ASPECTS OF THE MATING BEHAVIOUR OF *GLOSSINA MORSITANS MORSITANS* WESTWOOD AND *GLOSSINA PALLIDIPES* AUSTEN

BY

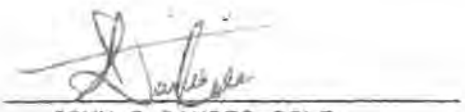
JOHN OLIVER ABIMBOLA DAVIES-COLE
B.Sc Hons, Dip.Ed, M.Sc.

A THESIS SUBMITTED IN ACCORDANCE WITH THE REQUIREMENTS OF THE UNIVERSITY OF SIERRA LEONE FOR THE DEGREE OF DOCTOR OF PHILOSOPHY

FEBRUARY 1990

DECLARATION

I declare that this thesis is my original work and has not been presented for a degree in any other University.



JOHN O DAVIES-COLE

We declare that this thesis has been submitted for examination with our approval as Supervisors.

PROFESSOR M O WILLIAMS
HEAD, DEPARTMENT OF ZOOLOGY,
FOURAH BAY COLLEGE,
UNIVERSITY OF SIERRA LEONE

PROFESSOR H MORGAN
ASSOCIATE PROFESSOR, DEPARTMENT OF ZOOLOGY,
FOURAH BAY COLLEGE,
UNIVERSITY OF SIERRA LEONE

DR B JAMES
LECTURER, DEPARTMENT OF ZOOLOGY,
FOURAH BAY COLLEGE,
UNIVERSITY OF SIERRA LEONE

TO

NANCY, EMLYN AND OLIVE

"....UNLIKE MOST BEHAVIOUR, COURTSHIP PATTERNS HAVE NO OBVIOUS RELATIONSHIP TO THEIR FUNCTION. IT IS NOT APPARENT WHY A COCKEREL SHOULD DROOP ONE WING AND 'WALTZ' AROUND A HEN, RATHER THAN SPREAD ITS TAIL LIKE A PEACOCK OR BOW LIKE A PIGEON".

AUBREY MANNING (1966)

I. C. I. T. E. LIBRARY
ACC No 6312
CLASS No 591.56 (P43.2) DAV
AUTHOR
TITLE

A C K N O W L E D G E M E N T S

I wish to express my sincere thanks to my Supervisors, Professor Hector Morgan, Dr B James, Drs M F B Chaudhury and G Kaaya for their guidance, patience and unreserved co-operation during the course of this work. My thanks also go to Dr Leonard Otieno, Programme Leader, Tsetse Research Programme, ICIPE, in whose laboratories this work was carried out. To Dr M Smalley and Professor Z Dabrowsky who as Coordinators of the African Regional Postgraduate Programme in Insect Science (ARPPIS) co-operated fully in order to make my tenure a rewarding one.

Special thanks go to Professor T R Odhiambo, Director, International Centre of Insect Physiology and Ecology (ICIPE) under whose auspices my tenure was made possible. To Drs Steve Mihok, R Saini and Professor Jan Zdarek for their valuable suggestions and comments. To Miss Nafisa Darji, Mrs Abigail Okumali, Mrs Mary Antao, Miss Esther Afande, Messrs M Opiyo, S Onyango and M Kotengo who assisted in various ways during the course of this work, and to my wife, for her assistance in typing this thesis. This work was carried out at The International Centre of Insect Physiology and Ecology in Nairobi, Kenya, under the sponsorship of Deutscher Akademischer Austauschdienst (DAAD) or German Academic Exchange Service.

A B S T R A C T

Sexual receptivity was investigated in Glossina morsitans morsitans and Glossina pallidipes by observing successful pairings of females for specified periods of time.

The duration of copulation, degree of insemination of females and male accessory reproductive gland secretions were not responsible for the termination of sexual receptivity in G. m. morsitans. However, mechanical stimulation due mainly to the onset of the 'male jerking phase' was the single most important factor responsible for refractoriness of females to further matings. Mechanical stimulation alone caused over 70% refractoriness within 72 hours. This result is comparable to that of normal matings where mechanical stimulation is accompanied by the introduction of male accessory gland secretion and spermatozoa. In G. m. morsitans, multiple matings were observed to occur early in life; however, once a female became refractory, it remained so for the rest of its life. Single matings were found to provide sufficient spermatozoa for fertilisation. Males as old as 60 days retained their virility.

G. pallidipes was reared on a small scale under controlled conditions of temperature (25° ± 1° C) and humidity (75 - 80%). Female sexual receptivity in G. pallidipes was highest when 9 - 13 days old but declined thereafter. Maximum mean spermathecal values (MSV) were recorded during the period of highest receptivity. The duration of copulation was comparatively short (mean of 24 minutes)

for all age groups. Though spontaneous ovulation occured in virgins, mating was generally the initiating factor. Eighty percent of females at the age of highest receptivity (9 - 13 days old) were inseminated when group-mated compared to 50% for single matings. Between 55 - 80% of females were inseminated when they were kept with males for 72 hours compared to 12 - 50% insemination when kept for 24 hours. The results also indicate that insemination rates are higher (60 - 100%) for females kept in smaller than in larger (60 - 80%) cages. There was no significant difference in the insemination of females kept together with males in continuous darkness (insemination of 50 - 88%) compared to those exposed to alternate 12 hour light and 12 hour dark regimes (insemination of 65 - 85%).

Males of G. pallidipes were most aggressive and made the greatest number of mating strikes when they were 11 days old. This age of male 'sexual appetitiveness' coincided with the maturation of their accessory reproductive glands, i.e. when the glands had reached 0.17 - 0.20mm in diameter. Males were, however, able to inseminate successfully from the age of 7 days onwards.

The demonstration that G. m. morsitans and G. pallidipes do not remate after several gonotrophic cycles would enhance the success of the use of the sterile insect technique in their control. The results also show that carefully controlled rearing and environmental conditions, adequate feeding, use of small cages, confinement of males and females for up to 72 hours and mating of

9 - 13 day-old females with over 11 day-old males would facilitate the successful rearing of a laboratory colony of G. pallidipes.

TABLE OF CONTENTS

PLATE 1

TSETSE FLIES IN COPULA

CHAPTER 1

GENERAL INTRODUCTION

The tsetse fly, Glossina spp. is restricted to tropical Africa from between approximately latitudes 15°North and 20°South, but extending to about 30°South along the eastern coastal area of Africa (Service, 1980). It covers about 11 million km of Africa and its limits are determined by climate, paleoecology, feeding habits and vegetation. Generally, areas that are devoid of trees due to the activities of man or otherwise are free of the insect. Near the equator, tsetse flies do not occur in areas about 1,800m, but the upper limit decreases with distance from the equator (Jordan, 1986).

There are about twenty-two different species of tsetse flies living in Africa today. Some of these are divided again into sub-species based on certain minor but constant differences in their anatomy. Generally, tsetse flies could be recognised by the rigid and forwardly projecting proboscis and a characteristic wing venation. In between veins four and five there is a closed cell which appears like a hatchet and termed a hatchet cell. Adults are yellowish or brown-black robust flies. According to Potts (1970), they were generally included in the dipteran family, Muscidae. Other authorities such as Brues et al (cited by Jordan, 1986) have regarded the genus as having very uncertain affinities with the Muscidae and have now placed it in a monogeric family, the Glossinidae. It is generally accepted that the nearest living relatives of the Glossinidae are the Hippoboscidae or louse flies.

Today, there are three groups of tsetse flies that are recognised; the fusca group, the palpalis group and the morsitans group. The fusca group is not well known, probably because none is of major economic importance. Most species in this group never feed on man neither are they attracted by his presence. The group includes such species as G. fusca Walker, G. brevipalpis Newstead and G. longipennis Corti. The palpalis group occupies river systems that drain into the Atlantic Ocean, Mediterranean Sea and the inland drainage systems of some of the great African Lakes, but not along river systems draining into the Indian Ocean (Jordan, 1986). This group includes species like G. palpalis Robineau-Desvoidy, G. fuscipes Newstead and G. tachinoides Westwood. These species are vectors of trypanosoma which cause sleeping sickness and animal trypanosomiasis or nagana. The G. morsitans group include: G. m. morsitans Westwood, G. pallidipes Austen, G. swynnertoni Austen, and G. austeni Newstead. G. morsitans and G. pallidipes are important vectors of trypanosomes which cause human and animal trypanosomiases. G. austeni is also an important vector of animal trypanosomiasis.

Sleeping sickness is a human disease which, when not treated in its early stages results in death. Animal trypanosomiasis or nagana in cattle also results in death if untreated. There are two species of trypanosomes causing African sleeping sickness in man, T. brucei gambiense Dutton, and T. b. rhodesiense Stephens and Fantham; those affecting domestic animals are T. vivax Ziemann, T. congolense Broden, T. simiae Bruce et al,

T. brucei brucei Plimer and Bradford and *T. suis* Ochmann. The debility caused by these diseases is tremendous. Although trypanosomiases were first discovered in 1841, human and animal trypanosomiases are ancient diseases that have scourged mankind in Africa for centuries (Cheng, 1973). Cheng states that, "to see Africans lying prostrate, drooling from the mouth, insensitive to pain, and later dying must have been a common occurrence to early slave traders". The presence of the flies not only makes it difficult to produce milk and beef, but also makes it difficult to optimise agricultural production as cattle play an important role as a source of draught power. For example, Jordan, (1985) stated that in the Zambezi Valley, more than half a million peasant farmers have been severely affected by sleeping sickness and many of their cattle died of nagana while surviving cattle could only be kept alive by the use of drugs. According to a report of the WHO Expert Committee (1986), African human trypanosomiasis is endemic in 36 countries of sub-saharan Africa where the tsetse fly occurs.

Many methods have been tried in the past to control this dreaded scourge. One of the first was the elimination of the fly by killing large numbers of wild animals, the natural hosts (Jordan, 1986). Later on, the methods were refined and only animals of a certain species (for example, warthogs) were shot. This method was abandoned because the flies could not be eliminated as they switched over to other animals such as reptiles which were not eliminated; such methods also resulted in indiscriminate destruction of wildlife. Another method on similar lines was the clearing of

vegetation which the tsetse flies normally frequent. Maintaining the cleared areas proved extremely costly apart from the fact that the clearing of vegetation also destroyed the environment. In rural development projects, the land is often cleared for various forms of agricultural practices; banana plantations have thus replaced the original vegetation in some instances. This has caused a resurgence of fly populations in these places as they have been known to harbour the fly (Mulder, 1989).

Insecticides have contributed immensely to tsetse control; DDT and dieldrin have been used successfully (Jordan, 1986). Recently, synthetic pyrethroids have been used because they appear to be less harmful to the environment than DDT, dieldrin and endosulphan. Trials were carried out with synthetic pyrethroids such as permethrin, cypermethrin and deltamethrin (Spielberger et al, 1979). Deltamethrin was found to be the most effective but the high cost of using these insecticides may prevent their widespread use.

Traps have been used for sampling Glossina spp. for many years. Early traps were not very efficient, because of their designs, but during the last decade trapping became the most favoured sampling technique owing to such advantages as the possibility of sampling populations at several places at the same time, suppression of human factors and the use of standard materials (Challier, 1982). Sometimes traps are impregnated with insecticides or baited with various chemicals to increase their efficiency. The biconical trap was developed in West and Central Africa and it proved to be more effective when

baited with attractants (Challier, 1977). Other traps such as the NG2B trap was developed for G. pallidipes; it caught 2-11 times more female G. pallidipes than a similarly baited biconical trap (Brightwell and Dransfield 1986). Other methods such as biological control (the deliberate use of predators, parasites or pathogens for control) have been tried. A few unsuccessful attempts have been made to release biological agents into habitats containing tsetse flies but these attempts have been unsuccessful (Jordan, 1986). Parasitoids are the only agents which show some promise and these include some members of the Bombyliidae and Mutillidae (parasitic flies and wasps).

Other methods of control such as genetic control have been suggested. For example, the sterile insect technique (SIT) was suggested by Von Borstel (1960) and Dame and Schmidt (1970). This method has been used with some success. For example, Van Der Vloedt et al (1980) were able to suppress the tsetse population in the Volta Noire tributaries of Upper Volta by the use of deltamethrin followed by the release of sterile males. They were able to demonstrate that sterile males can be used for eradication of low-level infestations. Takken et al (1986) also combined the use of traps, insecticide impregnated targets, and the SIT to eradicate G. palpalis in a 300km^2 area of Central Nigeria. They found that traps and screens reduced the population of flies by more than 90% but failed to eradicate it; but males sterilised by irradiation from a ^{60}C source and released at weekly intervals successfully eradicated the flies (at a ratio of 10:1 of sterile to wild male).

The SIT, therefore, has a lot of potential in the control of tsetse flies. The relative merits of the SIT developed are that the tsetse fly has a low reproductive potential, that once a fly mates with a sterile male, it is likely that it will not reproduce for the rest of its life. However, Curtis (1968b) found that when individual females were mated to both a sterilised and a fertile male, sperm from the male that mated first was used predominantly, but not exclusively for fertilisation. He concluded that the extent to which this behaviour would reduce the efficiency of the SIT would be relatively trivial. Dame and Ford (1968) also concluded that multiple mating was not expected to affect the SIT. However, the SIT requires the use of enormous numbers of tsetse flies and expensive mass rearing facilities.

Glossina pallidipes is difficult to rear under laboratory conditions, whereas G. morsitans has been successfully colonised in the laboratory (Azevedo and Pinhao, 1964). This difficulty in rearing coupled with expensive mass rearing conditions will make it very difficult to use the SIT to control or possibly eradicate this species from its habitats. Recently, some laboratories have reported successful colonies of G. pallidipes. For example, Leegwater-van der Linden (1980), Ochieng et al (1987) and Langley (1989) have all reported successful laboratory colonies of G. pallidipes. Gradually the battle is been won but for some strains of G. pallidipes, there are still problems. For example, Langley (1989) did not succeed with the Zimbabwean strain but succeeded with the Lugala, Uganda strain. Lack of information on the mating biology of this species has contributed to the rearing

difficulties (Jaenson, 1978b). Van Etten (1981) and Jaenson (1978a) emphasised the importance of studying individual populations as there are differences in mating behaviour between allopatric populations.

The objectives of this study are:

(a) to determine the time of female sexual receptivity and male sexual appetitiveness of G. pallidipes and compare these with those of G. morsitans;

(b) to determine the mechanisms of sexual receptivity and male sexual appetitiveness with reference to ovarian development, growth and development of the male accessory glands, and changes in behaviour pattern over physiological age;

(c) to identify components from male accessory glands capable of suppressing sexual receptivity and male sexual appetitiveness;

(d) to investigate the phenomenon of multiple mating in parous and nulliparous flies;

(e) to determine what controls refractory behaviour to prevent multiple-mating in female tsetse.

It is expected that the study will lead to an understanding of aspects of the biology and population dynamics of G. morsitans and G. pallidipes, assist in devicing better laboratory rearing techniques for G. pallidipes in particular, and contribute to the application of the SIT.

CHAPTER 2

LITERATURE REVIEW

2.1 SEXUAL RECEPTIVITY IN *GLOSSINA SPP.*

For successful reproduction in most insects, it is necessary that copulation occurs and male and female reproductive systems are prepared for this purpose in order to ensure insemination and fertilisation (Langley, 1977). Sexual receptivity in males and females therefore refer to the period when the sexes are ready for mating. In insects, the onset of sexual behaviour appears to be independent of the gonads. It has been shown in some insects that after castration in both males and females, normal copulation can still take place (de Wilde and de Loof, 1973; Tschinkel, 1985). It has also been shown in *Drosophila spp.* that males have an elaborate courtship display whereas in the Muscidae and Calliphoridae, males simply fly towards any object of the right size and attempt to copulate directly (Manning, 1966). These are not just chance encounters but are behaviour patterns under endocrine control. In *G. pallidipes*, Jaenson (1979a) found no distinct premounting courtship or other premounting sexual behaviour. He observed that on contact between a sexually appetitive male and a receptive female, the male mounted or landed on the female's back, while she opened her wings slightly and the male tried to grasp the tip of her her abdomen. If the female is unreceptive it usualy does not allow a hypopygial connection but instead bends its abdomen downwards and kicks violently.

In G. morsitans, Huyton and Langley (1982) reported that a receptive female remained immobile whilst the male moved into position over her with characteristic rapid movements until they were aligned head-to-head with the male opening the wings of the female to expose the tergum. They also observed that an unreceptive female vibrated her wings in the closed position with the abdomen flexed ventrally and the meso - and metathoriacic legs extended upwards to push against the body and wings of the male; if a male was unsuccessful his attempt was usually abandoned. In the laboratory G. morsitans readily mate after the first blood meal, usually 1-3 days after emergence (Saunders and Dodd, 1972; Tobe and Langley, 1978). Langley (1977) reported however that species differences exist and that in G. pallidipes, females became sexually receptive when they were 7-9 days old. Jaenson (1978b) and Leegwater-van der Linden (1982) reported peak receptivity for females of G. pallidipes at the age of 8-13 days, but Rogers (1972) reported this to be 7-9 days.

There are variations in the time when females are most attractive to males (Tobe and Langley, 1978). Nonetheless, whatever the preferred age at mating of female Glossina, there is no doubt that there is a period when virgin females are most receptive to males, and this is normally before the first oocyte has matured (Tobe and Langley, 1978). This may not be true for all species of Glossina; for example, Jaenson (1980) and Leegwater-van der Linden (1982) found that in female G. pallidipes, receptivity increases at the time of maturation of the first egg. Even though many males will

mate when quite young (for example, as seen in emergence cages of G. morsitans) there is likely to be a problem of insemination. Indeed, Nash (1955) found that females of G. palpalis 1-5 days old were much more willing to copulate than those 6-8 days old, but males must be 7 to 8 days old before their virility will approach that of older males. Rogers (1972) also found that female G. pallidipes mated when 3 or 5 days old gave lower production than those mated when 7 or 9 days old. The explanation was that those which mated earlier were probably not inseminated due to their failure to complete the process. In houseflies, Chaudhury and Ball (1974) found that attraction seemed to be maximum when females were 5 to 6 days old and males were 4 to 8 days old post-emergence. It is therefore necessary that the precise age at mating should be determined if large scale rearing is to be successful.

Although behavioural differences clearly exist between different species of organisms, in many cases, differences can also exist between members of the same species. Jaenson (1978b) reported that in East Africa, G. pallidipes appeared to exist in indiscrete populations and likely to be subjected to different ecological conditions. Different ecological strains could thus behave differently. Van Etten (1981) also observed such differences between the Nguruman and Mwalewa forest flies in Kenya.

What is it that makes a sexually receptive female attractive to males? Langley et al (1975) speculated that a sex pheromone might be present in Glossina which stimulates mating similar to

the short range sex pheromone in Musca domestica. This was confirmed by Langley et al (1975) and Langley (1974 and 1977) who reported that a hydrocarbon component of the adult female cuticle of G. morsitans acts as a contact pheromone and elicits copulatory behaviour in males, and that the male sense organs through which this operates was unknown. The existence of such a sex pheromone in G. pallidipes was also demonstrated (Langley et al, 1982) and a contact sex pheromone was identified in G. austeni by Huyton et al, (1980a and 1980b). In G. m. morsitans three components, 15, 19-dimethylheptatriacontane, 17, 21-dimethylheptatriacontane and 15, 19, 23-trimethylheptatriacontane were isolated from female flies (Carlson et al, 1978). These workers found that these three compounds independently caused release of mating attempts by the male at ultra - short range or upon contact; the third compound was the most active.

In G. pallidipes, 13, 23-dimethylpentatriacontane was found to be the most active compound (Carlson and Langley, 1983). It has been shown that differing responses of males of G. m. morsitans were largely the consequence of differences in composition of the female surface cuticular paraffins and not of female behaviour (Huyton et al, 1980b). It was later found that receptors arranged in two pairs on the inner and outer sides of the proximal end of the tibia of the legs of G. morsitans function in the perception of sex pheromones (Schlein et al, 1981b). It was reported by Huyton et al, (1980a) that the amount of sex pheromone in the cuticular surface of females of G. morsitans did not decline with

age and that there was no evidence that females became less stimulatory to males with age. However, there are hexane-soluble substances on the cuticle of male flies which terminate courting by other males on contact (Schlein et al, 1981a and 1981b; Coates and Langley, 1982).

It was suggested that receptivity in Glossina could be related to pheromone production. However, Langley et al (1982) thought this unlikely. These authors reported that sex pheromones of G. pallidipes are present in very young females in sufficient quantity to elicit full copulatory responses from males. The control of receptivity in tsetse flies is still largely unknown. Saunders and Dodd (1972) found that females of G. morsitans appear to be receptive after taking a blood meal. Gillott and Langley (1981) reported that loss of receptivity after mating in G. morsitans is due to the exchange of male accessory gland secretion as well as mechanical stimulation during mating; either factor on its own is ineffective in controlling receptivity. Dame and Ford (1968) had suggested that the relative amount of semen in the spermathecae may influence female receptivity to subsequent matings. This has not been confirmed. Samaranayaka-Ramasamy (1981) reported that in G. morsitans, the diameter of the male accessory gland increased with age; a clear correlation was seen between the increase in diameter of ARG and the amount of sperm transferred. Does it then follow that males become more aggressive at the time of maturity of their accessory gland? This has not yet been confirmed in other species of Glossina. The relatively long interval that must elapse before females can remember their mating experience suggests a hormonal

release or neural facilitation (Tobe and Langley, 1978). Recently, Chaudhury (1988) reported that male sexual maturation, mating behaviour and inseminating capability are all regulated by total blood intake, and by complex neuro-endocrine mechanisms involving both juvenile hormone and brain hormone.

2.2 LABORATORY REARING OF *GLOSSINA SPP.*

Laboratory colonization of *Glossina* was said to have been accomplished only on very few occasions and even then usually only partially and with difficulty (Lumsden and Saunders, 1966). The situation has changed very little with regards to the rearing of *G. pallidipes*. However, other species like *G. morsitans* are being reared successfully.

