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MULTIPLE MATING OF GLOSSINA MORSITANS WESTW. AND ITS POTENTIAL EFFECT ON THE STERILE MALE TECHNIQUE

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Promising advances in rearing techniques and the advent of insect sterilisation have refocused attention on the multiple mating of Glossina spp. Male multiple mating of Glossina has been demonstrated repeatedly by workers who used an individual male to inseminate several females. Vanderplank (1947) perhaps came closest to demonstrating multiple mating of females of Glossina when, during hybridisation trials with G. morsitans Westw. and G. swynnertoni Aust., a single female produced dissimilar male offspring, one, by morphological characters, G. morsitana, and the other a hybrid. Such a phenomenon could occur if the female accepted sperm from more than one male. All other reported observations relating to multiple mating of females are based either on observed coupling or on the quantity of semen in the spermathecae. For example, Jordan (1958) reported that young females of G. palpalis (R.-D.) paired with males repeatedly but older females did so less readily; Potts (1958) suspected that pupae produced by unirradiated females mated with irradiated males might have resulted from multiple matings in which unirradiated males had been concerned; Southon & Cockings (1963) found that spermathecae were more replete when older adults were mated and when the ratio of males to females was increased. Although these findings tended to indicate multiple mating in females, they did not demonstrate that the females actually accepted semen more than once.

It was suggested by Dame & Schmidt (1964) that labelling of insect sperm genetically or with radio-isotopes would provide a suitable tool for determining multiple insemination with certainty, and Dame & Ford (1966) have now reported male sterility as a technique of labelling Glossina sperm. The degree of fertility can be used as a positive criterion of multiple insemination of females. Studies were therefore made of multiple mating in females of G. morsitans by utilising sterile and fertile sperm as indicators. Also, the extent and effectiveness of male multiple mating was investigated, and the relationship between multiple mating in Glossina and population control by the sterile male technique was considered.

Methods

Standard test conditions

The tsetse flies (G. morsitans orientalis Vanderplank) were collected by hand as pupae in the Zambezi River valley near Kariba, Rhodesia. Emerging adults were separated within 24 hr., separately maintained at 79 ± 2°F. and 70 ± 2 per cent. relative humidity in 8 x 8 x 11-in. cages, and fed daily on guinea pigs. After the females had mated, the reproductive capacity was determined by collecting the resulting pupae and observing adult emergence as described previously by Dame & Ford (1966). When the adult males were two days old, they were permanently sterilised by exposure for one hour or four hours to tepa deposits of 10 mg./sq.ft. on glass. All tests were conducted with two- to

three-day-old virgin females and six- to seven-day-old virgin males unless otherwise indicated.

Tests of female mating behaviour

Initial matings were accomplished by placing single females in $2 \frac{1}{2} \times 1$-in. glass tubes with a sterile male and holding the pair until copulation was terminated naturally. Previous observations by the authors (unpublished data) had demonstrated that most mature females remaining *in opula* for 60 min. or more became inseminated; thus, females that copulated less than 60 min. were discarded. Immediately after copulation, 25 females were transferred to a cage containing 25 fertile (untreated) males. Other females were transferred to empty cages, and 25 fertile males were introduced 24 or 96 hr. later. Control cages were made up of 25 untreated pairs. Each test cage was held for 56 days, and the pupae produced during the first and last 28 days were collected and held separately. Adult emergence was determined six weeks after the collection of pupae.

In this type of experiment the males must be completely sterile, and the percentage of insemination resulting from the first series of matings must be known. Without such data, it would be difficult to interpret the results when progeny occur since they might originate from occasional fertile sperm from treated males or from females that were not inseminated during the first mating (and thus would not necessarily demonstrate a second insemination). Male sterility was therefore ascertained by placing the 25 treated males previously utilised for each replicate in a common cage and introducing 25 virgin females. After a 28-day holding period the cages were observed for progeny. Also, female insemination was checked indirectly by initiating more pairings than necessary for the first mating and determining the rate of insemination by dissecting the surplus females and examining the spermathecae microscopically. All percentages of insemination reported here are the result of determinations with a dissecting microscope followed, when they were inconclusive, with further observations by phase microscopy. By knowing the degree of both sterility and insemination, the number of pupae expected without a second insemination could be estimated, and the results could be interpreted accurately.

In another test, sterile or fertile males were caged with virgin females for 96 hr. and then replaced by fertile or sterile males, respectively. (Male sterility and rates of insemination were not determined.) Thus, repeated inseminations could occur during the first 96-hr. mating period; in the previous 96-hr. test, no matings were possible during the 96 hours after the initial pairings.

