimeter of the floor of

TABLE I. *Horizontal sector preferences* in still air shown by 10 mature, male* G. m. orientalis *in a glass tank*

	No. flies in indicated sector												
Sector	1	2	3	4	5	6	7	8	9	10	11	12	13
A	8	0	0	0	0	0	0	2	0	3	0	1	7
B	1	0	0	0	0	0	0	0	0	0	0	1	0
C	1	0	0	0	0	0	0	0	0	0	5	0	0
D	0/X	0	0	0	0	0	Z/0	0	0	Y/0	0	0	0
E	0	0	0	0	0	0	0	0	0	0	6	0	0
F	2	0	0	0	0	0	0	0	0	0	0	0	0
G	6	0	0	0	3	1	2	0	0	0	0	2	9

* Totals of two replicates each of five half-hour intervals between observations.
X is a condensate sample from nitrogen passed over virgin mature females.
Y and Z are control samples.

the tank. Only 60 out of 100 observed roosting positions during two tests were on the floor, and the remainder were on the glass walls. Sector preferences along the glass tube with the chloroform—methanol extract (X) in sector 5 and two control squares (Y & Z) in sectors 3 and 7 are shown in Table II. Male flies were released into sector 12 against an air flow from sector 1, and only one was seen to approach

TABLE II. *Observed sector preferences* shown by* G. m. orientalis *after a 4-h period in a 3·5×48-in. glass tube divided into 12 equal sectors and with an air flow from sector 1 to 12 over a sample of the chloroform—methanol extract (X) of virgin mature female flies*

Number of male flies in indicated sector											
1	2	3(Z)	4	5(X)	6	7(Y)	8	9	10	11	12
4	5	5	3	3(a)	2	5	8	2	4	4	5

* Total of three replicates with 25 male flies.
Y and Z are blank controls.
(a) sitting on glass wall

the extract during 4-h test periods. Similar results were obtained with the two condensate samples. The presence of eight virgin female flies across an air flow at sector 1 also resulted in apparently random distributions of males along the tube after 4 h similar to those shown in Table II. Every female fly subsequently released into the tube was approached by a male and 100% mating was obtained within 5 min of release.

Removal of the antennae and arista of the male or female fly showed that matings with untreated flies of the opposite sex produced somewhat lower insemination rates than in control tests with both sexes untreated (Table III). However, this result was probably due to the effect of the operation, and does not necessarily indicate that

TABLE III. *Insemination rates* obtained from matings between 25 pairs of* G. m. orientalis *where the antennae of one sex were removed*

Duration of test (h)	Treated sex	Female survival (%)	Insemination (%)
24	Male	100	68
48	"	98	61
24	Female	96	71
48	"	100	84
24	Neither	100	89
48	"	100	100

* Two replicates.

some chemoreceptors were removed to partially reduce the ability to detect a pheromone from the other sex.

A field trial with mature virgin female tsetse in a cage suspended just above a tethered ox supported laboratory results. Daily catches of wild male flies tended to

TABLE IV. *Daily totals of wild male and female* G. m. orientalis *caught off an ox tethered under a cage containing 50 virgin female flies and from a control ox*

	Ox plus virgin female flies		Control ox	
	Male	Female	Male	Female
	44	15	23	7
	47	15	25	8
	61	16	61	15
	21	24	93	40
	20	10	21	12
	9	10	36	14
	40	10	31	32
	32	18	35	10
	45	22	28	23
Totals	319	140	353	161

vary but similar total numbers were caught off the baited and unbaited ox (Table IV). These results indicated that the virgin females did not produce a pheromone attractive to male flies.