The maintenance of tsetse flies in the laboratory was begun early this century, but was on a small scale (Mellanby and Mellanby, 1937; Willett, 1953). Attempts to produce self-sustaining colonies were made by Willett (1953) in order to avoid the difficulties of obtaining pupae from remote districts by post, and the losses inherent in this method of supply. This attempt was partially successful with a few species such as *G. austeni* and *G. swynnertoni*. Azevedo and Pinhao (1964) were the first to report the results of a successful attempt to rear a single-line colony of *G. morsitans* at the Lisbon Institute of Tropical Medicine. The original pupae of *G. morsitans* were obtained from Mozambique. However, many problems arose with *G. pallidipes*, especially with fertilisation; the time of sexual receptivity had not been adequately studied.

In the 1960s, self-sustaining colonies of various species of Glossina were kept in many countries. The tsetse flies were fed mostly on Guinea pigs and sheep. Nash et al (1966a) reported a method for rearing G.austeni on a small scale using rabbits' ears for feeding. This was apparently a more simple and easy way of feeding tsetse. Their results showed that the full reproductive potential of G. austeni was reached and the insects did not appear to be physically inferior to those found in nature. The productivity of flies fed on lop-eared rabbits was also found to be better than any previously obtained for Glossina using other methods (Jordan and Curtis, 1968a and 1968b; Curtis and Jordan, 1968). It was concluded that the improvement was due to excellent nutrition of the flies. It was thought that for large scale rearing a much better way of feeding a large number of flies within a short time was desirable. Nash et al (1968) were able to arrive at such a method; they described a technique for a large scale rearing of G. austeni at the Tsetse Research Laboratory, University of Bristol. Goats were used for feeding. Using this method, it was found that the mean age at death of females was about 120 days, and the average yield was 9-10 pupae per female. This method proved successful and the colony became self-supporting and produced a surplus in excess of the demand by workers in the United Kingdom and elsewhere.

It appears however that the suitability of any feeding method depends not only on the species of tsetse flies but also on the animal used for feeding. For example, Nash (1968) reported that the performance of female G. morsitans fed on rabbits' ears was not so good as that of G. austeni. Jordan and Curtis (1968b) compared G. austeni

fed on rabbits with those fed on goats. They showed that the fecundity of the rabbit-fed females remained substantially constant whereas fecundity declined at an earlier age in goat-fed females. Curtis and Jordan (1970) also showed that rabbit-fed flies did slightly better than goat-fed ones. The merits of using goats in tsetse mass rearing was described by Nash and Jordan (1976). Nevertheless, Jordan and Curtis (1968b) suggested that, if mass rearing of Glossina were to be undertaken, the relative merits of goats and lop-eared rabbits as hosts would require investigation.

Various tsetse species have been reared successfully, for example, G. morsitans, G. palpalis, G. austeni and G. m. submorsitans. In spite of these successes some other species, for example, G. pallidipes and G.longipennis have still proved difficult to rear. Owaga (1981) tried to rear G. longipennis and found that this species could not breed easily in the laboratory. One problem was their refusal to mate. It was also found that there was a high rate of abortion in the laboratory reared females compared with the wild caught females. In Kenya and elsewhere in Eastern Africa, G. pallidipes is important in the transmission of both human and animal trypanosomiasis. There have been several attempts to rear this species in the laboratory with partial success. Van Etten (1981) tried to raise a colony from two allopatric populations of G. pallidipes in Kenya. Though he was unsuccessful, he arrived at the conclusion that the two populations differed in copulation time, pupal weight, age at which the first larva is produced and duration of interlarval periods; and therefore there was population diversity in this species.

Several studies have been carried out upon individual populations in order to understand other behavioural differences existing between different populations. Jaenson (1978b) found that the reproductive biology of G. pallidipes from Kibwezi forest in Kenya differed in some respects from that originating from the Lambwe Valley also in Kenya. In his studies, Van Etten (1981) found that the key factor which affected his colony from Mwalewa Forest was loss due to low production whereas in Nguruman colony, the key factor was pre-mating mortality. Rogers (1973a) and Rogers and Kinyanjui (1972) had earlier reported on a self-supporting colony of G. pallidipes originating from Tororo in Uganda using a 7-day-a-week feeding regimen. However, Leegwater-van der Linden (1981) thought this to be unnecessary, and reported that there was only a minor difference in interlarval period between flies fed daily and flies given a 4-day-a-week feeding regimen. It was however noted that the offspring of daily fed flies lived slightly longer and produced slightly heavier pupae. Madubunyi (1988) also reported that daily feeding had no advantage above feeding either every other day or once every 3 days (at least through the first pregnancy cycle) in G. morsitans. The conflict of views indicates that each population should be considered in its own perspective and generalisations should be avoided.

The problems of rearing G. pallidipes notwithstanding, in roads are being made and successful colonies of G. pallidipes have been reported by some workers. Leegwater-van der Linden (1980) described the successful rearing of G. pallidipes in Amsterdam

using lop-eared rabbits for feeding the flies. Ochieng *et al* (1987) also reported the successful rearing of *G. pallidipes* using a simple grass-thatched hut. The flies were offered blood every day (H.K. Banda, pers. comm.). Gradually, successful results are being reported in various places. Langley (1989) has described the successful laboratory colonization of *G. pallidipes* (Lugala, Uganda strain) at the Tsetse Research Laboratory, University of Bristol. In this colony, the flies were fed 6 days a week on defibrinated blood. It is however apparent that different methods will be necessary for the successful rearing of different strains of *G. pallidipes*.

CHAPTER 3

GENERAL MATERIALS AND METHODS

3.1 SOURCE OF TSETSE FLIES

G. morsitans used in this study were obtained from the ICIPE colony maintained at about 25°C and 65 - 75% relative humidity with a regime of approximately 12 hours dim light and 12 hours darkness, and fed on rabbit-ears 6 days a week. *G. pallidipes* was trapped at Nguruman in the Rift Valley of Kenya using biconical traps baited with cow urine and acetone. Females were fed on rabbits soon after collection (Plate 2) and were brought to the laboratory in Nairobi. Nguruman is semi-arid with an annual rainfall of 600-700mm. The annual mean maximum and minimum temperature is 30 - 34°C and 14 - 18°C respectively. The vegetation is dominated by *Acacia sp* and two tsetse species are found here: *G. pallidipes* and *G. longipennis* (Van Etten, 1981).

3.2 REARING AND MAINTENANCE OF TSETSE FLIES

In the laboratory, wild *G. pallidipes* trapped at Nguruman were maintained at 25±1°C, 75-80% relative humidity and LD 12:12 (12 hr light: 12 hr dark). Groups of 20 - 25 flies were held in PVC cages measuring 18 x 8 x 5 cm (Plate 3). Initially they were fed every other day on rabbit-ears but production was very low. This feeding regime was therefore changed and the flies were given

PLATE 2

FEEDING TSETSE FLIES ON LOP-EARED RABBITS

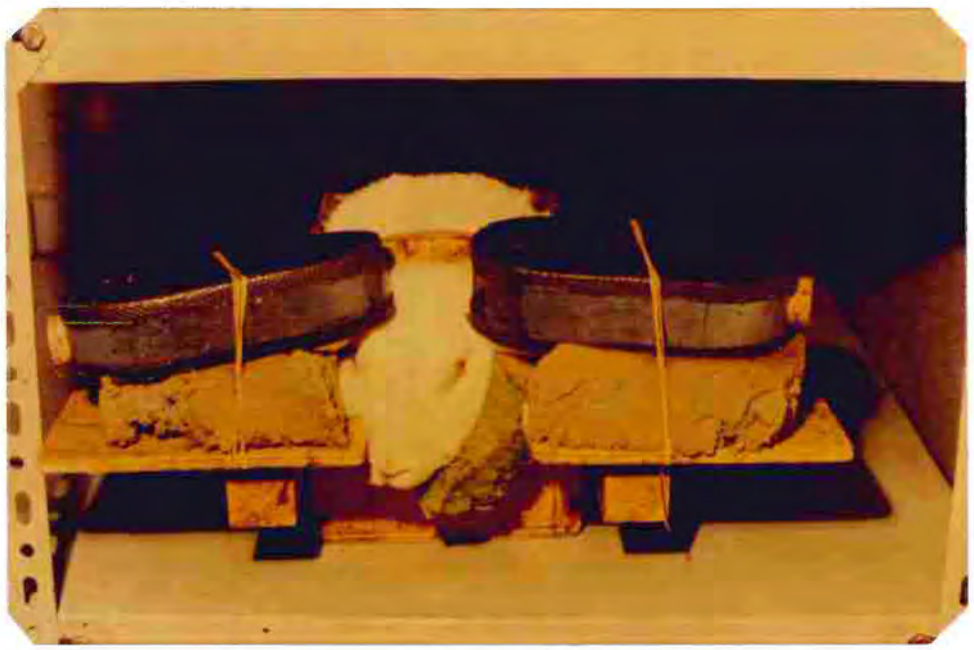


PLATE 3

LABORATORY PVC CAGES FOR HOLDING TSETSE

the opportunity to feed everyday (except on Sunday) which boosted productivity tremendously. Every morning, newly emerged flies were collected and sexed; they were considered to be one day old. According to Nash (1955), about three-quarters of the emergence of tsetse flies takes place before dusk so that 75% of the flies are 14 - 24 hours old. The F_1 flies were maintained under the same conditions as the wild flies and were kept until used in experiments. Wild flies were discarded when they were about two and half months old in the laboratory, and replaced by newly trapped ones.

3.3 MATING TSETSE FLIES

In mating experiments, the tsetse flies were either mated singly in transparent plastic vials (Plates 4 & 5) or group-mated in PVC holding cages (Plate 3) at a temperature of 25 ± 1°C after temperatures of 22°C, 24°C, 25°C and 26°C were tried using a few flies of G. pallidipes. G. morsitans was also mated at the same temperature. Receptivity in both species of flies was determined as follows: a female is regarded as receptive if she remains passive and opens its wings or the wings are pushed open by the male and hypopygial connection is made. It is refractory if she bends her abdomen downwards and closes her wings; if forced it reacts violently by struggling with the male. The females of G. morsitans used in the mating experiments were usually 3 days old and the male 7 - 10 days old, unless otherwise stated. For G. pallidipes the ages used are specified in individual sections. All mating experiments were started in the morning between 8.00 and 9.00am unless otherwise stated.

PLATES 4 AND 5

TRANSPARENT PLASTIC MATING VIALS

Plate 4

Plate 5

3.4 DISSECTIONS

The flies were killed by gently squeezing the thorax; the legs and wings were then removed. Dissections were carried out in 0.9% NaCl on a microscope slide, under a dissecting microscope. A fly was dissected by placing it on its ventral surface and held at the thorax with a pair of forceps and another pair of forceps used to hold the rear end of the abdomen and to pull the abdomen to reveal the reproductive system.

3.5 ESTIMATION OF DEGREE OF INSEMINATION

The degree of insemination was estimated using the method of Nash (1955). When the spermathecae are empty they appear transparent but when they contain sperm, the whole or parts of the spermathecae become opaque depending on the degree of insemination (Mellanby, 1936). If a female is fully inseminated, that is, when both spermathecal vesicles appear dark brown and completely opaque, the degree of insemination is expressed as: 1.00 + 1.00 = 2.00. In some cases, females are only partially inseminated; one vesicle may be full and the other empty, or one-half full and the other one-quarter full; in such cases the degree of insemination is recorded as 1.00 + 0.00 = 1.00, and 0.50 + 0.25 = 0.75 respectively.

3.6 ANALYSIS OF RESULTS

The results were analysed using the analysis of variance (Anova), multiple range, d Spearman rank correlation coefficient and χ^2 tests. These are all described in Parker (1979), except the Spearman rank correlation coefficient which is described in Lapin (1980). The formulae for the χ^2 test using Yates correction, the d test and Spearman rank correlation coefficient are given in the appendix. Tables for the Anova tests carried out in this work, and the data for all the figures presented in Chapters 4 and 6 are also shown in the appendix. Data for the figures in other Chapters are adequately displayed in the figures presented and are therefore not shown in the appendix.

3.7 IMPORTANT DEFINITIONS

Female Receptivity: The willingness to mate. Receptive females of Glossina spp. open their wings and keep their abdomen horizontal to allow a male to engage genitalia (Manning, 1967; Jaenson, 1978b & 1979b; Gillott and Langley, 1981).

Female Refractoriness: Refractory females of Glossina spp. bend their abdomen downwards so as to prevent males from engaging genitalia (Jaenson, 1978b and 1979b; Huyton and Langley, 1982).

Mating/Copulation: This refers to the pairing of a male and female with the occurrence of the male terminal jerking behaviour at the end (Jaenson, 1979a).

Duration of Copulation: The period between the engagement of male and female genitalia of Glossina spp. until they disengaged after the male jerking or ejaculatory period (Jaenson, 1978a).

CHAPTER 4

THE EFFECT OF VARIOUS FACTORS ON FEMALE RECEPTIVITY OF *GLOSSINA MORSITANS MORSITANS* WESTWOOD

4.1 INTRODUCTION

It has been shown that the willingness of virgin females of tsetse flies to mate decline with their age. Jordan (1958) found that virgin female *G. palpalis* up to 10 days of age will mate several times but the willingness to mate declines with age. Saunders and Dodd (1972) also found that, in the laboratory, *G. morsitans* mate readily after the first blood meal, usually 1 - 3 days after emergence. Females that had not taken a blood meal were often refractory to mating (Tobe and Langley, 1978).

Dame and Ford (1968) and Gillott and Langley (1981) have shown that after a female has mated its willingness to mate diminishes rapidly. It is not clear how soon this happens. Most of the work in this area have shown that within 24 hours a proportion of previously mated flies would have lost their receptivity to further mating. Gillott and Langley (1981) also showed that over 80% of female *G. morsitans* were refractory within 2 days postmating. This rapid decline in receptivity attests to the monogamous nature of tsetse flies; and probably the observation in the laboratory of multiple mating by females is not a common occurence in nature. In the confines of a small tube there could be forced matings.

The duration of copulation could affect the degree of insemination and correspondingly it could determine whether a previously mated female will accept a second male. Tobe and Langley (1978) suggested that this was so, but no experimental evidence is available. There is no evidence also that the duration of copulation can affect a second mating but the suggestion by Tobe and Langley (1978) that it could affect the degree of insemination suggests an indirect effect. According to Tobe and Langley, the number of matings is directly related to the amount of semen in the spermathecae because of Jackson's observation that the spermathecae of young female G. palpalis contained less semen than those of older females. Pinhao and Gracio (1973) also reported that in G. austeni, flies that had successfully copulated twice contained significantly more sperm in their spermathecae than those that had copulated only once. It is important to mention that these authors used males that were 4 - 5 days old and were therefore immature. Dame and Ford (1968) reported that females of G. morsitans accept multiple inseminations and become less receptive to subsequent insemination if the initial pressure is sufficiently heavy.

It has been reported that in Musca domestica, loss of receptivity to males by females after mating is caused by the presence in the female of the accessory material produced in the male ejaculatory duct (Riemann and Thorson, 1969). This could also be true of Glossina. In this regard, Gillott and Langley (1981) reported that in Glossina morsitans, loss of receptivity of females after mating is not caused by male accessory gland secretion alone but also

by mechanical stimulation acting jointly. These authors also reported that either factor on its own is not effective and that the nature of the receptivity inhibiting factor was unknown as well as its mode of action. Gillott and Langley did not observe any effect of the haemolymph of a mated female on receptivity in virgins. They stated, "the mated status of females does not result in the initiation of new physiological processes, but merely alters the competence of organ systems to respond to the demands of the reproductive process". The studies reported here were conducted so as to obtain more detailed information on those factors known to affect receptivity in other dipterans that have been suggested as possible causes of refractoriness in Glossina and also to identify the receptivity inhibiting substance present in male accessory gland secretion.

4.2 MATERIALS AND METHODS

4.2.1 EFFECT OF MATING ON FEMALE RECEPTIVITY AND REPLENISHMENT OF MALE ACCESSORY GLAND SECRETION

Two hundred and twenty-five female G. morsitans and an equal number of males were paired singly in mating tubes (or vials). They were allowed to mate only once. On successful completion of mating (i.e. there was male jerking at the end of the period), males were separated from females and groups of 25 females were presented with new males immediately after the first mating and after the following time intervals: 1, 4, 6, 24, 48, 72, 96 and 120 hours. The females were recorded as refractory or receptive according to their response to previously unmated males.

Replenishment of male accessory gland secretion (ARG) was determined by mating 60 males with an equal number of females. The males were divided into six groups of 10. Group 1 was dissected immediately and the diameter of the ARG was measured using an ocular micrometer; the remaining groups were dissected after 4, 24, 72, 96 and 126 hours respectively. Another group of 10 previously unmated males was dissected and served as a control. The mean of the diameter of a pair of glands per fly was determined. The above experiment was repeated but this time after being kept for 4, 24, 72, 96 and 120 hours after mating, the males were re-mated with fresh females. The aproximate size of the spermatophores formed was determined by dissection of their female pairs and calculating the mean size after taking two measurements at right angles. The control flies were mated with males that had never mated before and dissected immediately after separation.

4.2.2 EFFECT OF DURATION OF COPULATION ON FEMALE RECEPTIVITY

This was determined by recording the duration of copulation at a first mating and then recording whether a female accepted or refused a second male when presented with one (usually a previously unmated male) the following morning. The duration of the 'male jerking phase' was also determined. The last few minutes before the normal termination of copulation which is characterised by violent jerking movements by the male is referred to here as 'male jerking phase' and will be used throughout this thesis. In another experiment, females

were mated and separated after time intervals of 50, 60 and 80 minutes. Receptivity was determined after 72 hours by presenting them with previously unmated males.

4.2.3 EFFECT OF DURATION OF COPULATION ON THE DEGREE OF INSEMINATION

Groups of flies were mated and the duration of copulation was recorded. The corresponding value for the degree of insemination of the spermathecae was determined by recording the mean spermathecal value (MSV) after dissection the following morning.

4.2.4 EFFECT OF A SECOND MATING ON THE DEGREE OF INSEMINATION

One hundred and ninety-seven virgin females were paired singly in mating tubes. Upon separation, 66 were remated immediately; after 24 hours, another group of 32 was also remated. The duration of copulation during the first mating as well as the second was recorded. The MSVs were also recorded 24 hours after each mating period.

Two situations were compared. First, females were remated immediately after the termination of the first copulation and in the second, previously mated females were remated after 24 hours. In both cases, all males were 7-10 days old and previously unmated and the females were 3 days old at second mating.

In another experiment, a group of 28 females was mated once with 3-day-old males and another group of 21 females was mated once with mature males (7-day-old). These females were allowed to larviposit until they were 77 days old. Aborted larvae were also recorded.

4.2.5 EFFECT OF THE DEGREE OF INSEMINATION ON FEMALE RECEPTIVITY

Three day old females were paired with 5, 7 and 9 day old males to give varying degrees of insemination. After 72 hours they were presented with previously unmated males and recorded as refractory or receptive. They were kept for 72 hours before testing because previous observations showed that at this time only about 20% of females remain receptive after a previous mating. It was assumed that this percentage is arrived at only if the flies are successfully stimulated by the factor that switches off receptivity. In this case it will happen if the degree of insemination is high. It was also assumed that flies with low MSVs will remate in order to satisfy their normal requirement. In another experiment, males were mated several times until they became aspermic. This was determined by examination of the spermathecae of the last female they mated with. They were then presented with virgin females; 72 hours after mating, these females were then tested for receptivity. Other groups of females were mated until the start of the 'male jerking phase' when they were immediately separated; their receptivity was also tested after 72 hours.

4.2.6 EFFECT OF MALE ACCESSORY REPRODUCTIVE GLAND SECRETION ON FEMALE RECEPTIVITY

Seven to nine day old virgin males were dissected in sterile saline (0.9% saline was autoclaved a day before being used) to remove their accessory reproductive glands. Sterile conditions were maintained throughout the dissections. The slides, forceps, dissecting needles and eppendorf tubes used were autoclaved before use. After each dissection, the forceps and dissecting needles were dipped into 95% ethanol to ensure sterility. The glands were carefully dissected out with all adhering fat body removed. They were then transferred into an eppendorf tube containing sterile saline kept in a plastic bowl containing ice cubes in order to prevent any denaturing of the proteins.

The accessory reproductive glands were homogenised in saline using a hand homogeniser. The homogenate was then centrifuged at 5,500 g/min for 40 minutes in a refrigerated minifuge. The supernatent was taken and diluted to the required concentration with sterile saline. Concentrations of 0.5, 0.84 and 1.00 gland pair per microlitre were prepared. A Hamilton microlitre syringe (Hamilton Bonoduz AG, Switzerland) was used to inject 3-day-old previously fed females with 1μl of the above concentrations through the mesothoracic suture. The debris left at the bottom of the eppendorf tube was mixed with 20μl saline and injected into another group of females. Homogenate alone was also injected in another experiment. After time intervals of 3, 6, 12, 24, 48, 72 and 96 hours, the injected females were presented with 7-day-old males and recorded as receptive

females were presented with 7-day-old males and recorded as receptive or refractory. Any modification to the above procedure is given in individual sections of the results. Previously unmated females as well as saline-injected females served as controls.

4.2.7 EFFECT OF MECHANICAL STIMULATION ON FEMALE RECEPTIVITY

Seven-day-old males were surgically operated upon to sever their ejaculatory duct (Plate 6). A wax-bottomed petri dish was cleaned with 95% ethanol. A fly was placed dorsally in the petri dish and kept in position with blu-tack (Bostik Emhart, Leicester) which is a plasticine-like material but odourless. The set up is shown in Plate 7. The fly was immersed in cold sterile saline which served as an anaesthetic. The dish was then put on top of the stage of a dissecting microscope. A pair of Moria micro-scissors (manufactured by Moria-Dugast, S.A. of Paris, France) previously cleaned with 95% ethanol was used to make a minute cut on the ventral surface of the abdomen of the fly just in front of the hectors (Figure 1). A pair of fine forceps also previously cleaned with 95% ethanol was then carefully introduced into the incision to pull out the ejaculatory duct. When located two cuts were made just below the loop where the accessory reproductive gland and vas deferens bifurcate (Figure 2). Care was taken not to pull out much of the ARG or any other organ. Severing of the male ejaculatory duct prevents both accessory reproductive gland secretions and sperm from being transferred during copulation.