Tests of male mating behaviour

The mating behaviour of males was investigated in two series of tests. In the first, the ability of groups of males to inseminate larger groups of females was evaluated by caging five males with 25 females for three to four days. Then the surviving males were transferred to another cage containing virgin females at the same ratio; the surviving females were dissected, and their spermathecae were checked to determine whether normal amounts of sperm were present. This procedure was repeated until normal insemination ceased. The dissection microscope was used to detect normal amounts of sperm (ranging from the replete spermathecae to those only 10-15 per cent. full). Smaller amounts of sperm were more difficult to determine positively, and insemination was therefore confirmed with phase microscopy. When it was necessary to use the phase microscope, the insemination was recorded as subnormal.

In the second test series, the reproductive capacity of males involved in multiple mating was evaluated by caging 25 pairs of adults for one week. The
surviving males were transferred to a cage containing 25 fresh virgin females. The procedure was repeated for five weeks in four replicates and until no males survived in two replicates. Productivity of each group of females was determined after 28 days; also the rate of insemination in surviving females was determined.

Results and discussion

Mating behaviour of females

The results obtained when females were paired only once during the first mating phase are shown in Table I. The treated males in this group were completely sterile. A mean of six per cent. of these females were not inseminated during the first mating phase; but by the end of the test the rate of insemination in surviving females was 100 per cent. in all but one replicate (in which one female was uninseminated). Because male sterility was complete, not more than one or two progeny (six per cent.) should occur in any group if multiple insemination had not occurred, and these offspring would come from females that were not inseminated during the first mating. Thus, any group producing three or more progeny are indicative of multiple matings.

All groups given a chance at second matings produced an average of three or more progeny, and females allowed to remate immediately produced the most progeny (12-5) during the first 28 days; those held away from males for 24 or 96 hr. produced progressively fewer offspring. During the second 28 days, the number of offspring produced by all groups was essentially the same, regardless of the time between matings. Thus, females already inseminated with sterile sperm were able to reproduce sooner if the opportunity to accumulate fertile sperm was not delayed excessively, probably because fertile sperm was present in the spermathecae at the time of the first ovulation. In the group remated immediately, about 45 per cent. of the available ova were fertilised (12-5 offspring compared with the control mean of 28-0). Females whose opportunities for fertile insemination were delayed 24 or 96 hr. produced fewer offspring, presumably because the first ovulation and union of the gametes occurred before the introduction of fertile sperm. Thus, sterile and fertile sperm did not compete until the second ovulation 10-15 days later. The results of the second 28 days indicate that all groups were equally involved in second
matings since production was similar in the three categories. Therefore, some females accepted second or multiple inseminations, but the actual incidence of multiple matings is not known. However, since production during the second 28 days was about 31 per cent. that of the untreated controls, a third of the females were apparently inseminated more than once.

The results obtained when females had the opportunity for multiple mating during the first mating period also are shown in Table I. Although the degree of sterility of treated males and the rate of insemination of females was not determined, the lack of reproduction in the first 28 days by females caged originally with treated males suggests that 100 per cent. male sterility and insemination were achieved. During the second 28 days, females mated first with treated males produced only 25 per cent. as many offspring as females mated once in the first period. Production by females originally caged with fertile males and subsequently exposed to treated males was 67.86 per cent. as great as that of the untreated controls. Thus, the reproductive capacity of females was affected to a lesser degree by introducing new males after 96 hr. of continuous mating opportunity than that of females mated only once before the 96-hr. holding period. A logical explanation is that the females were inseminated more than once the first 96 hr. and, having satisfied their need for sperm, accepted further semen less readily.

The combined series demonstrates that females of *G. morsitans* accept multiple inseminations and suggests that they will, in time, become less receptive to subsequent insemination if the initial mating pressure is sufficiently heavy.

### Mating behaviour of males

The results of studies of the capacity of the males to inseminate are given in Table II. Normal inseminations occurred through the first six mating series, but the incidence of subnormal inseminations increased noticeably after the second series, and no sperm transfer occurred in the seventh series. The minimum mean number of normal inseminations achieved per male was 5.6; the maximum mean number of individual inseminations is unknown because some females may have been inseminated more than once. However, each male obviously had sufficient sperm to inseminate several females.