Vision

The behaviour of virgin male flies in a subsection of an 18×12×4-in. cage and apparently able to see 10 virgin females in the other section through the dividing

TABLE V. *Mean number* of 10 male and 10 female* G. m. orientalis *observed on either side of a cotton mesh screen dividing a cage into two sections*

Time (h)	Male		Female	
	End wall	Screen	Screen	End wall
0·5	1	1	2	1
1·0	2	1	2	1
1·5	2	1	3	2
2·0	2	3	2	5
2·5	2	0	2	3
3·0	1	1	3	0

* Three replicates

cotton mesh screen is indicated in Table V. The absence of higher numbers of males sitting on the screen indicates that they were not attracted to the female flies sitting passively in the other sector.

In a replicated test in which 20 male flies were released individually into a standard cage containing 20 female flies, 12·5% of the pairs were mating after 10 min. Releasing virgin females one by one into a cage containing male flies resulted in 32·5% of the pairs mating in 10 min. The enclosed flies were then activated by tapping the cages and the mean proportion of mating pairs increased within 4 min to 45% and 55% in the two sets of cages, respectively. Observations in non-replicated tests showed that individual male *G. m. orientalis* or female *G. pallidipes* Aust. released into a cage containing male *G. m. orientalis* were approached to within 1–2 cm and then ignored without any attempt to mate. Male *G. m. orientalis* paired with female *G. pallidipes* in 3×1-in. vials also made no attempt to mate. These results indicate that sex and species are recognised only at close range.

Mating tests in standard cages showed the relative importance of blinding the eyes of one sex with paint, and the effect of light or dark conditions on untreated tsetse. Painting the eyes of the male flies, or holding untreated pairs continuously in the dark

TABLE VI. *Insemination rates* obtained under various light conditions from matings between 25 pairs of* G. m. orientalis *where the compound eyes of one sex were painted*

Duration of test (h)	Treated sex	Light conditions	Female survival (%)	Insemination (%)
24	Neither	12 h	96	76
48	"	"	100	100
24	"	Dark	96	16
48	"	"	100	20
24	Male	12 h	96	25
48	"	"	88	12
24	Female	"	72	83
48	"	"	80	85

* Two replicates.

for 24 or 48 h, greatly reduced the proportion of females inseminated (Table VI). The few successful matings that did occur were probably due to accidental meetings between the sexes. Painting the eyes of female tsetse did not reduce mating success and insemination in trials with untreated males. These results indicate that the male fly is the aggressor but visual perception is normally a precursor to mating.

Sound

Vanderplank (1948) indicated that 'mating noises', due to vibration of the wings and halteres, were necessary for successful mating. This factor was investigated in tests where the wings and/or halteres of the male and/or female flies were removed prior to pairing in 3×1-in. vials (Table VII). Mating success apparently was not

TABLE VII. *Mating success* from 10 pairs of virgin* G. m. orientalis *where the wings, halteres or both were removed from one or both sexes prior to pairing in 3×1-in. vials*

	Wings removed	Halteres removed	Wings and halteres removed	Wings and halteres intact
		Male treated		
Mean time between pairing and mating (min)	0·62	0·25	0·37	—
Mean duration of mating (min)	189	216	199	—
Pairs mating (%)	100	100	100	—
		Female treated		
Mean time between pairing and mating (min)	0·10	0·80	1·85	—
Mean duration of mating (min)	300	200	129	—
Pairs mating (%)	100	90	100	—
		Both sexes treated		
Mean time between pairing and mating (min)	0·50	0·55	1·70	—
Mean duration of mating (min)	155	247	200	—
Pairs mating (%)	100	100	80	—
		Both sexes untreated		
Mean time between pairing and mating (min)	—	—	—	0·2
Mean duration of mating (min)	—	—	—	187
Pairs mating (%)	—	—	—	100

* Two replicates.

affected by removing the wings or halteres from either or both sexes compared with the results obtained from untreated flies, but the time between pairing and mating was usually increased. No sounds were heard from treated pairs though buzzing occurred not infrequently during control tests. Insemination rates were not checked in this study but observations by Dame & Dean (unpublished) showed that sperm transfer occurred with almost all periods of copulation exceeding 60 min.