PLATE 6

MALE ACCESSORY REPRODUCTIVE GLAND (ARG) AND

TESTES WITH EJACULATORY DUCT (ARROWED)

PLATE 7

TSETSE MALE HELD IN POSITION WITH

BLU-TACK READY FOR SURGICAL OPERATION

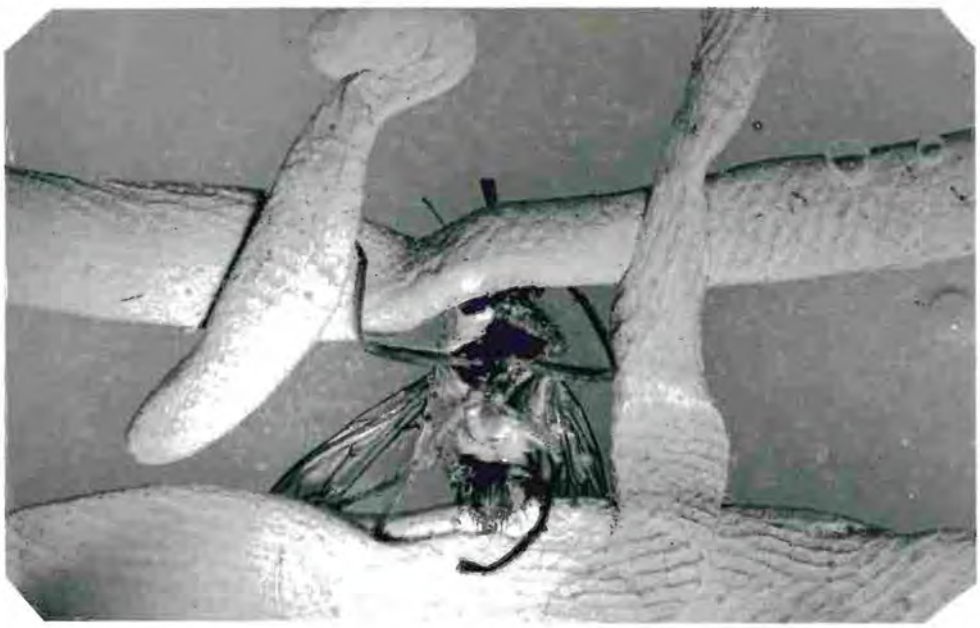


FIGURE 1

VENTRAL SURFACE OF THE TERMINAL SEGMENTS OF A MALE TSETSE FLY SHOWING WHERE A SMALL INCISION WAS MADE DURING SURGICAL OPERATION TO SEVER THE EJACULATORY DUCT

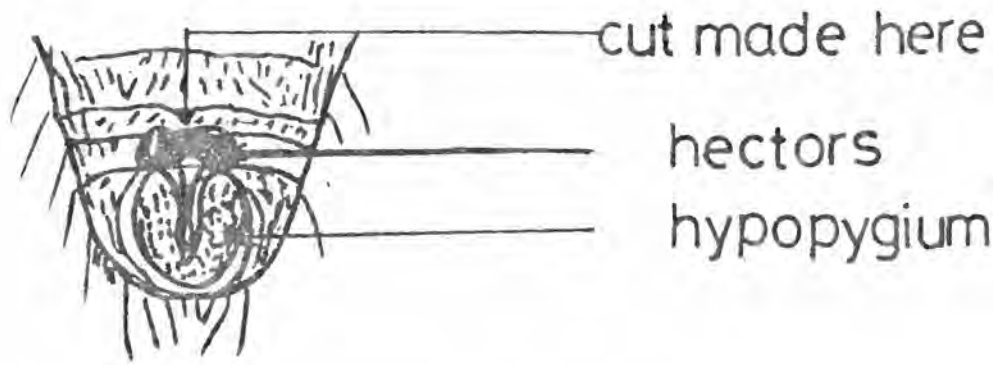
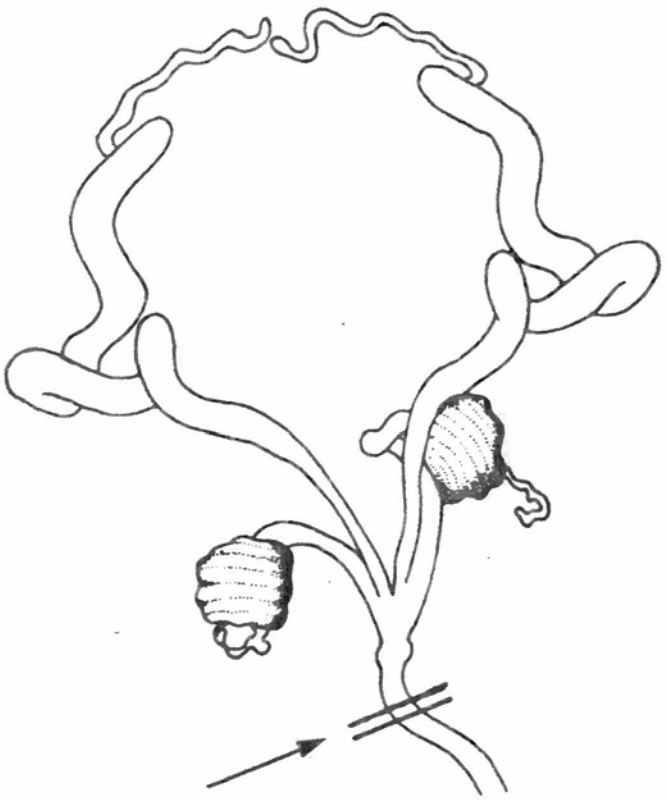

cut made here
hectors
hypopygium

FIGURE 2

MALE ACCESSORY REPRODUCTIVE GLAND AND TESTES

SHOWING WHERE TWO CUTS WERE MADE ON THE EJACULATORY DUCT (ARROWED)



The operation was generally successful if very little of the internal organs were pulled out. With practice, it was possible to pull out just the loop of the ejaculatory duct. Everything that was pulled out (fat body, ARG etc) was then pushed in again. The saline was disposed of and the blu-tack removed. The operated fly was then returned into a PVC holding cage where it recovered within a few minutes (about 3 - 5 minutes) if the operation was successful. After each operation the forceps and scissors were dipped into 95% ethanol and the saline poured out and replaced with fresh one. Any fly with too much of its internal organs removed during the procedure was discarded as such flies usually die within a few minutes. Successfully operated flies lived for over 5 weeks.

Operated flies were kept for about 3 days to fully recover and behave like normal flies before being used in experiments. These flies were mated singly with 3-day-old females and tested for receptivity after 72 hours. The females were dissected and their spermathecae examined to ensure that sperm were not transferred during mating. Sham-operated (simulated surgically operated males) were prepared in the same way but their ejaculatory ducts were only touched but not severed.

4.2.8 EFFECT OF HAEMOLYMPH OF MATED FEMALES ON RECEPTIVITY OF VIRGIN FEMALES

Three-day-old females were mated and after 72 hours, haemolymph was obtained from them by decapitation and withdrawing the haemolymph with a micropipette that was pulled out and calibrated to deliver 1 μl (Plate 8). This was used because it was difficult to get haemolymph out of a fly using a Hamilton microsyringe. It should be mentioned that decapitation causes contamination with other fluids, for example, saliva. However, this was the best method to obtain haemolymph since it was difficult to withdraw it from severed limbs; the microsyringe also got blocked easily. 1 μl of haemolymph so obtained was injected into previously unmated 3-day-old females. After 72 hours they were tested for receptivity.

4.3 RESULTS

4.3.1 EFFECT OF MATING ON FEMALE RECEPTIVITY AND REPLENISHMENT OF MALE ACCESSORY REPRODUCTIVE GLAND SECRETION

Receptivity declined rapidly after mating (Table 1). Immediately after mating, about 85% of females were still receptive. There appeared to be a time lag before the females realised that they had mated. Thus, after one hour about 50% were refractory. This trend continued up to 24 hours post-mating. The results also showed that up to 24 hours post-mating almost 60% of the flies remained receptive.

PLATE 8

CALIBRATED MICROPIPETTE TO

DELIVER 1 μl OF SOLUTION

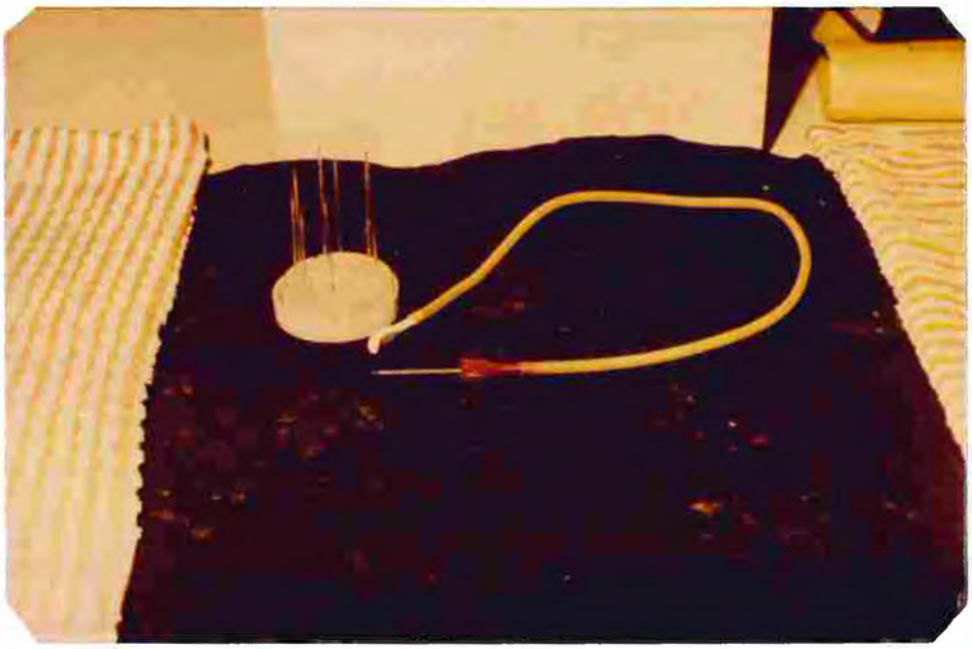


TABLE 1

PERCENT RECEPTIVITY OF FEMALE G. MORSITANS
AT VARIOUS INTERVALS AFTER MATING

Hours after mating	% Receptive	
	Pre-mated	Unmated(control)
0	85	100
1	54	97
4	40	100
24	61	100
48	35	84
72	33	97
96	11	84
120	22	90

n = 25 females in each group

However, up to 120 hours post-mating, about 20% of the flies were still receptive. Mating did not cause instantaneous refractoriness in females.

Soon after mating (0 hours), 4 and 24 hours, the ARG was small; there was a highly significant difference in ARG diameter when compared with unmated control males ($F = 10.97, P<0.01$). There was, however, no significant difference between the size of ARG at 0, 4 and 24 hours post-mating (Figure 3). This comparison is shown in Table 2 which is a multiple range test (Parker, 1979) comparing the mean diameter of ARG before and after mating. The rate of synthesis was slow at the beginning but increased thereafter. The ARG attained its normal maximum size after about 48 hours post-mating. It was difficult to show the effect of a small ARG on the size of the spermatophores formed because of their elasticity. On dissection, spermatophores of distorted shapes were visible which could not be easily measured. However, immediately after a first mating the spermatophores formed by males that remated appeared smaller than those produced by previously unmated males.

4.3.2 EFFECT OF DURATION OF COPULATION ON FEMALE RECEPTIVITY

Copulation for most flies lasted for between 2 to 3 hours. Most of them copulated only once irrespective of the duration of copulation (Figure 4). Of the flies that copulated only once, 23.6% copulated for one hour and over 60% copulated for a period between 2 and 3 hours (Table 3).

FIGURE 3

CHANGE IN DIAMETER OF THE MALE ACCESSORY GLAND (ARG) AND STANDARD ERROR AT VARIOUS HOURS AFTER MATING

C = CONTROL FLIES WHICH WERE UNMATED

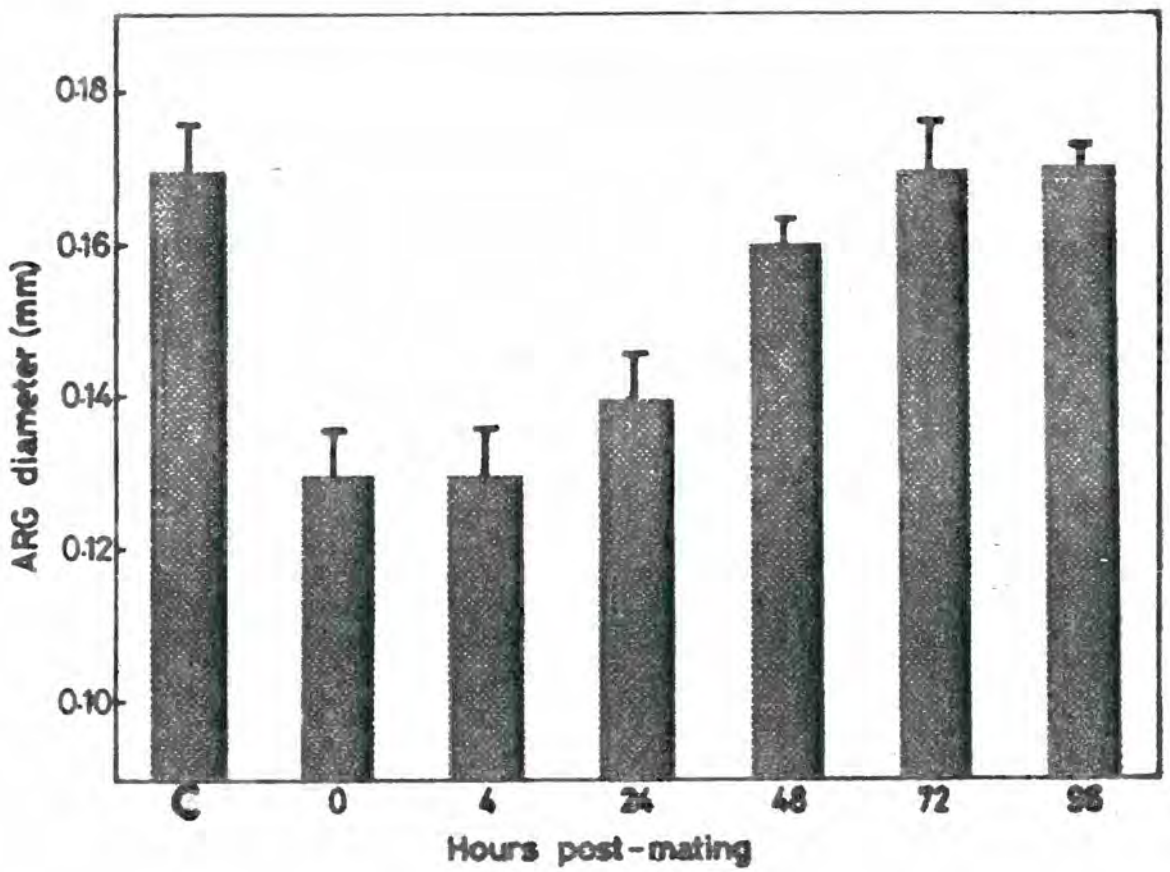

0·18
0·16
0·14
0·12
0·10
ARG diameter (mm)
C
0
4
24
48
72
96
Hours post-mating

TABLE 2

MEAN DIAMETER OF MALE ACCESSORY REPRODUCTIVE GLAND (mm) BEFORE AND AFTER MATING COMPARED USING A MULTIPLE RANGE TEST

Time (Hours)	Diameter of ARG(mm) Mean ± SD			
0	0.12	0.01		c
4	0.13	0.02		c
24	0.14	0.02	b	c
48	0.16	0.01	a b	
72	0.17	0.02	a b	
96	0.17	0.01	a b	
Unmated (control)	0.17	0.02	a	

Any two not underscored by the same letters are significantly different; any two underscored by the same letters are not significantly different.

FIGURE 4

PERCENT FEMALE FLIES MATING ONCE OR TWICE

RELATED TO THE DURATION OF COPULATION

FLIES UNWILLING TO REMATE

FLIES WILLING TO MATE TWICE

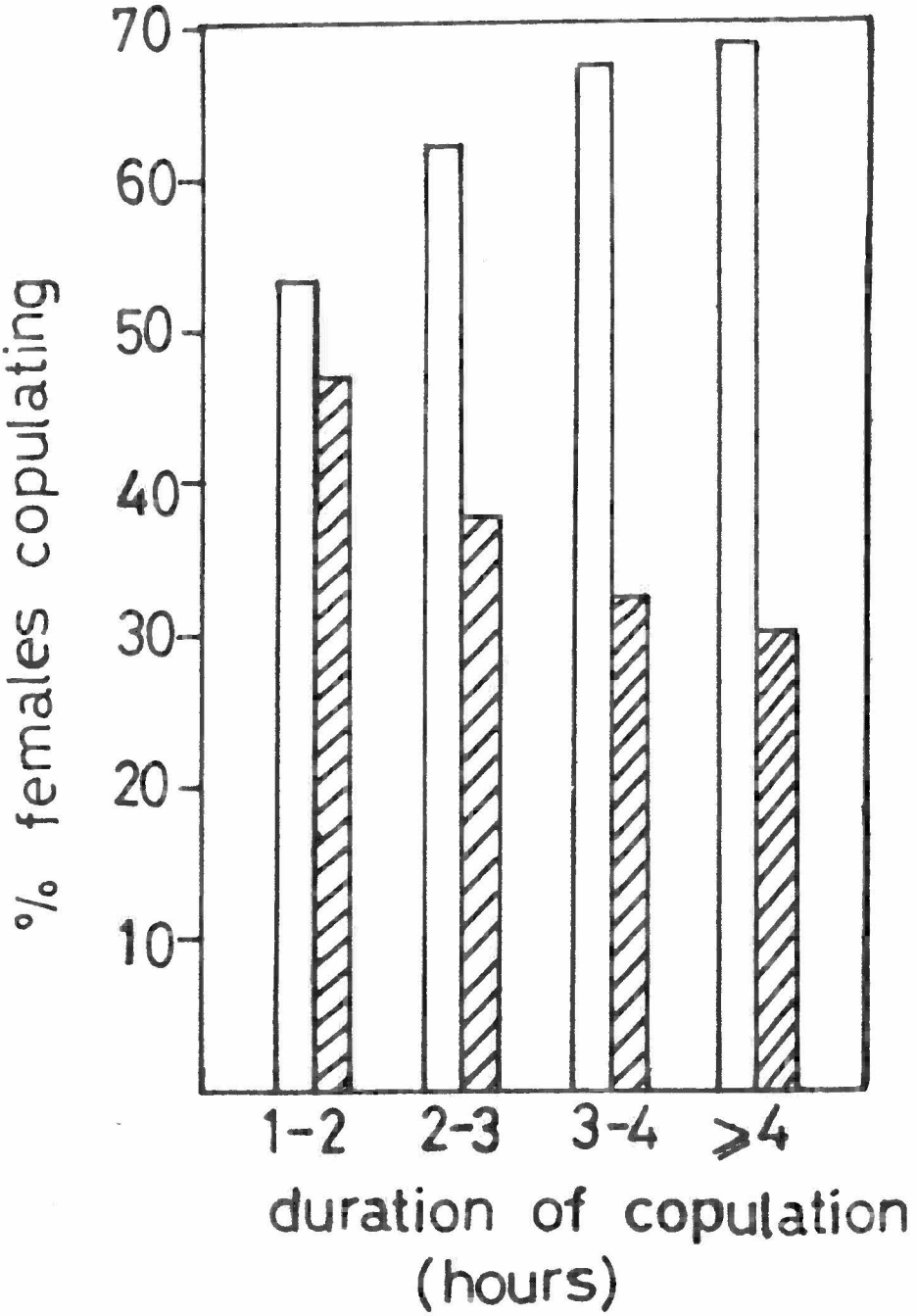

70
60
50
40
30
20
10
% females copulating
1-2
2-3
3-4
≥4
duration of copulation
(hours)

TABLE 3

PERCENTAGE OF FEMALES COPULATING ONLY ONCE OR
ATTEMPTING A SECOND RELATED TO THE DURATION OF COPULATION

Duration of Copulation (Hours)	% Females Copulating once	% Females attempting second copulation
1 - 2	24	34
2 - 3	32	32
3 - 4	32	25
>4	13	9

n = 116

Similarly, over 55% of those that attempted to copulate twice took 2 - 3 hours to complete the first copulation. There was no significant effect of the duration of copulation on receptivity (χ^2 = 1.82, P>0.05). It is noteworthy that these results apply to matings with mature males and females of the right age (>7 days and 3 days of age respectively). The general trend was towards a single copulation. It did not matter whether a female copulated for only one hour or more, most flies mated once than rather than twice. When females were given the opportunity to mate for 50, 60 and 80 minutes before being forcibly separated (male jerking phase was not reached) usually more than 90% attempted a second copulation (Figure 5). This confirms that the duration of copulation has no effect on receptivity. The jerking phase took an average of 2.81 minutes (n = 50).