The relationship between male mating behaviour and productivity is shown in Table III. Although no distinction was made between normal and subnormal amounts of sperm in the spermathecae and positive insemination was recorded if any sperm was detected, both percentage insemination and pupal production declined progressively after the third series of matings. Also, the drop in

### Table I. Consecutive insemination of females of Glossina morsitans caged for 3-4 days with males at a 5:1 ratio (mean of two replicates)

<table>
<thead>
<tr>
<th>Mating series</th>
<th>Male age (days)</th>
<th>Initial number of</th>
<th>Insemination</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Females</td>
<td>Males</td>
<td>Per cent.</td>
</tr>
<tr>
<td>1</td>
<td>6-10</td>
<td>27-5</td>
<td>6-5</td>
</tr>
<tr>
<td>2</td>
<td>9-18</td>
<td>22-5</td>
<td>4-5</td>
</tr>
<tr>
<td>3</td>
<td>12-17</td>
<td>20-0</td>
<td>4-0</td>
</tr>
<tr>
<td>4</td>
<td>15-21</td>
<td>17-5</td>
<td>3-5</td>
</tr>
<tr>
<td>5</td>
<td>18-24</td>
<td>16-0</td>
<td>3-0</td>
</tr>
<tr>
<td>6</td>
<td>21-28</td>
<td>15-0</td>
<td>2-0</td>
</tr>
<tr>
<td>7</td>
<td>27-31</td>
<td>10-5</td>
<td>0-5</td>
</tr>
</tbody>
</table>
Mating behaviour and the sterile male technique

Males and females of *G. morsitans* are each capable of mating more than once. Whether multiple mating occurs in nature is unknown, but it seems likely. However, mating pressure in the field is probably less intense than in the laboratory because of the lower population densities, and multiple mating may be much less common than that we experienced.

It should be noted, however, that multiple mating by females will not *per se* jeopardise a sterile male release programme so long as the sterile sperm are competitive with sperm from the wild population (von Borsiel, 1960). Our tests and others (Dame & Ford, 1966) have demonstrated that the treated sperm of *G. morsitans* are competitive with untreated sperm—the only difference is that dominant lethality has been induced in the treated sperm. In a wild population overcrowded by sterile males, females would have the same mathematical chance of encountering sterile males for second matings as they had at the time of the first mating. For example, in a population consisting of three times as many sterile males as wild males, a female has a 3:1 chance of pairing with a sterile male at each mating. Perhaps population control would take slightly longer because of the influence of normal sperm in females inseminated with both normal and sterile sperm, but control should not be seriously affected. The successful use of sterile melon flies, *Dacus cucurbitae* Coq., in an eradication programme (Steiner & others, 1965) demonstrated that multiple mating by females is not necessarily a deterrent to the control of natural insect populations by the release of sterile males. Indeed, multiple mating may sometimes enhance the rate of control achieved because the reproductive capacity of previously inseminated females may immediately be reduced when these females mate with sterile males (KnDistrict, 1964). If the females did not accept such second matings, only death would end their reproductive potential, and population control would be limited to sterile
matings with virgin females. Since females of *G. morsitans* are apparently more receptive to multiple matings when they are young, we may be dealing with a type of polyandry that occurs early in life and then ceases rather abruptly.

Multiple mating of male *G. morsitans* would be detrimental to the sterile male technique only if the sterile male behaved abnormally or failed to inseminate as many females as its wild counterpart. Our field cage studies and free flight releases (unpublished data) indicate that chemosterilised males mate and disperse normally in the natural environment. In studies of sterile males conducted with the same laboratory conditions described for the present tests, treated males inseminated an average of 7.7 females. Thus, male multiple mating would probably not have an adverse effect on control programmes utilising sterile male *G. morsitans*.

**Summary**

Virgin females of *Glossina morsitana* Westw. derived from pupae collected in the field were exposed in the laboratory to virgin sterile males and later to virgin normal males; the resulting productivity was an indication of multiple mating by females. Females inseminated once with sterile sperm and then held for 0, 24 or 96 hr. before being allowed to mate with fertile males produced about equal numbers of progeny 28–56 days later; however, subequal numbers of progeny were produced the first 28 days after the initial mating because of the relationship between the time of the second insemination and ovulation. Females given the opportunity to mate repeatedly for 96 hours with their first mates also mated again with new groups of males, but the incidence of this second mating was lower. Thus, multiple mating of females in the laboratory is established, but its frequency in nature is unknown.

When groups of males were exposed to successive new groups of virgin females, the minimum mean number of females inseminated per male ranged from 4.9 to 5.6. However, the later inseminations were progressively less effective, and once the complement of sperm of an individual male was depleted, fertility was not recovered. The multiple mating of male and female *G. morsitans* is not expected to affect the use of sterile males for population control.

**Acknowledgements**

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**References**


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