Behavioural response of female flies to repeated male mating attempts

Individual virgin female flies in 3×1-in. vials were given an opportunity to mate with a single virgin male on each of five successive days. The possibility of male impotency affecting the results was reduced by replacing the male fly whenever mating failed to occur within 60 min. The mean time between pairing the sexes and the start

TABLE VIII. *Mating success* in 3×1-in. vials with virgin female* G. m. orientalis *allowed to mate with new virgin males* ad libitum *on five successive days (A), compared with females mated for 30 min on day 1 and allowed either to mate* ad libitum *(B), or for 30 min only (C) on days 2–10*

	Initial no. females	Day no. 1	2	3	4	5	6	7	8	9	10	Mean no. females surviving test period	Insemination (%)
A Pairs mating (%)	10	100	65·0	11·1	5.5	0	—	—	—	—	—	9	100
Duration of mating (min)		101–193	65–201	54–230	87–240+	—	—	—	—	—	—		
Mean time (min) between pairing and mating		0·12	0·62	0·16	110·0+	—	—	—	—	—	—		
B Pairs mating (%)	20	100	91·7	25·3	31·7	18·2	12·5	8·4	15·7	13·4	25·0	9	100
Duration of mating (min)		30	60–270+	90–180+	45–240+	15–120+	120–210	120+	120–210	60–240	23–180		
C Pairs mating (%)	20	100	90·0	60·0	60·0	10·5	11·8	26·7	0	50·0	45·5	11	0
Duration of mating (min)		30	30	30	30	30	30	30	30	30	30		

* Two replicates.

of mating tended to increase after the first mating opportunity, while the percentage of pairs mating decreased to zero by the fifth day (Table VIIIA). All of the surviving female flies had been inseminated when examined after the last mating opportunity. In a further test, groups of 20 virgin female flies were mated for 30 min before the males were separated under CO_2 narcosis. On the second day 91·7% of the females given a chance to mate *ad libitum* did so for periods in excess of 60 min; the percentage of pairs mating decreased from the third day onwards but did not reach zero by the tenth day (Table VIIIB). Results from associated studies (Dame & Dean, unpublished) indicated that the majority of the females in this test were probably inseminated on the second day. Groups of females allowed to mate for only 30 min every day generally showed a higher proportion willing to accept a male fly during the 10-day test period (Table VIIIC). Dissection of the surviving females showed that none had been inseminated.

A similar test over a three-day period was varied by offering new males to the female flies two to four times a day. Table IX shows 100% mating with the first group of males, and none with the second and third groups during the first day.

TABLE IX. *Mating success* in 3×1-in. vials with 10 pairs of* G. m. orientalis *where each female was offered a succession of new males during the day*

Mating opportunities	Day 1			Day 2				Day 3	
	1	2	3	1	2	3	4	1	2
Time between mating and pairing (min)	0·1	—	—	37·5	1·7	—	—	—	—
Mean duration of mating (min)	223	—	—	127	12	—	—	—	—
Pairs mating (%)	100	0	0	30	5	0	0	0	0

* Two replicates.

On the second day, 30% and 5% of the females accepted males in the first and second groups, respectively; no mating occurred in subsequent groups or on the third day. The mean time between pairing and the start of copulation increased, and the duration decreased, after the first mating.

Male flies were always aggressive to both virgin and non-virgin females in these tests, and it was the female which prevented copulation after the first successful mating. A virgin female accepted a male passively and with partly open wings, but her

TABLE X. *Mating success* in 3×1-in. vials with 10 pairs of* G. m. orientalis *where the wings of the female flies were removed or glued in the partly open or closed positions*

	Mean time between pairing and mating (min)	Mean duration of mating (min)	Pairs mating (%)
A Virgin females with wings glued in closed position to the abdomen before mating	1·0	121	20
Virgin females with wings glued in closed position before mating but free from abdomen	5·5	144	25
Normal mating	0·1	187	100
B Normal first mating	0·4	197	100
Second mating with wings glued in semi-open position	0·2	189	15
C Normal first mating	0·2	159	100
Second mating with wings glued in closed position and tips cut off to expose the end of the abdomen	0·5	208	5
Normal second mating	0·4	147	25