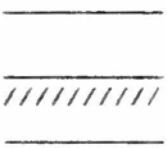
4.3.3 THE EFFECT OF DURATION OF COPULATION ON THE DEGREE OF INSEMINATION

The spermathecae were classified according to the size of the sperm mass using a modified version of the method of Pinhao and Gracio (1973). The following classes were obtained:

Classes	MSV
I	0
II	< 0.50 > 0
III	> 0.50 < 1.00
IV	> 1.00 < 1.50
V	> 1.50 < 2.00
VI	2.00

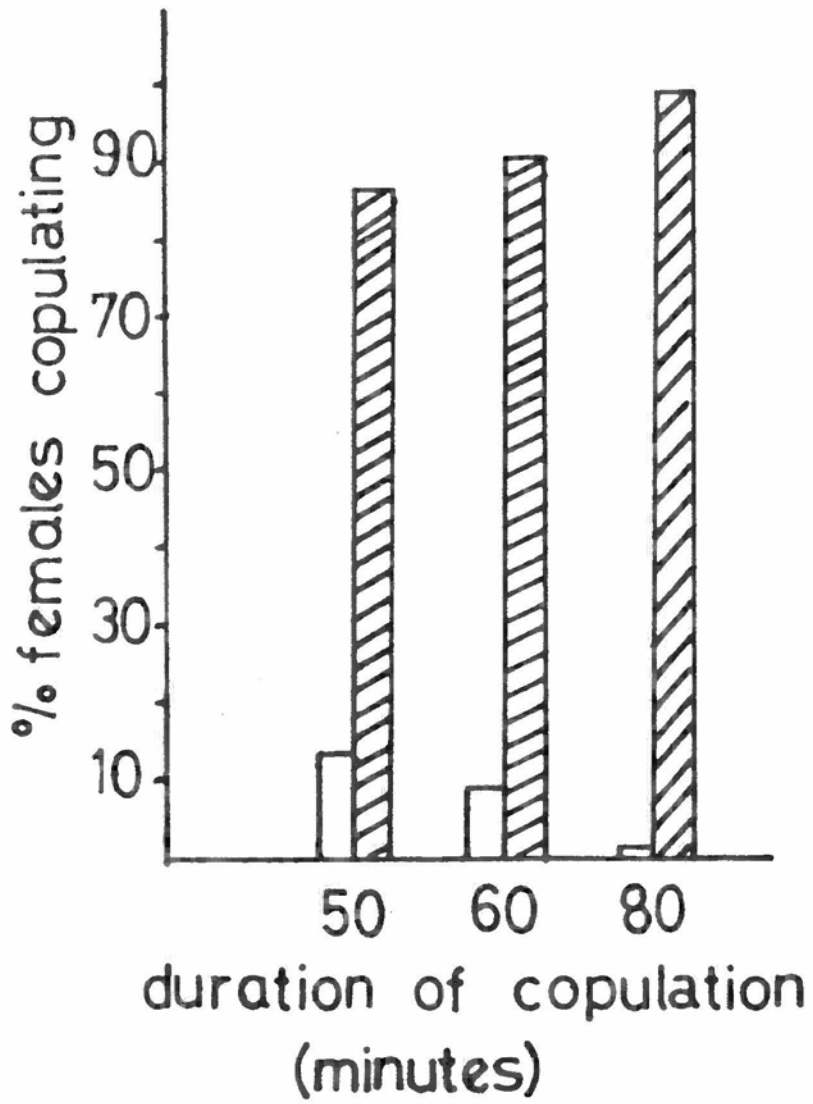
FIGURE 5

PERCENT FEMALE FLIES MATING ONLY ONCE OR ATTEMPTING A SECOND AFTER GIVEN THE OPPORTUNITY TO MATE FOR 50, 60 AND 80 MINUTES



FEMALES REFUSING TO REMATE

FEMALES WILLING TO MATE TWICE


% females copulating
90
70
50
30
10
50
60
80
duration of copulation
(minutes)

The results show that 96% of all the females had MSVs in classes V and VI (Table 4). Irrespective of the duration of copulation 3-day-old females mated to 7-9-day-old males had either full or almost full spermathecae. Only an insignificant percentage of flies were found with very low MSVs (classes I - III). The results did not suggest any effect of the duration of copulation on the degree of insemination. When copulation took one hour, 97% of the females had MSVs in classes V and VI, and when it lasted for 3 hours, 96% had MSVs also in classes V and VI. Furthermore, it was not indicative from the results that longer duration of copulation resulted in high MSVs or vice versa. Generally, duration of copulation lasted between 1 - 3 hours for over 85% of the flies (mean = 2hrs 51mins $\pm$ 54mins SD).

4.3.4 EFFECT OF A SECOND MATING ON THE DEGREE OF INSEMINATION

Figure 6 shows that when females were mated only once, about 90% were in the MSV classes V and VI. However, only 50% were in these two classes when females were remated immediately after the first copulation. Fifty percent of the remated flies had spermathecae that were either empty or partially full. Grouping the MSV classes into partial and full spermathecae, a χ^2 - test using Yates correction gave a highly significant difference between once mated and twice mated flies (χ^2 = 26.0, P<0.001).

TABLE 4

DEGREE OF INSEMINATION RELATED
TO THE DURATION OF COPULATION

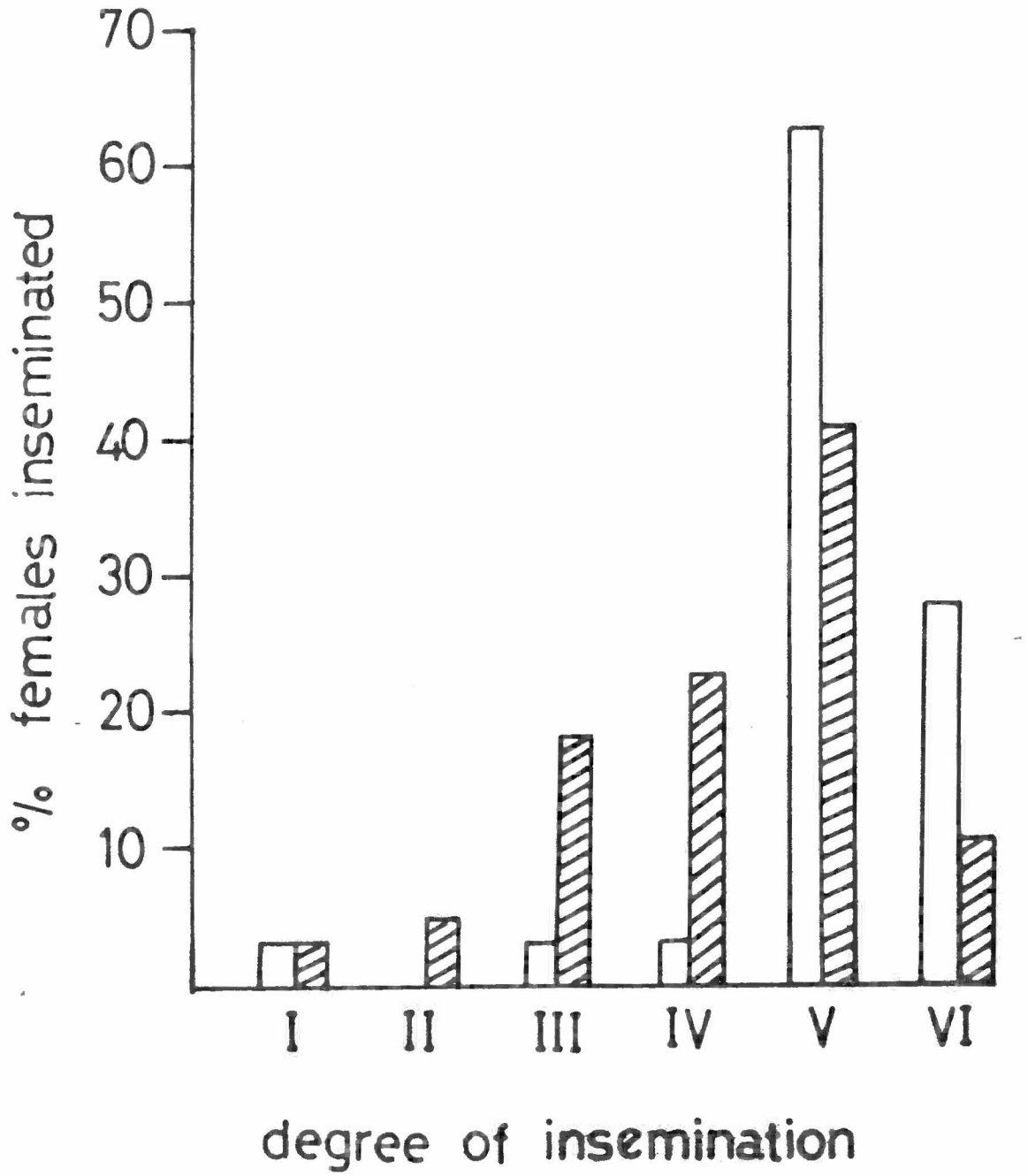
Duration of Copulation (Hours)	No of flies in indicated classes of insemination					
	I	II	III	IV	V	VI
1 - 2	0	0	1	0	32	3
2 - 3	0	0	2	0	22	3
3 - 4	0	0	0	1	25	3
4 - 5	0	0	0	0	8	0
5 - 6	0	0	0	0	4	0
> 6	0	0	0	0	2	0

n = 106

FIGURE 6

PERCENTAGE OF 3-DAY OLD FEMALE FLIES UNINSEMINATED WHEN MATED ONCE OR TWICE WITH 7-10-DAY OLD MALES AND THE DEGREE OF INSEMINATION

(THE TWICE MATED FEMALES WERE REMATED IMMEDIATELY AFTER THE TERMINATION OF THE FIRST MATING)


70
60
50
40
30
20
10
% females inseminated
I
II
III
IV
V
VI
degree of insemination

In the second situation (Figure 7), none of the flies that remated 24 hours after a first mating was found to be in a lower MSV class. One hundred percent of flies in this group were in classes V and VI. Insemination was therefore improved at a second mating 24 hours after the first. Class VI was predominant. However, grouping the classes as stated above and performing a χ^2 - test using Yates correction showed no significant difference between once mated and twice mated flies (χ^2 = 0.71, P>0.05). The duration of the first copulation was significantly different from the second using the d-test according to Parker (1979); *d = 548.8, P<0.001. The mean duration of the first copulation was 171.35 minutes and the second was 93.15 minutes (n = 66 in both cases).

When 3-day-old males were mated to 3-day-old females, almost 90% of these females were either uninseminated or had very low degrees of insemination (Figure 8). When such females were allowed to remate with mature males (7-10 days old) 24 hours after the first mating, there was a highly significant difference in the degree of insemination (χ^2 = 37.9, P<0.001). At the age of 77 days, females which were mated to 3-day-old males had produced 3.1 larvae (n = 28) whereas those that mated to mature males (7 days and over) produced 5.9 (n = 21) larvae.

*d = standard normal deviate

FIGURE 7

PERCENTAGE OF 3-DAY OLD FEMALE FLIES
INSEMINATED WHEN MATED ONCE OR TWICE WITH
7-10-DAY OLD MALES AND THE DEGREE OF INSEMINATION
(THE TWICE MATED FEMALES WERE REMATED
24 HOURS AFTER THE FIRST MATING)

———— FEMALES MATED ONCE

////////// FEMALES MATED TWICE

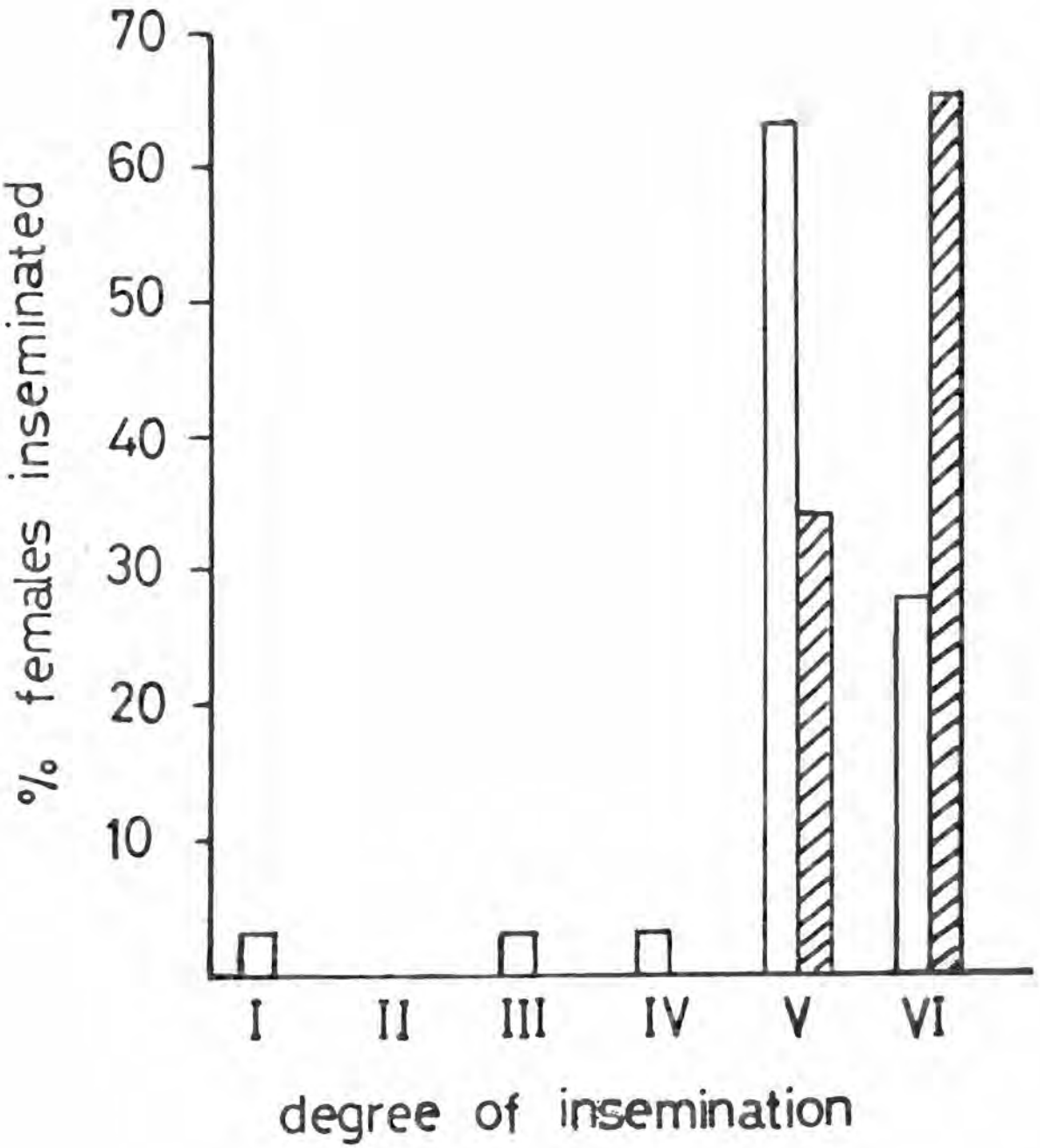
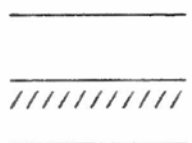

70
60
50
40
30
20
10
% females inseminated
I
II
III
IV
V
VI
degree of insemination

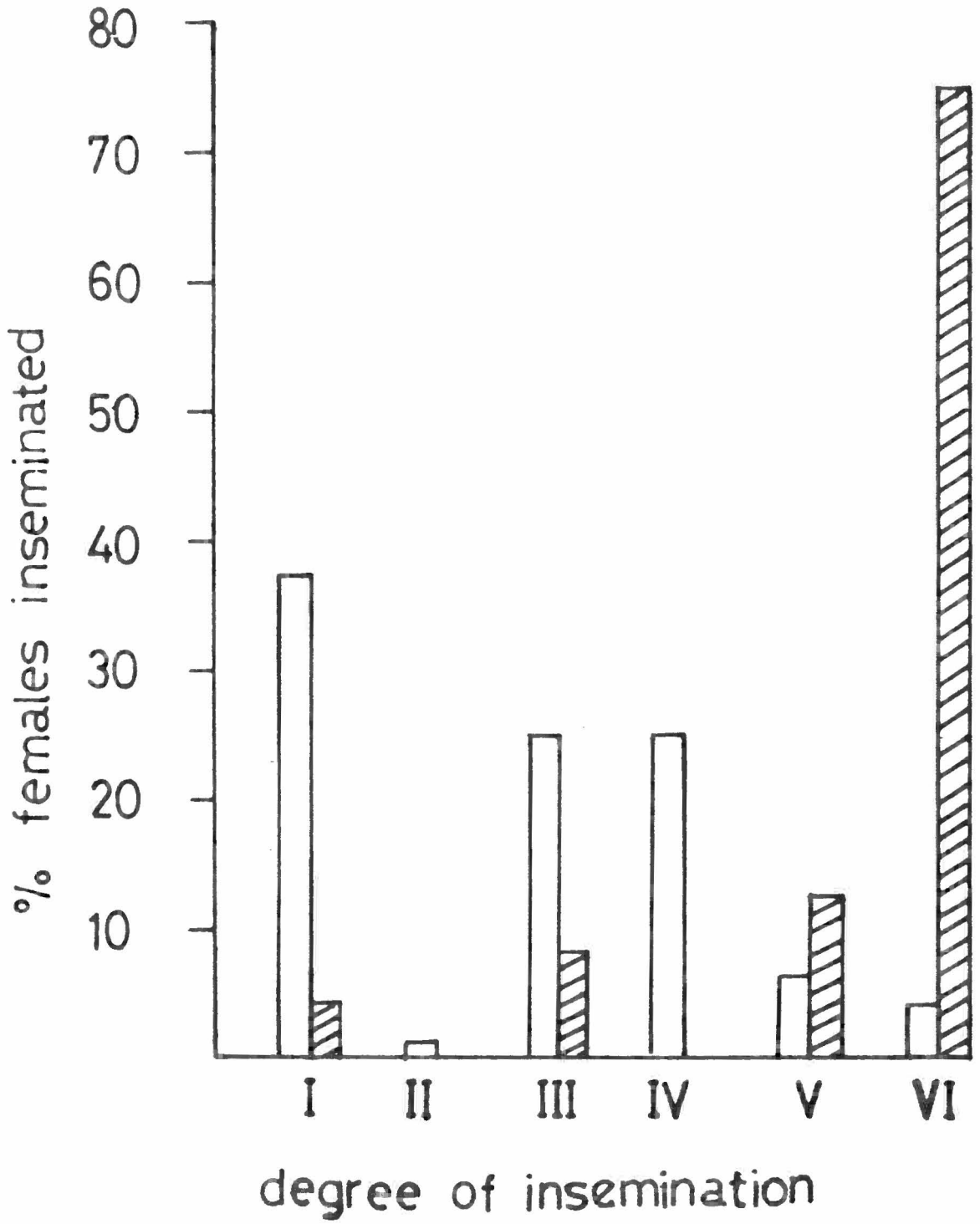
FIGURE 8

PERCENTAGE OF 3-DAY-OLD FEMALES INSEMINATED FOLLOWING MATING WITH 3-DAY-OLD MALES COMPARED WITH PERCENT FEMALES INSEMINATED WHEN REMATED WITH MATURE MALES AND THE RESULTANT DEGREE OF INSEMINATION



FEMALES MATED ONCE WITH 3-DAY-OLD MALES

FEMALES MATED ONCE WITH 3-DAY-OLD MALES AND THEN REMATED WITH 7-10-DAY-OLD MALES 24 HOURS AFTER THE FIRST MATING


80
70
60
50
40
30
20
10
% females inseminated
I
II
III
IV
V
VI
degree of insemination

4.3.5 EFFECT OF DEGREE OF INSEMINATION ON FEMALE RECEPTIVITY

One hundred and thirty-two 5-10-day-old males were paired with 3-day-old females. Five-day-old males were used in order to obtain varying degree of insemination. Table 5 shows that even with 5-day-old males about 95% of the flies showed a high degree of insemination. Those showing very low degrees of insemination did not necessarily show a willingness to re-mate. About 95% of females that mated only once were in classes V and VI and about 91% of those that mated twice were also in classes V and VI. This means that those with full spermathecae did not mate only once as was once thought, instead over 90% mated twice even when they had very high degrees of insemination.

When 3-day-old males were mated to 3-day-old females, 69% were still receptive after 72 hours post-mating (Table 6), whereas only 24% were receptive when they were mated to 7-10-day-old males for the first time. Three-day-old males could not inseminate well and as such gave very low degrees of insemination (Figure 8). However, Table 7 shows that females that were mated to aspermic males lost their receptivity and only 16% were receptive after 72 hours. This is indicative of the fact that a low insemination is not necessarily the cause of high receptivity. Aspermic males were between 20-25 days old when they were used in this experiment whereas the females were 6 days old when they were presented with normal males (7-10 days old) 72 hours after mating with aspermic males.

TABLE 5

THE PERCENTAGE OF FEMALES WITH DIFFERENT DEGREES OF INSEMINATION FOLLOWING MATING ONCE OR TWICE WITH 5 TO 9-DAY-OLD MALES

Degree of Insemination	PERCENT FEMALES SHOWING INDICATED DEGREE OF INSEMINATION	
	Attempted only one mating n = 74	Attempted second mating n = 58
I	0	2
II	0	0
III	4	5
IV	1	2
V	85	84
VI	10	7

TABLE 6

PERCENTAGE OF FEMALES SHOWING RECEPTIVITY
72 HOURS AFTER THE FIRST MATING WITH
3 OR 7-10-DAY-OLD MALES

Mated first time with males of indicated age	n	% Receptive at second mating with mature * males
3-day-old	35	69
7-10-day-old	71	24
Control(unmated)	38	100

*Female age = 6 days; male age = 7-10 days

TABLE 7

PERCENT RECEPTIVITY OF FEMALES 72 HOURS AFTER MATING WITH ASPERMIC MALES

Treatment (First mating)	n	% Receptive at second mating
♀♀ x aspermic ♂♂	25	16
♀♀ x normal ♂♂	60	27
Virgin ♀♀	40	88

4.3.6 EFFECT OF MALE ACCESSORY REPRODUCTIVE GLAND SECRETION ON FEMALE RECEPTIVITY

ARG secretion did not show any effect on receptivity (Table 8). after 72 hours, 100% of the injected flies remated. When the concentration was increased to 1.5 gland pair per microlitre, mortality was very high (Table 9). There was 100% mortality of flies injected with this increased concentration as well as those that received injections of particulate matter. Injections of homogenate also caused a high mortality (over 80%). Five flies that survived in this group mated when presented with males 24 hours after injection. Injections of 1 µl of 1 gland pair per microlitre of the supernatent of ARG extract after centrifugation caused very little and in a few cases, no mortality.

4.3.7 EFFECT OF MECHANICAL STIMULATION ON FEMALE RECEPTIVITY

Females lost their receptivity 72 hours after mating with males with severed ejaculatory duct (Table 10). A small percentage (about 30%) of females remained receptive but this was also true of normal-mated flies where almost 18% remained receptive 72 hours after a previous mating. With sham-operated males, a small percentage of females also remained receptive. However, when males with severed ejaculatory ducts were mated and separated at the jerking phase all the females were refractory. The reason for this could be because these males tended to pair for much longer periods and held tightly on when any attempt was made to separate them.

TABLE 8

PERCENT RECEPTIVITY OF VIRGIN FEMALE
AT VARIOUS TIME INTERVALS AFTER INJECTION
WITH 1 μl OF MALE ARG SECRETION

		Percent receptivity following indicated hours after injection					
Injections	n	3	6	12	24	48	72
ARG Secretion	25	100	100	96	100	96	100
Saline	20	100	95	100	100	100	100
Uninjected	30	100	97	100	100	93	100

TABLE 9

PERCENT RECEPTIVITY AND MORTALITY 24 HOURS AFTER INJECTION OF 1.5 ARG PAIR PER μl, PARTICULATE MATTER, HOMOGENATE AND SALINE

Injection	n	% Receptive*	% Mortality
High Conc. of ARG	17	-	100
Particulate matter	20	-	100
Homogenate	**30	100	83
Saline	30	100	0

*Percent of females survived

**Only 5 flies were alive and all remated when tested 24 hours post-injection

TABLE 10

RECEPTIVITY IN FEMALES 72 HOURS AFTER BEING MATED TO MALES WITH EJACULATORY DUCT SEVERED AND ALLOWED TO COMPLETE THE ACT OR SEPARATED AT THE JERKING PHASE OR SEPARATED AFTER 1HR AND 20 MINUTES

Males	n	% Receptive after 72 hours
* d- (mating complete) (mean dur of cop = 2hr 52 min)	24	29
d- (separated at jerking phase)	6	0
** d- (separated after 1 hr 20 minutes but before jerking phase)	14	50
Sham	20	20
Normal	17	18
Unmated	23	87

* d- = severed ejaculatory duct
** It takes a minimum time of about 1 hour for normal copulation

The extra force needed to separate them might have caused trauma in the females. The number of females so treated was however very low (6).

When operated females were separated after 1hr and 20 minutes (with no jerking phase), 50% of the flies were still receptive. This suggests that the onset of refractory behaviour is associated with the completion of mating.

4.3.8 EFFECT OF HAEMOLYMPH OF MATED FEMALES ON RECEPTIVITY IN VIRGIN FEMALES

There was no effect of haemolymph of mated females on receptivity in virgins (Table 11) as shown by a χ^2 - test (χ^2 = 1.19, $P<0.05$). Over 60% of previously injected virgin females remained receptive 72 hours after receiving an injection of 1 μl of haemolymph.

TABLE 11

RECEPTIVITY IN VIRGIN FEMALES 72 HOURS
AFTER INJECTION WITH HAEMOLYMPH FROM MATED FEMALES

Treatment	n	% Receptive
Haemolymph	32	69
Thorax pierced but uninjected	15	87
Uninjected control	26	77

It is well established in many dipterans that female receptivity or the willingness to mate diminishes rapidly after the first mating; for example Musca domestica, Riemann et al (1967) and Culex tarsalis, Young and Downe (1983), show this behaviour. These species have been described as monogamous. Gillott and Langley (1981) showed that receptivity in G. m. morsitans declined rapidly so that over 80% of the mated females were refractory within two days. In the present study, receptivity was found to decline rapidly but immediately after mating over 80% of females were still receptive (Table 1). It took between 48 - 72 hours for about 80% of mated females to lose their receptivity. At least during the first few hours after mating there is the likelihood of females mating several times since almost 60% of mated females were receptive after 24 hours post-mating. It was also reported by Jordan (1958) and Dame and Ford (1968) that females were more receptive to multiple matings when they were young. Multiple mated females must therefore benefit from this behaviour, in terms of their reproductive capacity.

Jordan (1972b) showed that males can mate up to six times in the laboratory but their inseminating ability is improved if rest periods are allowed in between to enable them to replenish their accessory reproductive gland secretion. In G. austeni, Pollock (1974) showed that it takes about 48 hours for the ARG secretion to be replenished and in this study it has been shown that it takes between 48 - 72 hours for the ARG secretion to be completely replenished. Since previously mated males can deplete their ARG secretion which can

become more acute if they mate several times in succession resulting in small spermatophores, it seems logical that females mated to males with depleted ARG secretion would need to mate again. A second mating can also increase genetic diversity. Curtis (1968a and b) working with G.austeni had reported that sperm from whichever mating was made first was used predominantly but not exclusively for fertilisation, showing that there is a possibility of females benefitting from multiple matings.

Though multiple mating can occur within the first 24 hours post-mating without much hindrance, the willingness to remate diminishes thereafter. This behaviour though occurring naturally in virgins is accelerated in mated females due to various factors associated with mating. The duration of copulation, however, did not cause monogamy. Whatever the duration of copulation, the general trend was usually towards a single mating shown by the high percentage of flies willing to mate only once (Figure 4). This behaviour was usually observed when females were mated to mature males. It must be emphasised that a different behaviour could result when females mate with young males (\< 3 days old). Incomplete copulation could also result in such females remating; this suggests that whatever the stimulus, it must be associated with the completion of mating. This appears to be the 'male jerking phase'. It was often observed that most flies which did not reach this stage usually remated. Commonly observed is the fact that some flies remained in copula for very long periods, sometimes for over 6 hours. Why these flies would want to remain together for

so long is not entirely clear. However, when couples were closely observed with a dissecting microscope, they were found to be relatively inactive. The males appeared to be sitting on the females without any apparent action. Male ARG secretion which could be faintly observed entering the female in more normal copulations was not seen. These males were probably abnormal in some respect.

Irrespective of the duration of copulation, mature males of G. morsitans (>/7 days old) mated to females of the right age (3 days old), always resulted in over 90% fill up of the spermathecae in over 95% of the cases. There was nothing to suggest that when the duration of copulation was low, the degree of insemination was low. In fact very low durations (less than 1 hour) were hardly recorded unless it was a second copulation (Section 4.3.4). These results also agree with Pinhao and Gracio (1973) and Jaenson (1979b) who did not observe any effect of duration of copulation on insemination. However, Tobe and Langley (1978) stated that the duration of copulation has a direct influence on the insemination rate in Glossina. Short durations could result if the male is abnormal and could not complete the process; this will definitely affect the rate of insemination. The duration of copulation is however influenced by the number of times the female had mated. Usually it is long during the first mating (over one hour) and short in the second (less than one hour); two spermatophores could be seen in females after their second mating. Therefore, though these second matings are of short duration, there is usually insemination but whether these low durations result in low inseminations is not clear.

According to Tobe and Langley (1978), the number of matings appear to be directly related to the amount of semen in the spermathecae. They quoted the experiments of Pinhao and Gracio to explain their statement. Pinhao and Gracio (1973) concluded that in G. austeni, flies that had copulated successfully twice had significantly more sperm in their spermathecae than those that had mated only once. This is partially true according to results obtained in this study. Generally, when mature males mate with females of the right age, 90% of them showed relatively full spermathecae (classes V and VI). Thus whether these females mated once or twice was immaterial as there was no significant difference between the two groups. However, Pinhao and Gracio used males that were 4 - 5 days old which were probably immature. It has been shown in this study that if females mate with young males larviproduction was low compared with those that mate with mature males. This reflects poor insemination; thus a second mating should improve the degree of insemination. The situation is not so simple because it was also found that when a female remated immediately after a first copulation, the degree of insemination was significantly lower than if mated only once. Indeed, Dame and Ford (1968) found that once mated females produced more progeny than multiple mated ones. They concluded that once mated females accepted semen more readily than multiple-mated. When a female is carrying a spermatophore, it does not readily accept semen from another male.

It is also known in many species of insects that the male ARG secretion can prevent reinsemination (Leopold, 1976). There could be some rejection of sperm or probably the presence of a second spermatophore causes a constriction of the spermathecal duct or some other mechanical distortion. These factors could impede the normal flow of sperm into the spermathecae. Pollock (1974) suggested that multiple mated flies could be carrying spermatophores at the time of mating with fertile males which could prevent insemination by the fertile males. After about 24 hours when the spermatophore would have been ejected, there was an increase in the degree of insemination. In females mated to mature males, this second insemination did not appear to be necessary because it has been shown that a single mating is enough for most flies to be properly inseminated. It is doubtful whether such females (females in classes V and VI) will ever exhaust this quantity in a life time (this is further discussed in Chapter 5). Mellanby (1936) found that one female after producing many pupae and abortions for 200 days after fertilisation contained living sperm in the spermathecae.

The degree of insemination did not affect receptivity in females mated to mature males. Dame and Ford (1968) had suggested that the relative amount of semen in the spermathecae may influence the female's receptivity to subsequent matings. Matings with young males usually resulted in low degrees of insemination (Figure 8) and over 60% of such females usually remated. This could be because males at this age are very young and lack the necessary vigor to stimulate the females at the jerking phase (weak jerks may have been produced). Such females therefore failed to remember they had mated.

It has already been shown that flies with very low degrees of insemination did not necessarily mate twice. In fact, females mated to aspermic males also lost their receptivity thus eliminating the importance of spermatozoa in this behaviour. These results disagree with those of Jaenson (1978b) who suggested that the degree of insemination may affect receptivity. These results however agree with those of Gillott and Langley (1981) who thought this was unlikely.

In a number of species, the accessory reproductive glands serve multifunctional roles that appear unique to insects (Leopold, 1976). Male ARG secretions may inhibit remating by altering the mating behaviour of the female. In some dipterans, e.g. Musca and Aedes, ARG secretion has been found to inhibit mating. It is not known whether or not Glossina spp show comparable behaviour. It has been clearly shown here that the ARG secretion was not effective in preventing multiple insemination, though Gillott and Langley (1981) suggested that both ARG secretion and mechanical stimulation in combination were the factors responsible for refractoriness. In Culex, a peptide component of low molecular weight from the accessory gland is effective in suppressing receptivity (Young and Downe, 1987). Likewise in the stable fly, Stomoxys calcitrans, injection of ARG extract even in concentration as low as 0.25 gland pair per female prevented insemination (Morrison et al, 1982) and in Musca domestica, injections of extracts of male ARG were effective in preventing mating (Adams and Nelson, 1968). Results from this study show that injections of male ARG into female G. morsitans did not cause refractoriness. These results therefore differ from the findings of Gillott and Langley (1981).

In female dipterans known to be affected by ARG secretion, the secretion alone is effective in suppressing receptivity. Therefore Glossina cannot be similar to other Diptera as claimed by Gillott and Langley (1981).

Mechanical stimulation caused an appreciable loss in receptivity comparable to that obtained during normal matings (Table 10). However, in all cases receptivity did not usually drop to zero percent until very late thereafter. At least up to 72 hours post-mating, a small percentage of females was receptive as Gillott and Langley (1981) also observed. Haemolymph from mated females did not affect receptivity in virgins. However, because of the possibility of dilution of the haemolymph by other secretions from the neck region where the sample was taken, 1 μl might not have contained 1 μl of haemolymph, thus it could not be truly concluded that nothing was released into the haemolymph to prevent remating.

It is, therefore, suggested that mechanical stimulation is the only factor to be responsible for the onset of refractory behaviour in Glossina, and in addition it is the jerking phase which is the crucial element in the act of mechanical stimulation. Probably, the terminal setae are so vigorously stimulated that a chemical of very low concentration is released which consequently influences receptivity to further matings. If anything is released into the haemolymph, it could not be detected in 1 μl of haemolymph as this quantity was not observed to affect receptivity. Gillott and Langley (1981) also did not observe any effect of haemolymph of mated

flies on virgin females. They suggested that there was probably too little male material present in 1 µl sample of haemolymph or it was inactivated 24 hours after mating.

CHAPTER 5

THE MATING BEHAVIOUR OF OLD *G. MORSITANS*

5.1 INTRODUCTION

The behaviour of old or sexually experienced males as well as of inexperienced males that are over 7 days old (the age when they are fully potent) has not been adequately investigated. This is also the case with females that have gone through several gonotrophic cycles (old females). In *G. palpalis*, Mellanby (1936) reported that sperm introduced at one mating does not become exhausted. She found that 200 days after fertilisation a fly had produced many larvae but still contained living sperm in the spermathecae. Remating later in life does not therefore appear to be necessary. However, Nash and Kernaghan (1965) found occasional uninseminated females of *G. austeni* aged over 64 days old. Nash *et al* (1966b) also reported that the spermathecae of a female which died at the age of 131 days was found to be devoid of sperm.

In *Aedes aegypti*, it is widely accepted that matrone (a pheromone) causes refractoriness in fully inseminated females. However, Williams and Berger (1980) found that some fully inseminated females of *Aedes aegypti* were inseminated a second time after a number of gonotrophic cycles. Young and Downe (1982) also showed that 75 - 90% of previously inseminated females were inseminated again prior to the second gonotrophic cycle. The ability to replenish the sperm therefore exists among dipterans and is usually necessitated by

sperm exhaustion. Whether this happens in all species of Glossina is not clear. There are few reports mainly on G. palpalis in this area which does not indicate that this behaviour is generally present in Glossina.

Multiple mating does occur in Glossina but seems to be confined to early life (Jordan, 1958). According to Jordan (1958), Squire suggested that there are safe periods when females will allow a male, one being when a third stage larva was present since it would be protected from the claspers of the males; or, in-between larvipositions. Jordan (1958) did not find evidence of extensive re-mating in females older than 10 days old, although a very small number up to 70 days of age re-mated. The attractiveness of old females to males as well as their willingness to re-mate is variable (Nash, 1955). Nash found that in one case, females 65 - 71 days old were attractive but unwilling; in another case, females that were 73 days old were attractive and fairly willing; in a third case, females that were about 123 days old were not even attractive.

Rogers (1972) speculated that lower fecundity in females mated to old males of G. pallidipes could be due to loss of virility with increase in age rather than sperm exhaustion. If females can re-mate in their early days of life and re-mate later in life, this could seriously affect the sterile insect technique. According to Jordan (1958), re-mating amongst older flies is not usual in G. palpalis. Dame and Ford (1968) have shown that re-mating early in life will not adversely affect the SIT.

It is well established that males of Glossina can mate several times in succession. Curtis (1968a) showed that a single male of G. austeni is capable of inseminating 9 - 15 females. It was also shown by Jordan (1972b) that much-mated males were unable to inseminate well due to sperm depletion. "In nature there is an absence of old males from the following swarm" (Jordan, 1958). It is probable that why this is so is because the ability to inseminate is extinguished at an old age.

From the aforementioned, there appears to be some confusion as to what controls mating activity in old age. Apart from casual observations, there is little or no detailed experimental evidence to confirm or deny whether there is a renewal of mating in females at a certain stage in life or the ability of males of various species to mate at an old age.

Challier (1977) reported that in G. m. morsitans survivorship curves from different seasons indicated a decreasing survival rate from the warm rainy season to cold dry season to the hot dry season, and expected maximum longevities were 160, 110 and 50 days respectively. Phelps and Vale (1978) found that during the hot season, the mean age of males of G. morsitans was 14.7 days; in females, it was 48 days and the maximum age was 226 days. The purpose of this study was therefore to re-examine the possibility of re-mating in old females of G. morsitans as well as the mating potential of old males and their contribution to the overall population dynamics, including the SIT.

5.2 MATERIALS AND METHODS

Sixty 3-day-old virgin female G. morsitans were mated singly in mating tubes (Plate 4, Chapter 3). They were allowed to mate only once and then divided into two groups. One group of 31 females was allowed to larviposit and the number of larvae produced per fly was recorded (abortions were also counted). The other group of 29 was also allowed to larviposit, but after various time intervals of larvipositions namely: 2, 4, 6 and 8 larvipositions, they were tested for re-mating by presenting them with males. In this case some could be carrying a newly ovulated egg or a developing larva. Another set of twenty-five 3-day-old females were also mated once and after the seventh larviposition, they were closely observed. Just before the eighth larviposition when the late third instar was recognised (by the black polypneustic lobes visible through the abdominal sternite) the larva was gently squeezed out. Each of such females was then placed with a mature mature male and observed for about 4 hours to see whether it will will re-mate in-between larvipositions.

In another experiment, females of various ages were taken from the ICIPE G. morsitans colony when they were 80, 86 and 90 days old. These females were flies that had been group-mated and had larviposited several times. Males that were 7 - 9 days old were introduced into cages containing about 15 females per cage. They were closely observed to see whether pairs were formed. Those that paired were given a few minutes and were then isolated into mating tubes. Those that successfully copulated were dissected immediately after

they separated in order to see if spermatophores were formed. The status of the uterus (for example, whether it contained a larva, an egg or was empty) was recorded. The duration of copulation and the MSV were also recorded.

Virgin male G. morsitans of various ages ranging from 7 - 60 days were mated with females that were 3 days old. The MSV of the recipient females was also recorded after 24 hours. Immediately after the males were introduced into female cages, they were closely observed to determine whether they appeared reluctant or willing to mate. Virgin males of various ages ranging from 3 - 60 days were dissected and the size of their ARG was measured using an ocular micrometre.

5.3 RESULTS

5.3.1 MATING BEHAVIOUR OF OLD FEMALES

When females were mated only once and observed until they were 200 days old, over 80% died before they reached this age with many of them appearing black in the abdomen. However, before their death, over 80% of them had produced over 7 larvae (Table 12) and over 50% of them had produced over 10 larvae. The average number of larvae produced per fly was 10. Very few flies (about 19%) produced less than 6 larvae. Of the 4 flies that remained alive at the age of 200 days, 3 had produced over 15 larvae (Table 13) with one producing 19. These flies would probably have remained fertile if the observation had

TABLE 12

LARVAE PRODUCED PER FLY BEFORE
NATURAL DEATH OR AFTER 200 DAYS

Larvae Produced	% Flies Producing
0	6 (2)
1 - 5	13 (4)
6 - 10	23 (7)
11 - 15	35 (11)
15 - 20	23 (7)

n = 31

Numbers in parenthesis indicate the number of flies observed to larviposit.

TABLE 13

FEMALES ALIVE AT THE AGE OF 200 DAYS
AND THE NUMBER OF LARVAE PRODUCED

Fly	No of Larvae
1	18
2	10
3	16
4	19

continued until death as there was enough sperm found in the spermathecae (MSVs were not determined). These results show that one mating was usually sufficient for the flies to produce larvae throughout their lives. Flies presented with males after various periods of larviposition did not re-mate during the observation period.

Gently squeezing the abdomen of pregnant flies to facilitate birth did not show that when the uterus was empty they could re-mate. Out of 20 flies, 2 yielded to male attempts to copulate after very strong resistance. They were, however, not able to complete the action. Very old females did not show that after several larvipositions they needed to re-mate in order to replenish their sperm supply. Table 14 shows that very few flies were willing to re-mate even after several larvipositions. Twenty percent of 90-day-old females coupled but most of them were reluctant initially and struggled with the males for a few minutes; this was also observed with 80-day-old females where 44% paired but only 3 flies successfully copulated. This behaviour could not be regarded as a renewal of receptivity as pairing was achieved mainly through coercion. Amongst those that successfully completed copulation, some were inseminated as a spermatophore could be found in the uterus. Thus, insemination did take place later in life in a few flies.

When 20 virgin females all of which were 16 days old were given the opportunity to mate, only 3 (15%) copulated. Most of the old females that paired when presented with males, only paired for a short

TABLE 14

OLD FEMALES REMATING AT VARIOUS AGES

Age (days)	n	% Females in copula	Mean MSV
16 (virgin females)	20	15	-
80	30	3	-
86	20	44	0.43 (3)
90	30	27	1.15 (5)

Numbers in parenthesis indicate the number of flies that successfully copulated and were inseminated.

time (10 - 20 minutes in most cases) indicating that they were forced. It was only those that paired for a much longer period (about 1 hour or more and in some cases over 2 hours) that were inseminated (indicated by the presence of a spermatophore in the uterus). Table 14 shows that the MSVs were, however, low. Sperm found in the spermathecae were probably the remnant from an original mating or some that trickled into the spermathecae during insemination or soon after separation.

5.3.2 MATING BEHAVIOUR OF OLD MALES

The willingness of males to mate appeared undiminished even when the flies were 60 days old. Old virgin males inseminated well (Table 15), and compared favourably with younger males (7 - 8 days old). Old virgin males also appeared to be willing to mate and showed even more aggressiveness than 7-8-day-old males. Many old males persisted for very long periods even when rejected. The MSV of females mated with such males was high in all cases reaching a value of over half and in many cases were either full or about three-quarter full. The difference between the age groups shown in Table 15 was significant ($F = 2.72$, $P<0.05$). When the means MSVs were compared using the multiple range test, the age group 26 - 35 was significantly different from 15 - 17 down to age group 7 - 8, though not significantly different from age groups 18 - 25 and 36 - 60. However,

TABLE 15

AGE OF MALE AND ABILITY TO INSEMINATE

Age (days) of males	n	MSV of Female Mean ±	SE	
7 - 8	25	1.73	0.01	b
9 - 11	42	1.67	0.01	b
12 - 14	32	1.71	0.01	b
15 - 17	11	1.70	0.03	b
18 - 25	18	1.78	0.01	ab
26 - 35	9	2.00	0.00	a
36 - 60	24	1.85	0.01	ab

Means with the same letters are not significantly different

the sample size for age group 26 - 35 was very small and the discrepancy could have arisen because of this. With the exception of age group 26 - 35, all other age groups were not significantly different from each other. This was also expected of age group 26 - 35.