* Two replicates.

rejection of a second mating was associated with closed wings, a downward curved abdomen and some activity to shake off the male. The male usually ignored the female after making repeated attempts during the first 5 min of pairing. The effect of wing position on the success of mating was assessed by glueing or removing them. Glueing together the wings of virgin female flies in the closed position decreased the proportion of pairs mating and increased the time between pairing and the start of copulation (Table XA). Thus, wing position appeared to obstruct the repeated mating attempts made by the males even though the females were passive and apparently willing to accept copulation by maintaining their abdomen in a normal position. Fixing the wings of female flies, immediately after the end of the first *ad libitum* mating, in either the semi-open position, or in the close position with tips cut off to expose the abdomen, did not increase the percentage of second matings (Table X, B & C). Observations during these second mating attempts indicated that failure to copulate was due mainly to the downward curve of the female's abdomen when approached by a male fly.

Groups of virgin females were mated with virgin male flies in 3×1-in. vials for periods of one to 45 min and then separated. Table XI shows that the proportion of these females accepting new males *ad libitum* on the following day, and the percentage finally inseminated, were reduced compared with normal first matings

TABLE XI. *Mating success* with 10 virgin female* G. m. orientalis *mated for various periods, then separated from the males and remated* ad libitum *with new males 24 h later*

Duration of first mating period (min)	Females accepting second mating (%)	Time between pairing and mating (min)	Mean duration of mating (min)	Females inseminated (%)
1	50·0	I†–2·5	>120	35·0
	10·0	1·0	>180	10·0
15	35·0	I†–1·0	121	20·0
30	33·5	I†–0·5	117	13·3
45	10·0	0·8	>180	10·0

* Two replicates.
† I=Instant mating.

(Table X). However, the time between pairing and the start of mating, and the duration of copulation on the second day, were not affected in these tests. An initial mating period of one minute appeared to have less effect on the success of the second mating than the longer periods. The percentage of females accepting a second mating after a 30-min first mating period was lower in this test than that in Table VIIIC. The reason for this difference is not known since experimental conditions were apparently similar, though the observations were made about a year apart.

Age of the female fly

A possible effect on the success of mating due to the age of the virgin female fly was investigated in both 3×1-in. vials and in standard cages after a 24-h mating period. The percentage of females mating and receiving sperm decreased with age between 7 and 14 days after eclosion and none of the 83-day-old females were inseminated. The time between pairing and mating also increased with the age of the female (Table XII). A comparable range of mating tests with male flies of different ages was not done. However, in a replicated test with fifteen 37-day-old males and 4-day-old virgin female flies the mean insemination rate was 93·3%, thereby indicating that male age probably does not affect mating success.

Discussion

The existence of a pheromone was not established, and successful mating of *G. m. orientalis* was not assisted by the production of 'mating noises'. Under laboratory conditions, the male fly is visually and actively attracted to a moving object,

TABLE XII. *Mating success* with ten virgin female* G. m. orientalis *of various ages paired with 7-day-old males in vials or a standard cage*

Female age (days)	Glass vials (3×1 in.)			Standard cage (8×8×11 in.)		
	Mean time between pairing and mating (min)	Pairs mating (%)	Insemination (%)	Mean time between pairing and mating (min)	Pairs mating (%)	Insemination (%)
3	0·07	100	100	1·5	100	100
7	0·6	100	100	1·2	100	95
14	3·0	25	25	4·8	20	20
21	8·0	20	15	3·0	35	35
28	4·0	20	20	2·0	5	5
83	—	0	0	—	—	—

* Two replicates.

while the female fly does not appear to seek the male. The male does not seem to definitely identify the female fly until within 1–2 cm, but these studies do not indicate how the male differentiates between another male or another species.