The male ARG increased in size with increase in age of the males (Table 16). There was no evidence of shrivelling due to old age. A large size was maintained at all ages except when very young. For example, at 3 days of age, the mean ARG diameter was 0.11 $\pm$ 0.001 SE and at 40 - 60 days of age the mean was 0.18 $\pm$ 0.0006 SE. At the age of 7 days, it is normal to record a value of 0.16mm or more.

TABLE 16

AGE OF MALE AND SIZE OF
ACCESSORY REPRODUCTIVE GLAND (MM)

Age(days)	n	Size of ARG Mean ±	SE
3	20	0.11	0.001
7 - 12	47	0.15	0.0004
13 - 18	10	0.15	0.002
19 - 24	6	0.16	0.003
30 - 40	6	0.16	0.003
40 - 60	33	0.18	0.0006

5.4 DISCUSSION

One mating appears to be sufficient in order to satisfy the reproductive needs of a female for the rest of her life. An average of 10 larvae were produced per female whereas in individual cases some produced between 0 - 19 larvae. This is in agreement with Mellanby (1936) who observed that the spermathecae of one female still contained living sperm 200 days after insemination. It also appears that sperm from one mating is sufficient to produce reasonably full spermathecae. Indeed, Pollock (1970) observed that the spermatophores dissected from the uterus of G.austeni usually contained more sperm than necessary to fill the spermathecae of a recipient female.

Nash (1955) predicted a desire of female G. palpalis to re-mate later in life. It has, however, been shown that even after 19 larvipositions such females still retained the ability to continue larvipositing as indicated by the fact that enough sperm was found in the spermathecae to secure her fertility. While it cannot be denied that females can re-mate several times early in life (Jordan, 1958; Dame and Ford, 1968), there was no willingness to re-mate after larvipositions. In his work on G. palpalis, Jordan (1958) found that re-mating among old flies was unusual. The suggested 'safe periods' when it is possible for females to re-mate (for example, when the uterus is empty) mentioned by Squire (cited by Jordan, 1958) therefore seem unlikely. Most of the pairings that took place which resulted in insemination were forced as the females were observed to actively resist the courting males.

This cannot therefore be regarded as true renewal of receptivity since receptivity implies active participation of the female, namely: opening of her wings or they were pushed open by the male when she kept her abdomen horizontal to allow the male to make a hypopygial connection. This forced mating may be due to mating pressure in the small cages where a large number of aggressive males were present. Probably in the field, this pressure is less intense because of the lower population densities, and multiple mating may be much less common than is observed in the laboratory (Dame and Ford, 1968).

If some old females were found with empty spermathecae as could be observed occasionally, then they probably were not well inseminated at a first mating. Nash et al, (1966b) reported that they found a female at the age of 131 days with empty spermathecae after producing 6 larvae. This is not unexpected since occasionally even with 7-day-old males, recipient females could be found with a low degree of insemination (Chapter 4). It cannot, however, be said that a high percentage of flies behave in this manner. In the field, females will occasionally encounter very young males as well as males which have experienced several matings. Very young males (1 - 4 days old) cannot inseminate well (Nash, 1955); males also lose their ability to inseminate after repeated copulation (Jordan, 1972b). Thus, both cases will lead to inadequate insemination. If such recipient females failed to re-mate soon after the first mating, they may therefore lose their ability to re-mate at older ages and as such could exhaust their sperm supply. Given the ability to re-mate at the early ages, it is

clear that only a small percentage of flies would eventually exhaust their sperm supply. Since old females rarely re-mate later in life the SIT will not be affected.

Females that mated twice when kept together with males for 24 hours before separation can produce about the same number of larvae per female as those that mated only once. For example, Nash et al 1968) reported that female flies fed on goat blood lived for about 120 days and produced an average yield of 9-10 pupae per female (these females were usually left with males for 24 hours). According to these authors, by day 103, 7.1 larvae per female were produced. In the present study, an average of 10 larvae per female were produced in 200 days.

Male Glossina seem to be quite active and willing to mate even when over 60 days old. Can old males live so long in the field? There are indications that they do. For example, in G. pallidipes in Western Uganda, Kangwagye (1971) found that the longest life span was 134 days. In G. m. morsitans, Challier (1977) reported that maximum longevities were 160, 110 and 50 days during the warm rainy season, cold dry season and hot dry season respectively. The above-mentioned account shows that males can live for long periods and can contribute to the mating process. Old males showed even more aggressiveness than young males and did not lose the ability to mate and to inseminate. Their agility might only be affected by excessively frayed wings or loss of wings as they do not usually occur in 'following swarms'. However, external signs of aging such as damaged and frayed wings are not always good indicators of

of aging (Sohal, 1985). Jordan (1972b) reported that poorer performance of females mated to much-mated males was not caused by the males being older and less able to inseminate; rather, the reason is due to the testes of such males containing few or no sperms. He also observed that the fecundity of the mates of males first mated when 30 days old was comparable to those mated to much younger males.

Pollock (1974) showed that the accessory glands of unmated male flies increased in width. In the present study, the accessory glands of very young flies (3 days old) had a smaller diameter than those of older flies. The size attained when the male was mature was generally maintained without any decrease observed. It was also found that these males correspondingly inseminated well. Samaranayaka-Ramasamy (1981) also showed that by day 3, the increase in diameter of the ARG of G. morsitans was highly significant. The absence of old males from 'following swarms' could not be due to their inability to inseminate or lack of 'libido' but probably due to loss of flying power caused by extensively frayed wings. However, because of the possibility of sperm exhaustion (Jordan, 1972b), their contribution to the overall population dynamics may not be significant.

It is therefore concluded that, old females do not regain their ability to mate at a later stage in their life after several larvipositions. Once a female has mated with a mature male, it generally loses its receptivity. There does not exist any period

later in life when a female is likely to re-mate. The sperm from one mating was usually sufficient to fertilise the number of eggs produced by a female in her life time. Males lost neither their libido nor their ability to inseminate. The reason why much mated males failed to inseminate well must be only due to accessory gland depletion or to sperm exhaustion. The SIT is not likely to be affected since females do not acquire the ability to re-mate later in life.

CHAPTER 6

MATING BEHAVIOUR OF FEMALE G. PALLIDIPES AUSTEN

6.1 INTRODUCTION

The rearing of tsetse flies is cumbersome and expensive. Glossina pallidipes is particularly difficult to rear under laboratory conditions when compared with other savanna species such as Glossina morsitans. For example, Willett (1953) reported problems in attainment of adequate fertilisation rate among mated females. Leegwater-van der Linden (1983) also described a successful protocol for the rearing of G. pallidipes in the laboratory of Experimental Entomology, University of Amsterdam. This protocol is also being used at ILRAD, Nairobi with some success (Dr S K Moloo, pers. comm). At the University of Bristol in Langford, United Kingdom, G. pallidipes from Lugala in Uganda has been successfully colonised (Langley, 1989). Recently, Ochieng et al (1987) described the successful rearing of G. pallidipes from the Lambwe Valley in Western Kenya by using simple grass-thatched hut without any climatic control. However, at other laboratories, G. pallidipes is being reared but with little success.

In the laboratory G. morsitans readily mates after the first blood meal, usually 1 - 3 days after emergence. Mating can also occur between newly-emerged unfed Glossina in laboratory emergence cages

when the flies are crowded together (Tobe and Langley, 1978). In G. pallidipes, the age at which females are receptive seems to depend on the origin of laboratory populations. For example, Van Etten (1981) compared the performance of two laboratory species of G. pallidipes originating from Nguruman in the Rift Valley and Mwalewa Forest in the coastal area of Kenya. Although he was not successful in establishing colonies, he discovered inbreeding characteristics between the two populations. He concluded that there was considerable population diversity in this species.

As there are still gaps in our knowledge of the mating behaviour of G. pallidipes, a study of sexual receptivity in females was undertaken to find the factors responsible for reported cases of failures in colonising G. pallidipes.

6.2 MATERIALS AND METHODS

Tsetse were trapped at Nguruman in the Rift Valley of Kenya using biconical traps baited with cow urine and acetone. Female flies were brought to the laboratory in Nairobi where they were maintained at $25^{\circ} \pm 1^{\circ}C$, 75 - 80% relative humidity and LD 12:12 (12 hr light: 12 hr dark) until used in experiments. Groups of 20 - 25 flies were held in PVC cages measuring 18 x 8 x 5cm. They were given the opportunity to feed everyday except on Sundays. Every morning newly emerged flies were collected and sexed; they were considered to be one day old.

For mating experiments, groups of 10 - 14 virgin female flies of known ages (4-18 days) were put in holding cages measuring 21 x 8 x 6cm and mated with twice the number of males aged between 12 - 30 days old at a temperature of 26°C after temperatures of 22°C, 24°C, 25°C and 26°C were tried with a few flies. According to Jaenson (1978b), males of this age are sexually active. Some males were used twice twice after resting for about 3 days. Males that were 20 - 30 days days old were those that were re-used especially if they were first first mated when about 20 days old. Flies that paired within the first 3 hours were isolated in single tubes so that the duration of copulation could be recorded. The rest of the flies were left together till the following morning.

All flies were dissected 24 hours after pairing to determine the mean spermathecal value and also record whether ovulation had taken place. Females that paired during the first 3 hours were presented with fresh males immediately after they separated to determine the extent of multiple mating. They were observed for a further period of 5 hours. However, it is possible that if kept together for longer periods remating could take place, but this needs other methods like the use of genetic markers. Ovaries of unmated flies of various ages were dissected in 0.9% Nacl under a dissecting microscope. Follicle lengths were measured using a calibrated eye-piece micrometer. The extent of ovulation in virgin females was also determined after dissection.

6.3 RESULTS

6.3.1 SEXUAL RECEPTIVITY

In the Nguruman population, female sexual receptivity increased from day 6 on; no fly below 6 days of age was observed to copulate within the 3 hour observation period (Figure 9). Peak receptivity was reached at days 9-13 (60 - 80%) and dropped thereafter. Flies rarely paired immediately and hence these maximum rates were achieved only if they were left together for 24 hours. Temperature at the time of mating was also crucial as no flies were observed mating below 24°C (room temperatures are usually lower than 24°C in the morning). At older ages, receptivity dropped but never fell to zero (Figure 9).

6.3.2 OVARIAN DEVELOPMENT

The rate of ovarian development in groups of virgin females aged 1 to 15 days was studied. A total of 225 flies with 15 flies per age group were dissected. Development of follicles A_1 and C_1 progressed with increase in age (Figure 10). Between ages 4 and 6, development of follicle A_1 was accelerated and by the age of 10 days follicle A_1 had reached maturity (about 1.6mm) corresponding to the onset of peak receptivity in female (Figure 9). Follicle C_1 had a more gradual development; the highest lengths (about 1.6mm) were reached at the time of high receptivity.

FIGURE 9

% G. PALLIDIPES FEMALES COPULATING WITHIN 3 HOURS
AT VARIOUS AGES, AND TOTAL % INSEMINATED WITHIN 24 HOURS

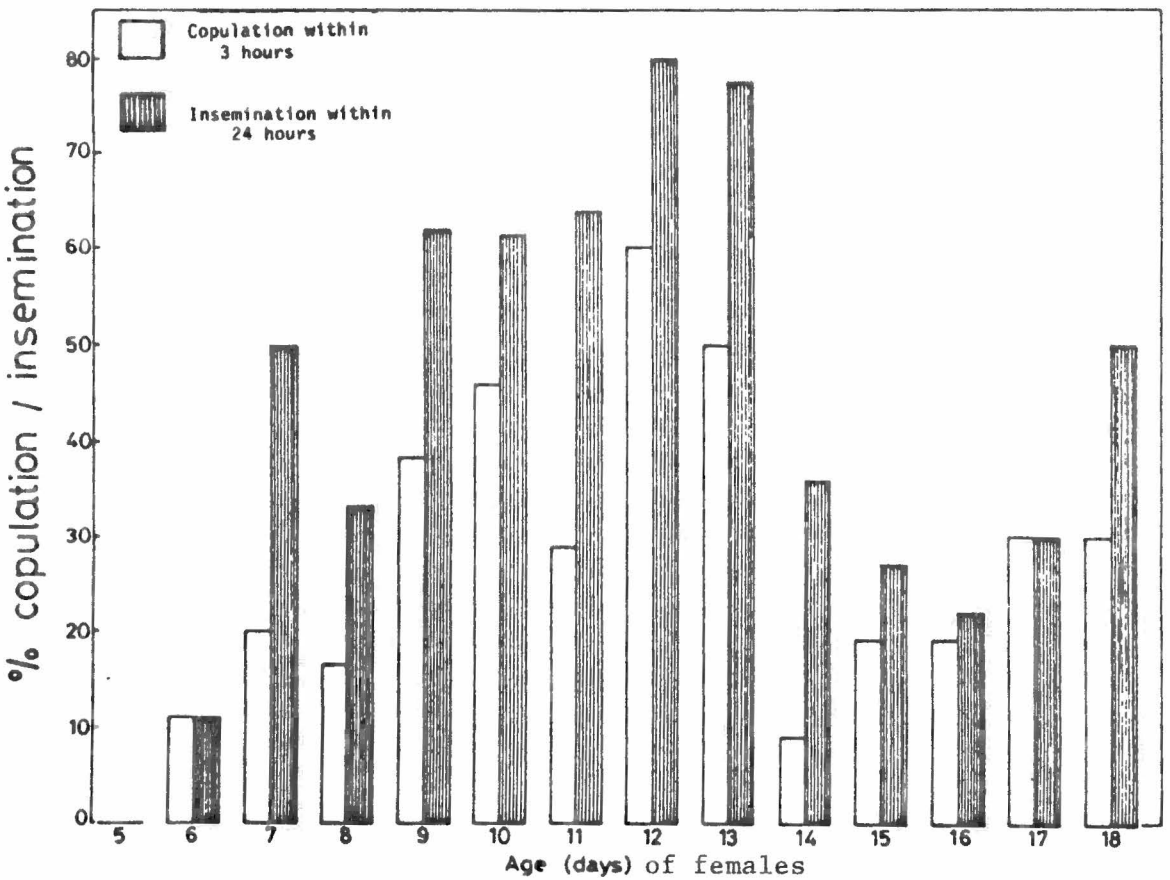
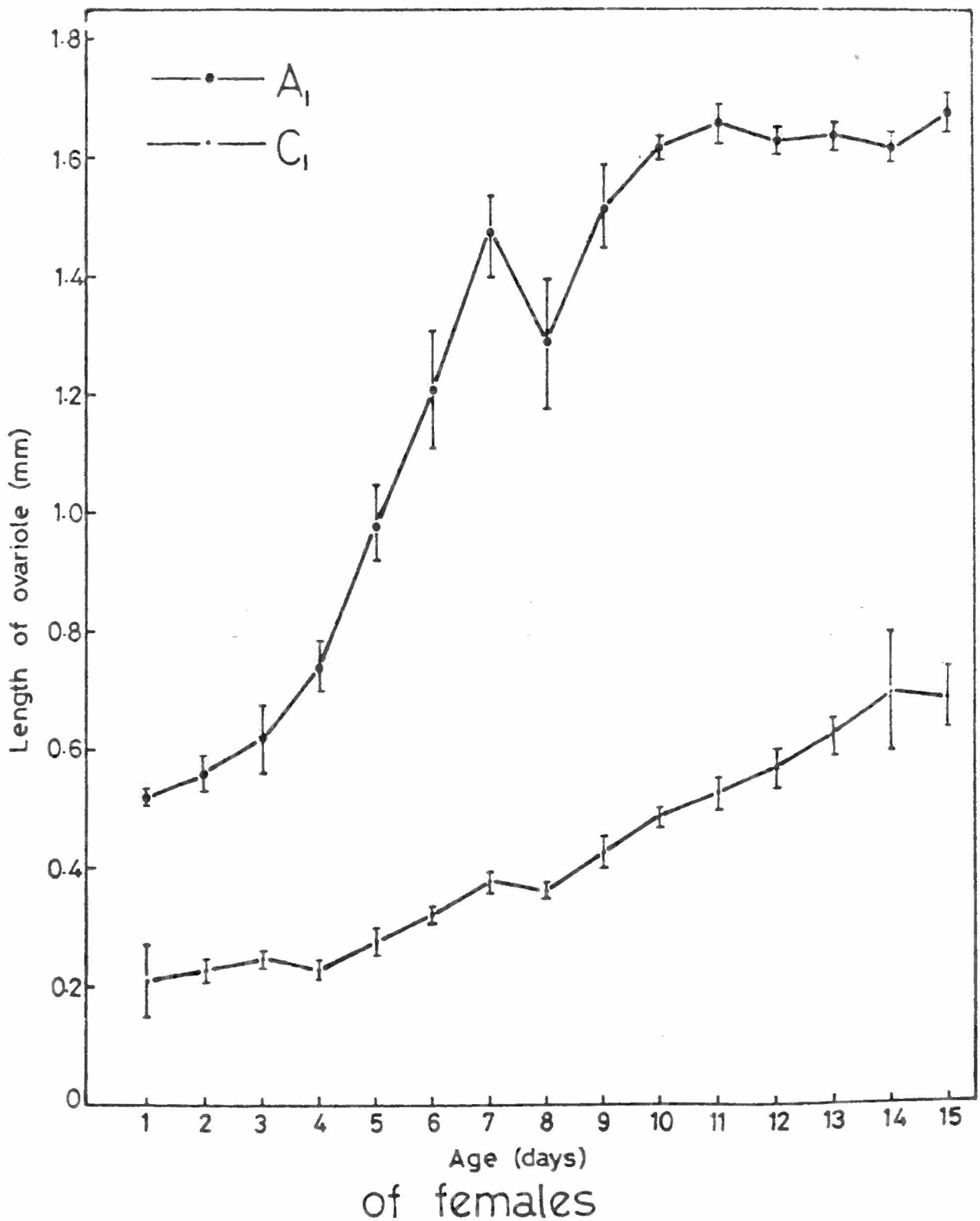

Copulation within
3 hours
Insemination within
24 hours
% copulation / insemination
0
10
20
30
40
50
60
70
80
5
6
7
8
9
10
11
12
13
14
15
16
17
18
Age (days) of females

FIGURE 10

LENGTH OF OVARIOLE A_1 AND C_1 (MEAN ± STANDARD ERROR)
OF VIRGIN FEMALE *G. PALLIDIPES*
(FIFTEEN FLIES WERE DISSECTED IN EACH GROUP)

— • — Ovariole A_1

— . — Ovariole C_1


A1
C1
Length of ovariole (mm)
1·8
1·6
1·4
1·2
1·0
0·8
0·6
0·4
0·2
0
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15
Age (days)

of females

6.3.3 DURATION OF COPULATION

The mean duration of copulation was comparatively short ranging from 23.3 minutes to 24.8 minutes (Table 17). When the ages were grouped into three categories, 6-9 days, 10-13 days and 14-18 days which represent respectively the ages at which the flies were less receptive, highly receptive, and when receptivity was low again (Table 18), it was found that there was no significant difference between the age groups ($F = 0.52$, $P>0.05$).

6.3.4 MEAN SPERMATHECAL VALUE (MSV)

Mean spermathecal value was determined to test if short copulation time affected the degree of insemination. When females were mated at the time of high sexual receptivity, the mean spermathecal value was overall high (Table 19). Most flies were found to have MSV over half (>1.00). It was only when receptivity dropped that the MSV dropped as well. During periods of peak receptivity (ages 9-13 days) the average MSV was very high and ranged from 1.66 to 1.95. High receptivity corresponded with high MSV values and low receptivity with low MSV values. There was a significant correlation between receptivity and MSV as shown by the Spearman rank correlation coefficient ($r_s = 0.63$, $P<0.05$).

TABLE 17

MEAN DURATION OF COPULATION OF FEMALE
G. PALLIDIPES AT VARIOUS AGES

AGE(DAYS) OF FEMALES	DURATION OF COPULATION (Minutes)			NO OF FLIES OBSERVED
	MEAN	±	SD	
6	24.5		-	2
7	19.0		-	2
8	28.7		10	3
9	24.0		4	5
10	23.7		5	3
11	23.3		7	7
12	25.5		7	8
13	20.8		5	10
14	22.0		5	7
15	28.0		14	3
16	23.0		-	2
17	28.7		8	3
18	22.5		-	2

TABLE 18

MEAN DURATION OF COPULATION OF FEMALE
G. PALLIDIPES AT VARIOUS AGES

AGE(DAYS) OF FEMALES	DURATION OF COPULATION (Minutes) MEAN ± SD	NUMBER OF FLIES
6 - 9	23.7 6	12
10 - 13	23.3 6	28
14 - 18	24.8 8	17

(F = 0.52, P>0.05)

TABLE 19

MEAN SPERMATHECAL VALUE OF FEMALES OF *G. PALLIDIPES* AT VARIOUS AGES

AGE(DAYS) OF FEMALES	NO OF INSEMINATED FEMALES DISSECTED	MEAN SPERMATHECAL VALUE MEAN ± SD
7	7	1.64 0.56
8	8	1.88 0.35
9	8	1.66 0.55
10	6	1.79 0.33
11	4	1.75 0.50
12	8	1.69 0.53
13	5	1.95 0.15
14	6	1.42 0.75
15	3	1.17 0.57
16	3	1.50 0.50
17	4	1.43 0.51

6.3.5 MULTIPLE MATING

No previously mated female was observed to mate a second time within 5 hours after separation, from observations of 40 females.

6.3.6 OVULATION IN MATED FEMALES

Table 20 shows the percent ovulation occurring in mated females 24 hours after mating. Sixty-one percent of females mated on days 9-16 ovulated within 24 hours. Flies less than 9 days did not ovulate within this period. It appeared that they needed a day or two before they could ovulate.

6.3.7 OVULATION IN VIRGINS

Plate 9 shows ovaries from a newly mated female soon after ovulation. Six percent of flies that were 11-13 days old had ovulated when dissected (Table 21). Below 25 days of age a small percentage of flies ovulated (usually about 20%). Over 70% of flies in the age bracket 43 - 52 days had ovulated and it appears that ovulation in virgins is not very common unless the flies are really very old when a high percentage of ovulation can be found. In the age bracket, 43 - 52 days, most flies were found to have mature eggs in their ovaries and many had ovulated two or more eggs. Some eggs had been resorbed in the ovaries or the uterus and others showed signs of degeneration while others were retained (Plate 10).

TABLE 20

% OVULATION IN MATED FEMALES OF *G. PALLIDIPES*
24 HOURS AFTER MATING

	AGE(DAYS) OF FEMALES	% OVULATION
	7	0
	8	0
	9	60
	10	60
*	11	-
**	12	-
	13	40
	14	60
	15	50
	16	100

*
** OVULATION WAS NOT DETERMINED FOR THESE TWO AGES

PLATE 9

OVARIES OF AN INSEMINATED FEMALE G. PALLIDIPES
SOON AFTER OVULATION

A: Spermatheca

b: Ovary

c: Ovulated egg in uterus

d: Uterus

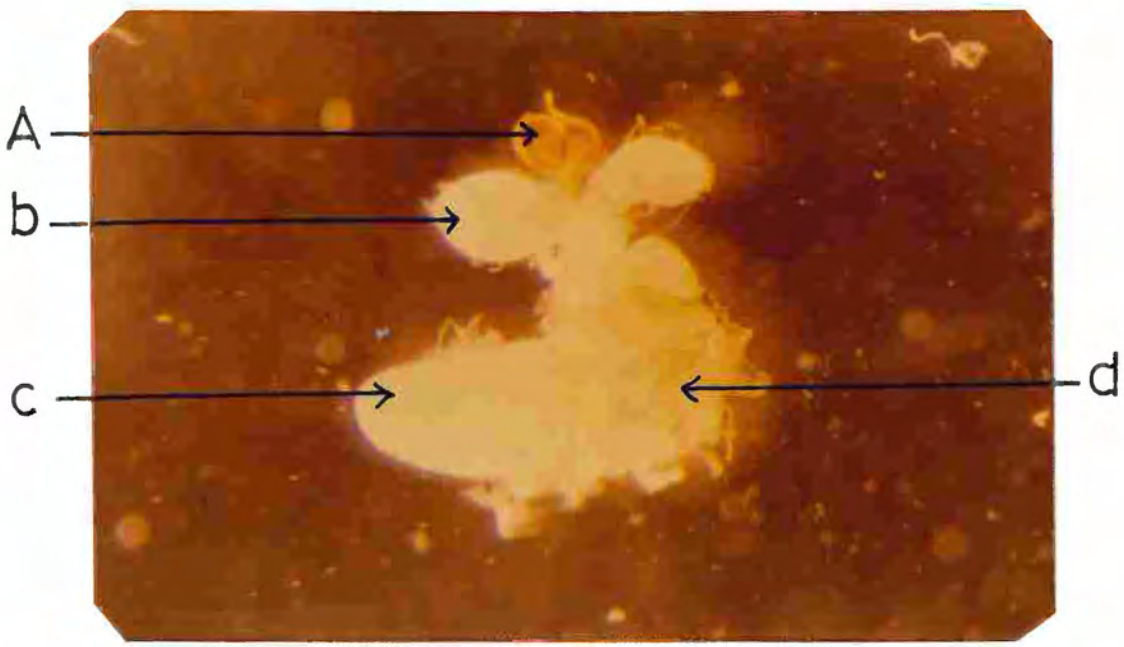

A
b
c
d

TABLE 21

% OVULATION IN VIRGIN *G. PALLIDIPES*

AGE(DAYS) OF FEMALES	% OVULATION	NO DISSECTED
7 - 10	0	30
11 - 13	6	35
14 - 16	21	24
20 - 25	21	28
43 - 52	76	25

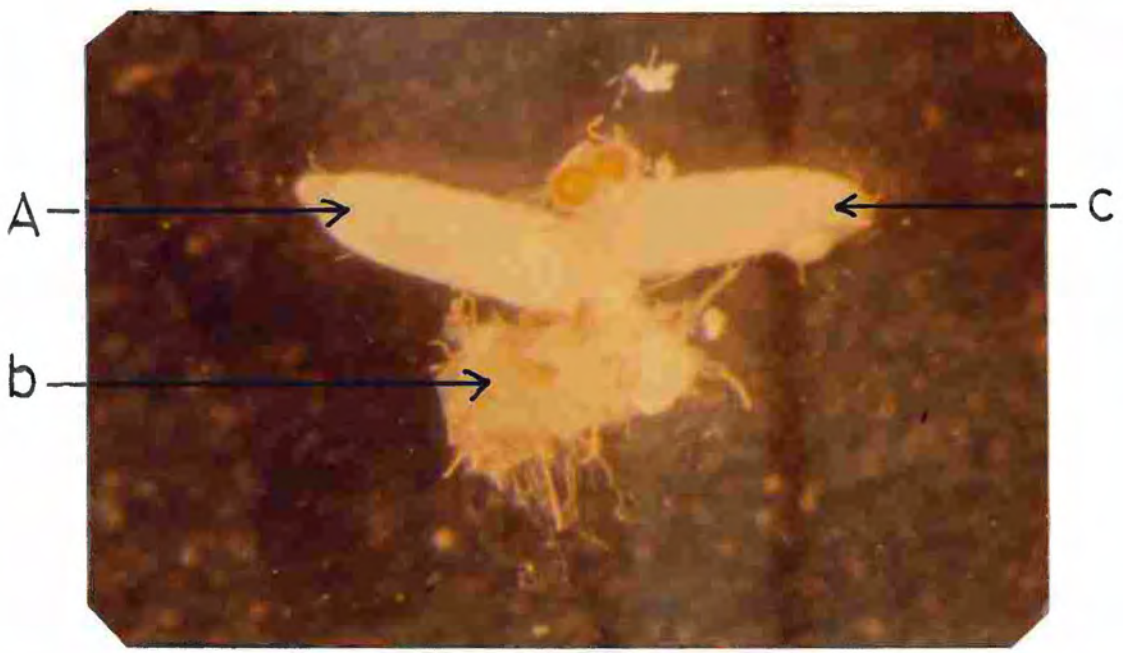
PLATE 10

OVARIES OF AN INSEMINATED FEMALE *G. PALLIDIPES* SHOWING RETAINED EGGS

A: Ovariole C_1 retained in the ovary

b: Ovariole A_1 retained in the ovary

c: Uterus


A
b
c

6.4 DISCUSSION

Tsetse females of most species normally mate within a few days of emergence (Jordan, 1958, Langley, 1977, Tobe and Langley, 1978). This has been demonstrated for G. morsitans (Foster, 1976), G. palpalis (Mellanby, 1936, Nash, 1955) and G. austeni (Foster, 1976). However, the results presented here indicate that mating in females of G. pallidipes does not occur until they are at least 6 days old. Also, high levels of receptivity are observed to occur around age 9-13 days. These results are in agreement with the findings of Rogers (1972), Jaenson (1980) and Leegwater-van der Linden (1982). Leegwater-van der Linden (1982) found that the receptivity of females increased from day 5 to day 8 and was highest on days 8 - 13. Peak receptivity appears to coincide with maturation of the first egg (Figure 10), which Adams and Mulla (1968) also found was the case in the eye gnat. However, the size of the egg follicles in newly emerged Glossina is correlated with the size of the fly (Saunders, 1961) and depends on the food intake. Occasionally small flies which do not feed well produce correspondingly small eggs.

Jaenson (1978b) reported that few females were receptive until 4 - 5 days of age; about 50% copulated on day 5. Leegwater-van der Linden (1982) reported nearly 80% insemination at this age. In the present study, no female less than 6 days old was found to copulate, and none was inseminated even when left for 24 hours. These differences confirm Van Etten's (1981) and Jaenson's (1978a) conclusion that there are differences in mating behaviour

between populations. Langley (1989) also found differences between his Ugandan and Zimbabwean strains of G. pallidipes. Although he succeeded in maintaining a colony of the Ugandan strain, he could not do so with the Zimbabwean strain. The results obtained from this study also agree with those of Odhiambo (1968), who did not observe mating in females less than 6 days old. Chaudhury et al (1984) reported that at Nguruman, field observations showed that females of physiological age group Oa (age 1 to 5 days) and a substantial number of the age group Ob (age 6 to 10 days) were not inseminated. Many of the uninseminated Ob females possessed a developing follicle as long as 1.4mm but not 1.6mm recorded in the present study for most inseminated females.

The observation that female G. pallidipes does not immediately accept a mate or is reluctant to mate, even at the time of optimum receptivity is puzzling. For whatever reasons, the readiness of G. pallidipes to go in copula appears to be different from that observed in other species such as G. morsitans. Female G. pallidipes appear to require some stimulation by the male before they are pacified; this could be a form of courtship (this is further discussed in Chapter 8). Males, however, rarely persist when rejected by females. This behaviour must have been observed by Langley et al (1982) who reported that the copulatory responses of male G. pallidipes were often not as vigorous as those of G. morsitans. Hence, even though these flies were not observed to copulate initially, high rates of insemination were achieved when they were left together long enough (e.g. overnight).

Manning (1966) quoting Barass reported that in the Pteromalid, Mormoniella vitripennis Walker, a single courtship, either successful or unsuccessful, depresses a male's response to other females and that recovery is achieved after an hour's rest. It is possible that male G. pallidipes also recovers after a period of rest. However, this was not investigated. In other groups of flies such as Drosophila, a dance courtship is necessary before copulation can proceed (Smith, 1956; Spieth, 1974). However, with the kind of behaviour shown by G. pallidipes it appears that group-mating is best in order to obtain high insemination rates in the laboratory.

Mating strikes by males of G. morsitans are visually mediated (Wall, 1989a). Nevertheless, it is not clear if light is necessary for successful mating. For example, Leegwater-van der Linden (1983) has shown that there is no significant difference between percent insemination of flies mated in light and in darkness even though light promotes female and male encounters. As Romoser (1973) explains, a wide variety of mechanisms function in bringing together the sexes; these usually involve visual, olfactory and auditory cues occuring singly or in combination. Temperature is, however, important in this respect. Laboratory temperatures are usually lower than 24°C in the mornings and when matings were tried at temperatures lower than 24°C, no pairing was observed. When the temperature was increased (between 25°C and 26°C), the number of pairings were observed to increase. It is therefore important that for purposes of laboratory rearing, the temperature of the mating room should be properly controlled.

Whereas G. morsitans will mate for over 1 hour and in a few cases between 4 and 6 hours (Chapter 4), G. pallidipes takes a very short time, usually less than 30 minutes (Table 17). Saunders and Dodd (1972) reported that the minimum time for successful completion of mating in G. morsitans orientalis (= G. m. morsitans) was 45 minutes and about 3 hours for over 50% of the females to get inseminated; Rogers (1973b) also reported a short mating time in G. pallidipes. In G. pallidipes the short mating time did not appear to affect the degree of insemination and during periods of peak receptivity (age 9 - 13 days) the average MSV was high and ranged from 1.66 to 1.95. Nash (1955) found that the degree of insemination among fertilised females of G. palpalis was uniformly high at all ages (MSV ranging from 1.8 to 1.9). Similar results were obtained in this study. Individual values for G. pallidipes showed that many flies had full spermathecae (MSV = 2.00). Older females (females over 13 days old) showed a drop in MSV coincident with a drop in receptivity. Therefore, a second mating appears to be unnecessary and keeping flies together in mating cages for up to one week with the hope that multiple mating will occur to improve insemination seems to be redundant. Keeping males and females together for a much shorter period is desirable and could improve longevity (Leegwater-van der Linden, 1983). A period of 3 days appears to be sufficient to allow enough time for all females to be inseminated.

No previously mated female was observed to mate a second time immediately after the first copulation. Whether females will re-mate after a much longer period, for example 24 - 72 hours was not determined. However, this phenomenon may not be a common occurrence in the field as Rogers (1973c) reported for the Ugandan strain of G. pallidipes. Probably, the females that seem to copulate more than once in the field are "taken advantage of" by being forced into mating by a few very aggressive males. Jordan (1958) stated that, apparently a considerable proportion of young females are likely to mate in nature on a number of occasions, but that the desire for mating is extinguished much sooner in life in mated females. Gillott and Langley (1981) showed that soon after mating the desire of females of G. morsitans to re-mate rapidly disappears. According to Mellanby (1936), one fertilisation of G. palpalis is sufficient to allow the female to produce larvae over a period of many weeks. Similarly, dissections of old females gave no indication that the sperm introduced at one pairing ever became exhausted. Leegwater-van der Linden and Tiggelman (1984) also reported that multiple mating in G. pallidipes rarely occurs. In some other dipterans such as the housefly, most mated females are monogamous (Riemann et al, 1967). Similarly, once a virgin female Drosophila has mated, it is unwilling to accept another male for sometime (Manning, 1967). This phenomenon is also known in mosquitoes (Craig, 1967), and may be the normal pattern for Glossina.

The observations on spontaneous ovulation in the present study is in agreement with Leegwater-van der Linden (1981) but differs in that the phenomenon is not as common as she reported. It is only when the flies were very old that over 70% ovulation was observed to have occurred. These results also agree with those of Wall (1989b) who found that by 15 days of about 20% ovulation was observed. Ovulation increased thereafter. These results differ from those of Odhiambo (1971) who reported that ovulation did not take place in virgins. Ovulation in virgins is not only peculiar to G. pallidipes, it happens also in G. morsitans (Saunders, 1961). The receptivity and fecundity of flies could be affected by spontaneous ovulation as about 5% of flies aged 11-13 were observed to have ovulated. Mating in the laboratory should best be done before 11 days of age. When timing of ovulation in mated females was compared with ovulation of virgins, it was clear that generally, ovulation was accelerated by mating. When 9-day-old females mated, over 60% ovulated within 24 hours. It was also shown in G. morsitans by Saunders and Dodd (1972) that mating accelerated ovulation. If mating is delayed until the moment of ovulation of the first egg, it is likely that such females will fail to produce an offspring in the cycle immediately following mating (Langley, 1989). Therefore it may be sensible to mate female G. pallidipes at the age of 7 - 8 days.

In conclusion, it is important to emphasise that before attempting to rear G. pallidipes, it is necessary that any previously uninvestigated population be studied independently to

ascertain differences in mating behaviour, whether minor or major. Mating at the right age, carefully controlling temperature and humidity to prevent wide fluctuations, as well as keeping males and females together in mating cages for the shortest time necessary to result in successful copulation and adequate insemination are desirable for mass rearing of *G. pallidipes*.

CHAPTER 7

SOME FACTORS AFFECTING RECEPTIVITY AND INSEMINATION IN G. PALLIDIPES

7.1 INTRODUCTION

Laboratory conditions do not simulate natural conditons. It has been observed that several species of Glossina could be easily reared in the laboratory but some have proved very difficult. G. pallidipes can mate though not readily in the laboratory as observed in other species such as G. palpalis and G. morsitans. Several factors could be responsible for this. For example, Dean et al (1968) found that G. morsitans could not be bred in large cages. They also concluded that the flies lived longer and were more reproductive in small cages. Some other insects can mate only in conditions of high light intensities while others can only do so in the dark (Romoser, 1973).

Peters and Barbosa (1979) have reviewed the influence of population density on various factors such as size, fecundity etc. in insect colonies. They quoted Nighigaki who showed that copulation frequency increased exponentially but fecundity increased only proportionally with increasing density in Callosobruchus chinensis. Snow (1980) also found that in G. pallidipes the insemination rate was correlated with the population density; it was lower in low-density populations, than in high density populations. However, high density could also affect longevity by causing high mortality.

The effects of some of these factors are not well-known in Glossina as very few studies have been carried out in this area. Leegwater-van der Linden (1983) reported that a comparison of groups of 3 and 6 females of G. pallidipes in a cage with the same number of males showed that groups of 6 was better. However, when groups of 6 and 9 where compared, there was no significant difference between the two. She also pointed out that shortage of mating encounters in the groups of 3 females and 3 males could impede accumulation of sperm through multiple mating. The latter has, however, been found to be infrequent in the Nguruman strain of G. pallidipes (chapter 6).

According to Lumsden and Saunders (1966), most workers who attempted to colonise Glossina accepted 12:12 hour light regime of tropical zones in which they worked. They explained that since Glossina are generally diurnally active insects, most manipulation of them has been done in light; but low light intensities may be desirable. Leegwater-van der Linden (1983) reported that light was necessary to activate males and females of G. pallidipes when they are ready for mating. Mellanby (1936) also found that when G. palpalis settled near the edge of sunlight, they appeared to be more comfortable. However, she (Mellanby, 1937) also observed that using larger cages and many different light intensities showed little effect on reproduction.

Sex ratio at mating could also affect receptivity. Low fecundity may be due to inadequate insemination because adequate numbers of males were absent. However, excessive copulatory attempts by males

could either be favourable, or cause mortality if large numbers of males are present due to loss of energy by females continuously struggling with males. Leegwater-van der Linden (1983) found that under conditions of surplus males, performance was slightly better if the flies were left together for 24 hours.

It is unusual in tsetse mating experiments and even in laboratory rearing procedures to mate the flies singly in mating vials. Other workers have used group mating because it is faster but individual flies cannot be easily followed. G. morsitans mated singly does not cause problems because it mates readily. However, G. pallidipes does not readily mate when a male and a female are introduced singly into a vial. Sometimes after several attempts to mount a female the male may become unwilling to mate.

G. austeni was found to perform well when mated singly in tubes (Nash et al, 1968) and an insemination rate of about 99% was achieved. Jaenson (1979b) also obtained high fecundity in single matings with G. pallidipes but only when the flies were allowed to mate twice. However, Van Etten (1981) could not succeed when males and females of G. pallidipes were allowed to mate singly but Langley (1989) obtained good results when he allowed them to group-mate and were and kept together for 40 - 48 hours. It is clear therefore that different populations or strains of the same species of G. pallidipes could behave differently and more work needs to be carried out in order to fully understand the behaviour of this fly.

This study was therefore undertaken in order to determine the best conditions for maximum insemination of G. pallidipes in the laboratory.

7.2 MATERIALS AND METHODS

7.2.1 GROUP MATING COMPARED WITH SINGLE-MATING

Groups of flies were allowed to mate in batches with at least 8 females per cage and a maximum of 12 at a ratio of 1:1 (1 female: 1 male). All males were over 12 days of age. Another batch of flies were allowed to mate singly by introducing a single female and male into a plastic vial and leaving them together for a period of 72 hours in a light regime of 12 hr darkness: 12 hr light. After this period the females were dissected and their spermathecae were examined for sperm. Females between ages 9 - 12 days (period of highest receptivity) were compared.

7.2.2 MATING PERIOD (24 HOURS VERSUS 72 HOURS)

Groups of females were allowed to mate in batches in standard PVC cages in a ratio of 1 male: 1 female. All males were over 12 days of age. One group of females was left together with males for 24 hours while the other was left for 72 hours. At the end of each period, the females were dissected and their spermathecae were examined for sperm.

7.2.3 CAGE SIZES

It was thought that mating flies in a slightly bigger cage while still maintaining the same number (8 - 12 females per cage) would improve insemination because the males would have a better opportunity to fly and make mating strikes. Therefore, our standard PVC cages (18 x 8 x 4cm) used in all of the mating experiments carried out with G. pallidipes was compared with a bigger sized cage (21 x 16 x 18cm). This cage is usually used as an emergence cage in the laboratory (Plate 11). Flies, 8 - 12 in number, were introduced into each cage and kept together for 72 hours (keeping flies together for longer periods has been observed to improve insemination); see Chapter 6.

Various ages of females from 7 - 10 days of age were compared. Ages 7 and 8 days represent a period of relatively low receptivity and 9 and 10 days represent a period of high receptivity. The males were over 12 days old. After a period of 72 hours together, the females were dissected and their spermathecae were examined. Other cage sizes could not be compared because of limited time and also because of the unavailability of adequate numbers of flies.

7.2.4 L D 12:12 VERSUS DD

L D 12:12 was compared with continuous darkness (DD) to determine whether the flies preferred to mate in dark conditions than bright conditions. This is because of the more crepuscular habit of

PLATE 11

THE TWO DIFFERENT CAGES USED TO MATE *G. PALLIDIPES*

standard laboratory cage

emergence cage

G. pallidipes which Willett (1953) suggested could be the reason why this species failed to mate under laboratory conditions.

The flies were introduced into PVC cages (18 x 8 x 4cm). Eight to ten virgin females were introduced into the cages with the same number of males aged 12 days or more. Various age groups of females, namely: 5 - 8, 9 - 13 and 14 - 18 days were compared. One group of flies was covered with a double piece of black cotton cloth throughout the day and as well as during feeding. The other group was uncovered and so was maintained in about 12-hour light and 12-hour dark conditions. All groups of flies were kept in the insectary for a period of 72 hours. After this period, the females were dissected and their spermathecae were examined for sperm.