The apparent absence of a mating stimulus other than visual is probably offset by this insects' behaviour towards host animals. Tsetse flies in the field are visually attracted to large moving objects (Buxton, 1955; Glasgow, 1963) which can act as foci for meetings between the sexes. Swynnerton (1936) first mentioned the "following swarm" of male flies behind a moving object, and Bursell (1961) showed that those flies were almost all in their "sexual appetitive" phase and were reluctant to feed. Buxton suggested that this behaviour might be similar to the nuptial dance of certain other Diptera, but was not necessarily obligatory in this genera since mating can occur in a small glass tube. It has been generally accepted that males are attracted to a host animal for both feeding and the chance of mating with a female fly, and this normally occurs before or during her first meal since it is uncommon to find a virgin non-teneral female fly. More male than female flies are usually caught during surveys and it has been assumed that this was due to the females arriving only to feed, and that the fertilised females were avoiding harassment by the males which might cause abortions (Glasgow, 1963). However, Mellanby (1936) with *G. fuscipes*, and Buxton (1955) concluded that an excess of males in laboratory cages does not harm the female flies. It seems likely, therefore, that the present techniques sample only segments of the fly population and that differences in the sex ratio, which, in emerging flies, is close to equality (Dean *et al.*, 1968), are due to a combination of behavioural characteristics. In fact, Phelps (1968) has shown that female *G. morsitans* do not go to an ox only to feed, though his results do not indicate what else attracts them. Thus, the specific feeding behaviour of tsetse flies, and in particular the sexual appetitive behaviour of males around visibly moving objects, would seem to eliminate the need for a special sex attractant between the sexes when they must meet regularly at specific foci, the host animal.

Observations on multiple mating opportunities confirm that some female tsetse will accept more than one male. Dame & Ford (1968) demonstrated that females can be inseminated by sperm transferred from two or more male *G. morsitans*, and Squire (1951) reported that female *G. palpalis* (R.-D.) may be mated many times. However, a single fertilisation is sufficient for the whole life of the female (Buxton, 1955) and multiple mating may be uncommon in wild populations. Female tsetse refuse to mate with males immediately after copulating, whether sperm has been transferred or not, but a proportion of them tend to recover their willingness to mate a second time during the next day. Repeated successful or unsuccessful matings over several days also appear to decrease the female's willingness to accept a male. The passive acceptance with partly open wings during the first mating changes to a more active state with closed wings and downward curved abdomen (Mellanby, 1936), and it appears that the position of the abdomen is probably more important than wing position in preventing copulation. This resistance by the female fly after the first mating, even in the absence

of sperm transfer, may be due either to mechanical stimulation or to physiological activity of hormones released by males prior to sperm transfer. Craig (1967) with *Aedes aegypti* (L.) and Riemann *et al.* (1967) with *Musca domestica* L. found that a component of the seminal fluid inhibits insemination from later matings. If this mechanism operates in *G. m. orientalis*, it is obviously not so efficient since second matings are only partially suppressed. Both responsive virgin, and non-responsive fertilised, female tsetse are attractive to the male, but interest in non-responsive females tends to disappear within a few minutes of pairing. Similarly, insemination of virgin females older than 7–14 days was reduced and observations indicated that they were less attractive to male flies than younger females; copulation was rarely seen after an initial brief period of inspection by the male. Mellanby (1936) also found that insemination of virgin female *G. fuscipes* declined if they were older than eight days.

The lack of response by tsetse flies under laboratory conditions does not entirely prove the absence of a pheromone, but chemical attraction between the sexes over large distances is considered to be unlikely. The mechanism governing the females' behaviour immediately after the first mating, and the characteristics by which the male fly recognises or rejects other males, or other species, are subjects for further study.

Summary

Laboratory and field investigations into sex attraction and mating behaviour of *Glossina morsitans orientalis* Vanderplank have been described. Male flies were not attracted to volatile and soluble compounds from two- to three-day-old mature virgin females, or to virgin females in a simple olfactometer. Removal of antennae from either sex did not appreciably reduce insemination rates after 24–48-h mating periods compared with normal flies. Similar numbers of wild male flies were caught off oxen baited or non-baited with mature virgin females. *G. m. orientalis* probably does not produce a pheromone.

Male flies appeared to be sexually activated only after movement by the female. Mating success was reduced in the dark and when the male's eyes were painted, but blinding the female fly did not reduce insemination. Mating was similar with or without wings and halteres and the sounds produced by them.

Time between pairing the sexes and the start of mating tended to increase, and the percentage of inseminated females accepting two or more matings decreased, after the first copulation. Female flies rarely re-mated within a day. Virgin female flies accepted males passively with partly open wings, and mated females rejected further copulation with closed wings, activity to shake the male off, and primarily a downward-curved abdomen. Virgin female flies mated for periods of 1–45 min did not receive sperm but successful *ad libitum* mating was reduced a day later. Insemination rates decreased with females older than seven days and reached zero 28–83 days after eclosion, while 37-day-old males inseminated 93% of young females.

Acknowledgements

The authors wish to thank staff of the University College of Rhodesia and the Agricultural Research Council of Central Africa for their assistance and advice.

References

BURSELL, E. (1961). The behaviour of tsetse flies (*Glossina swynnertoni* Austen) in relation to problems of sampling.—*Proc. R. ent. Soc. Lond.* (A) 36, 9–20.

BUXTON, P. A. (1955). The natural history of tsetse flies.—*Mem. Lond. Sch. Hyg. trop. Med.* no. 10, 816 pp. London, H. K. Lewis.

CRAIG, G. B. (1967). Mosquitoes, female monogamy induced by male accessory gland substance.—*Science, N.Y.* 156, no. 3781, 1499–1501.

DAME, D. A. & FORD, H. R. (1966). Effect of the chemosterilant tepa on *Glossina morsitans* Westw.—*Bull. ent. Res.* 56, 649–658.

DAME, D. A. & FORD, H. R. (1968). Multiple mating of *Glossina morsitans* Westw. and its potential effec. on the sterile male technique.—*Bull. ent. Res.* 58, 213–219.

DEAN, G. J., WILSON, F. & WORTHAM, S. (1968). Some factors affecting eclosion of *Glossina morsitans* Westw. from pupae.—*Bull. ent. Res.* 58, 367–377.

GLASGOW, J. P. (1963). The distribution and abundance of tsetse.—241 pp. Oxford, &c., Pergamon Pr.

MELLANBY, K. (1936). Experimental work with the tsetse-fly, *Glossina palpalis*, in Uganda.—*Bull. ent. Res.* 27, 611–632.

PHELPS, R. J. (1968). A falling cage for sampling tsetse flies (*Glossina*, Diptera).—*Rhod. J. agric. Res.* 6, 47–53.

RIEMANN, J. G., MOEN, D. J. & THORSON, B. J. (1967). Female monogamy and its control in houseflies.—*J. Insect Physiol.* 13, 407–418.

SQUIRE, F. A. (1951). Observations on mating scars in *Glossina palpalis* (R.-D.).—*Bull. ent. Res.* 42, 601–604.

SWYNNERTON, C. F. M. (1936). The tsetse flies of East Africa. A first study of their ecology, with a view to their control.—*Trans. R. ent. Soc. Lond.* 84, 579 pp.

TEESDALE, C. (1940). Fertilization in the tsetse fly, *Glossina palpalis*, in a population of low density.—*J. Anim. Ecol.* 9, 24–26.

VANDERPLANK, F. L. (1948). Experiments in cross-breeding tsetse-flies (*Glossina* species).—*Ann. trop. med. Parasit.* 42, 131–152.

(*Received 25 April 1969*)

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