7.3 RESULTS

7.3.1 GROUP MATING COMPARED WITH SINGLE MATING

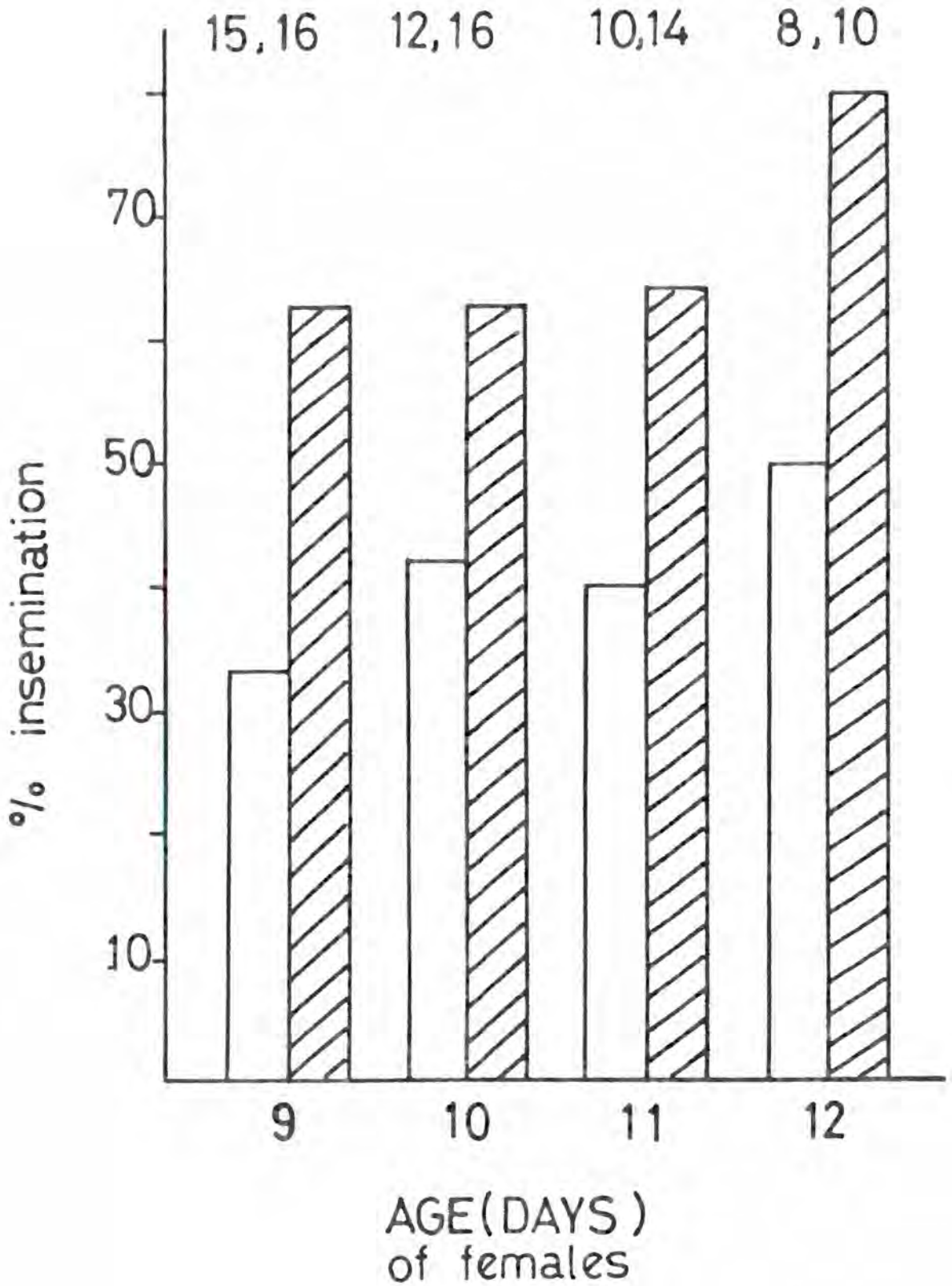
Figure 11 shows that 80% of the females aged 12 days old were inseminated when group-mated, compared with only 50% at the same age when singly-mated. It can be seen that irrespective of the age of the fly, in single matings the percentage of flies inseminated was quite low (usually below 50%). This was even so when females were left together with males for a period of 72 hours. However, in group-mated flies there was over 60% insemination at all ages (9 - 12 days of age). Thus, group-mating was better than single-mating. Note that although the flies used in this and subsequent experiments were several days

FIGURE 11

PERCENT FEMALES INSEMINATED WHEN GROUP-MATED
OR SINGLY-MATED

SINGLY-MATED FEMALES

GROUP-MATED FEMALES


15,16
12,16
10,14
8,10
70
50
30
10
% insemination
9
10
11
12
AGE(DAYS)
of females

older at the end of the mating period, they were generally referred to by their original ages at the time of mating, for convenience.

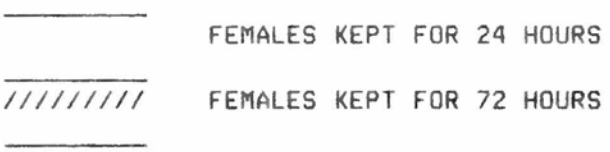
7.3.2 MATING PERIOD (24 HOURS VERSUS 72 HOURS)

Insemination was generally improved when females were allowed to stay together with males for a period of 72 hours (Figure 12). It was even more difficult to get high insemination rates at a ratio of 1:1 when the flies were left together for 24 hours. Figure 12 thus shows a distorted picture. There was no trend; at ages 9-13 days when high receptivity was expected, only about 16% insemination rate was achieved for a period of 24 hours compared with over 60% for 72 hours. There was therefore a highly significant difference between a 24-hour period and a 72-hour period at the age of 9-13 days (χ^2 = 10.04, <0.01).

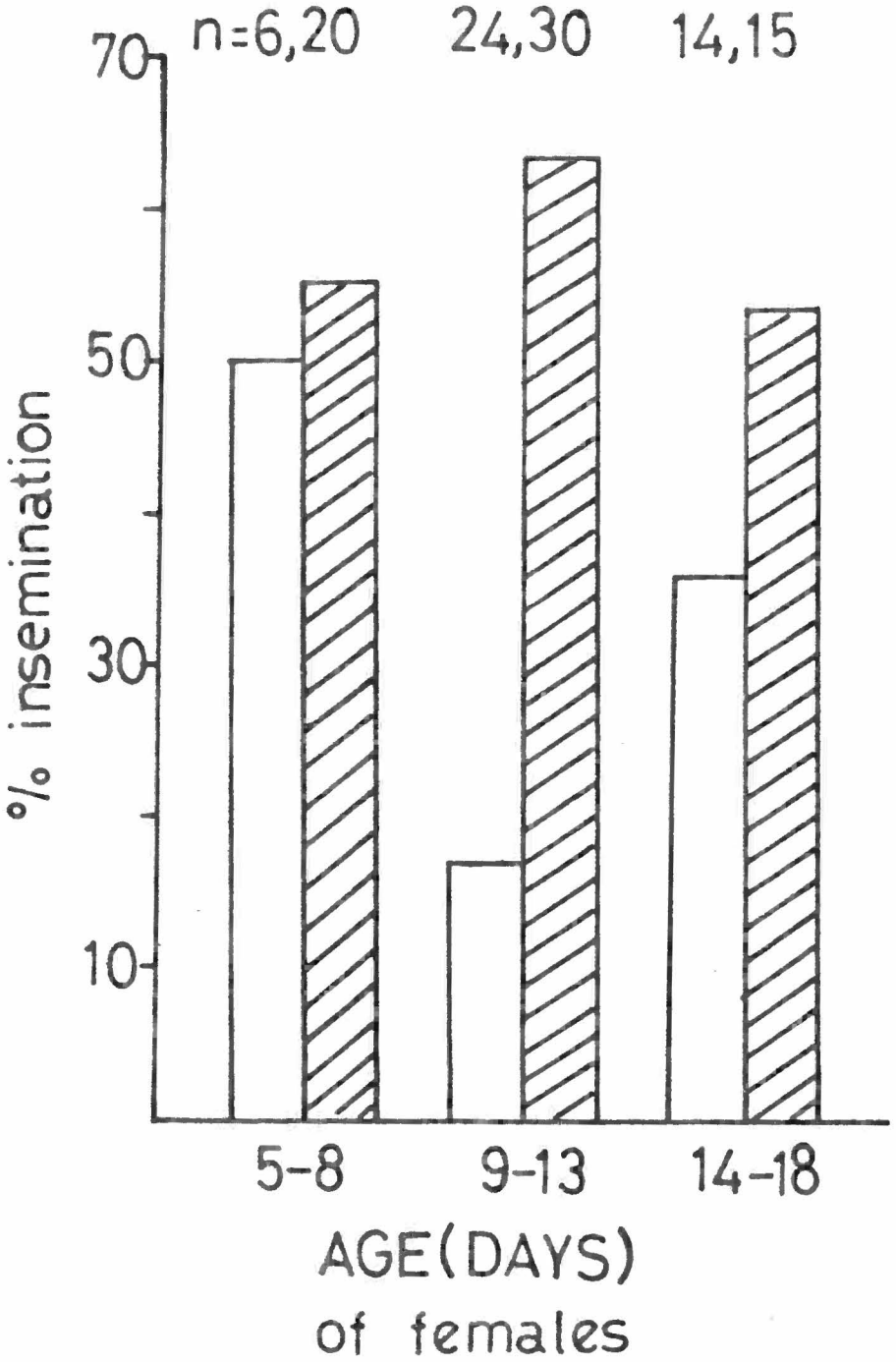
If Figure 12 is compared with Figure 9 (in Chapter 6), it can be seen that a much better rate of insemination was achieved for a period of 24 hours. This is because, in the experiment presented in Figure 9, mating was carried out at a ratio of 2:1 (2 males: 1 female) when excess males were available for courtship. Keeping males and females together for 72 hours appears to be long enough to achieve a high insemination rate at a ratio of 1:1. At the age of 14-18 days when receptivity was expected to be low, over 50% of the females were found to be inseminated when they were kept together with males for 72 hours. This compares with about 30% insemination rate during a 24-hour period.

FIGURE 12

PERCENT FEMALES INSEMINATED WHEN KEPT WITH MALES
FOR PERIODS OF 24 HOURS AND 72 HOURS



n = NO. OF MATED FEMALES


n=6,20
24,30
14,15
70
50
30
10
% insemination
5-8
9-13
14-18
AGE(DAYS)
of females

7.3.3 CAGE SIZES

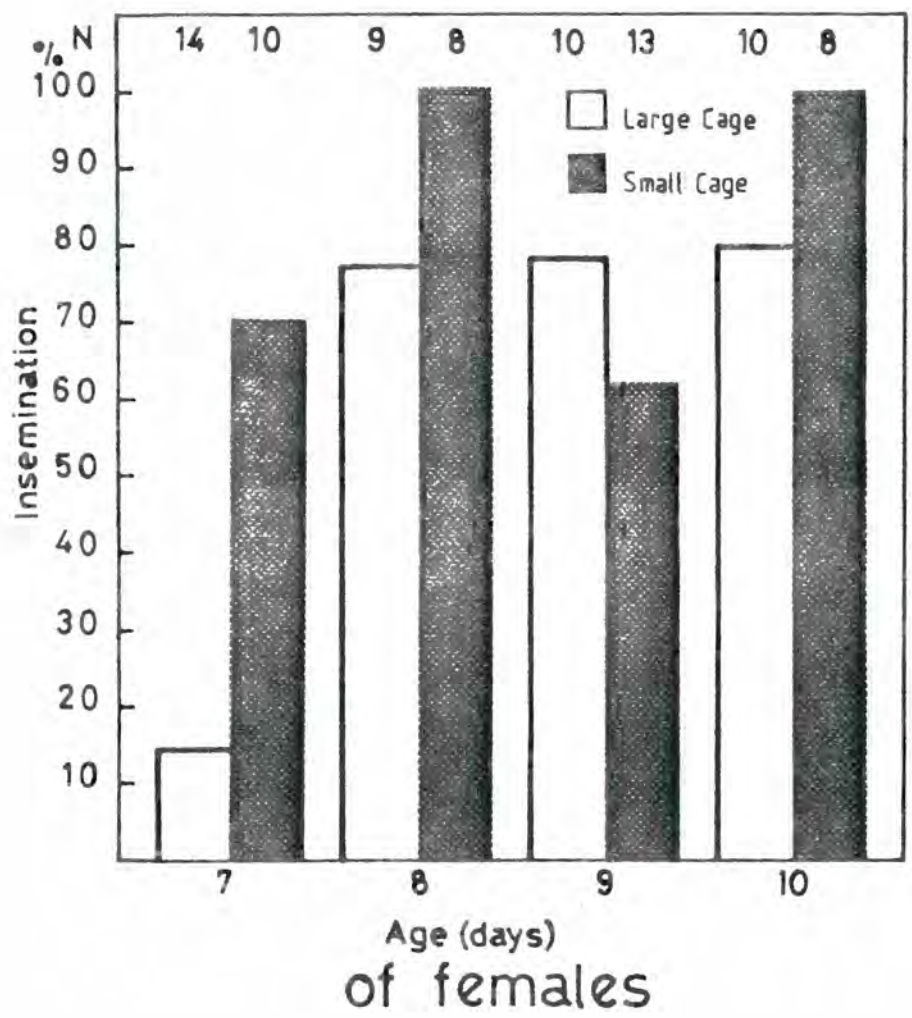
Though the small cage did relatively better than the large one (Figure 13), over 60% insemination was generally achieved with both cages. The exception was with females that were aged 7 days; at this age just over 10% insemination was achieved with the large cage. With the small cage, 100% insemination was achieved in some cases. It was considered unnecessary to determine whether there was any significant difference between the cage sizes because the attainment of 100% insemination clearly showed that the small cage was better. Under the circumstances, a small cage is preferred because it is easy to use especially when feeding the flies.

The very high insemination rate observed was achieved with flies that were 8 and 10 days of age. It should, however, be noted that at these ages, the number of flies mated was 8 (Figure 13). This number is the least preferred of flies per cage in mating experiments of of *G. pallidipes*, because preliminary observations had showed that high insemination rates are achieved when mating is carried out at a ratio of 1:1.

FIGURE 13

PERCENT FEMALES INSEMINATED WHEN KEPT WITH MALES
IN SMALL OR LARGE CAGES FOR 72 HOURS

N = NUMBER OF MATED FEMALES

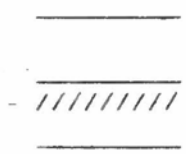

% N
14 10 9 8 10 13 10 8
100
90
80
70
60
50
40
30
20
10
Insemination
Large Cage
Small Cage
7
8
9
10
Age (days)
of females

7.3.4 L D 12:12 VERSUS DD

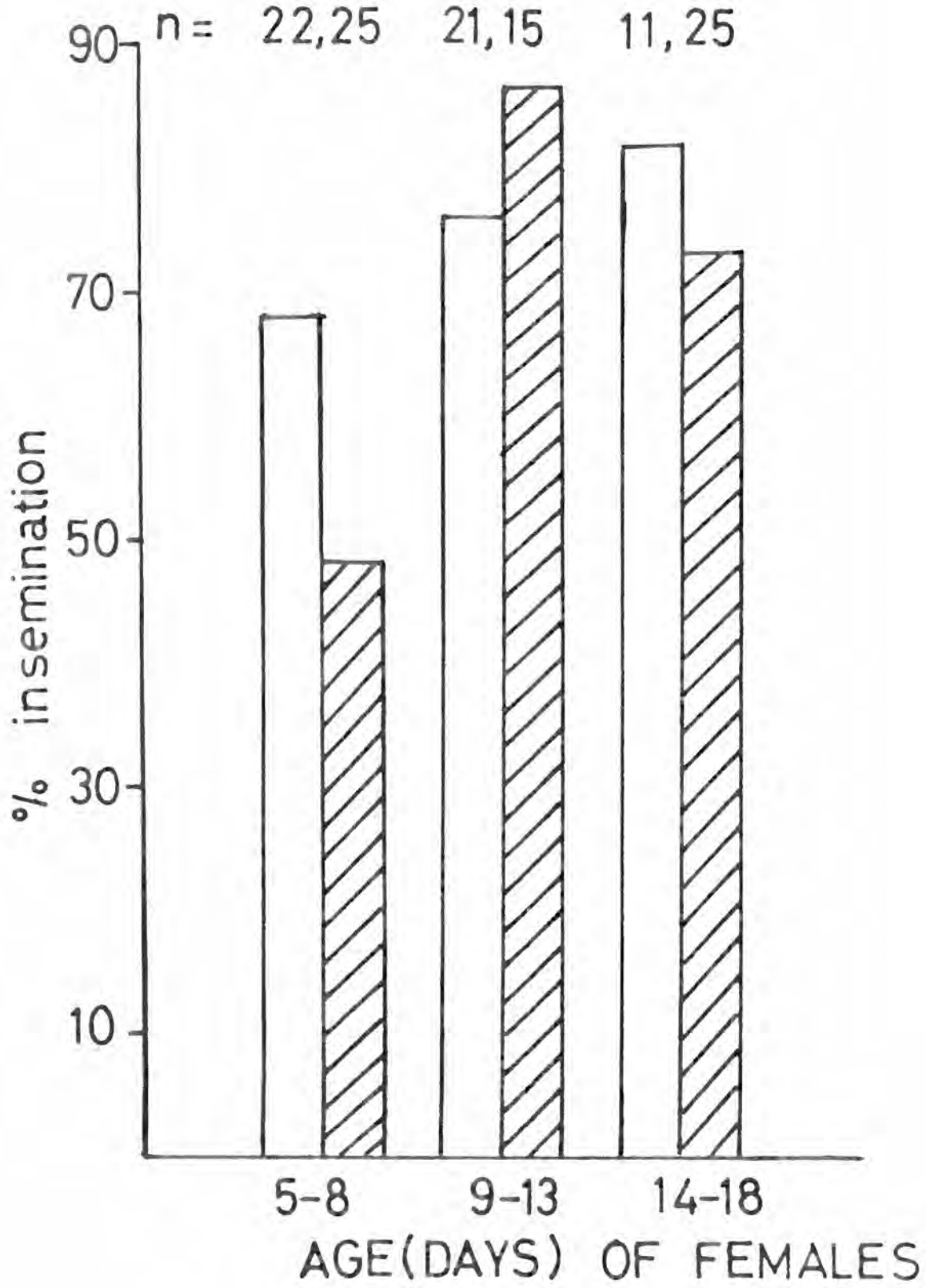
There was no significant difference between flies kept under a regimen of 12-hour dimlight and 12-hour darkness and those kept under continuous darkness throughout the observation period ($\chi^2 = 0.13$, $P>0.05$). Over 70% insemination was achieved at ages 9-13 days in both cases (Figure 14). At the end of the experiment, flies that were 5-8 days old were instead 8-11 days and were therefore expected to show high insemination rates. This was generally so as over 60% of those kept under normal conditions were inseminated. However, less than 50% insemination was achieved with flies that were kept in continuous darkness. Once the flies were kept together for 72 hours, the presence or absence of light was insignificant.

FIGURE 14

PERCENT FEMALES INSEMINATED WHEN KEPT WITH MALES
IN CONTINUOUS DARKNESS OR IN 12 HOUR LIGHT AND
12 HOUR DARK REGIME FOR 72 HOURS



n = NUMBER OF MATED FEMALES


n = 22,25 21,15 11,25
90
70
50
30
10
% insemination
5-8
9-13
14-18
AGE(DAYS) OF FEMALES

7.4 DISCUSSION

The capability of producing an insect species easily, efficiently and in large numbers is a prerequisite for considering a program of sterile male release for any insect (Chambers, 1977). The first problem is that of rearing the insect. Problems could arise if high receptivity and thus high insemination rates cannot be achieved in the laboratory. Since different species of tsetse require different climatic and other conditions to reproduce well, it was necessary to obtain information on factors that could improve the receptivity of G. pallidipes in the laboratory. Getting G. pallidipes to mate in the laboratory has proved to be a problem for many workers in this field.

In the studies reported here, a high insemination rate was achieved when the flies were group-mated compared to single-mating. Owaga (1981) could not get G. longipennis Corti to mate when females were kept singly with males. Probably, group-mating would have led to some success. According to Leegwater-van der Linden (1980), a mating technique which offers many mating opportunities appears essential if high insemination rates should be achieved. This will be possible only when group-mating is carried out. In group-mating, females have the opportunity of being courted by a number of males and unwilling males can be easily excluded. In single-mating, however, close monitoring of the pairs and selective removal of unwilling males will be necessary, which,

needless to say, will be cumbersome and laborious if large numbers of females are to be mated. In Lucilia cuprina (a blowfly), Barton-Browne and Von Gerwen (1988) reported that, when the females were housed in groups, receptivity was low but high when they had been housed singly. This behaviour is similar to swarming behaviour in some insects,for example, Anopheles gambiae which as Charlwood and Jones (1980) explained, tends to increase male and female contact. Flies mated singly also have a problem of being properly fed since each vial will have to be fed at a time, or 3 or 4 vials will have to be put together. For group-mated flies in mating cages, two cages containing probably 10 females each can be fed at a time, compared to 2 or 6 females in vials. Group-mating therefore presents a number of advantages.

The percent insemination of mated females increased significantly when the flies were kept for 72 hours compared to 24 hours (Figure 12) and for 24 hours compared to 3 hours (Figure 9). As has been mentioned, the females probably need several minutes of courting before they are appeased and keeping them for 72 hours probably gives sufficient time for this to happen. In other words, the females became more receptive as they received adequate stimulation for appeasement. According to Manning (1966), some virgin female insects are totally receptive to males and accept their first partner, but others reject males until they have received an adequate amount of stimulation. Manning also explained, that a mature virgin female Drosophila melanogaster Meigen requires an average of three minutes courtship before she accepts a male.

According to Chapman (1982), once a male has recognised a female he may mount her and attempt to copulate immediately as is the case in Musca (Diptera) and some Odonata but in some cases she may be unreceptive. He stated that in the latter case, she is said to be coy and needs to be stimulated by the male before she will permit him to mount. Bastock and Manning (1955) explained that coyness could be regarded as a way by which disturbance is reduced to a minimum and probably to facilitate correct identification of a mate so that fewer mistakes are made.

A ratio of 2:1 (Chapter 6) proved better as more flies were inseminated within a 24-hour period. More encounters were possible in this case. However, for economic reasons it is more advantageous to mate at a 1:1 ratio and keep the flies together for 72 hours. The results in this study therefore differ from those of Leegwater-van der Linden (1983) who reported that a mating opportunity of 24 hours was effective for insemination.

Small cages were more effective than large cages in improving insemination rates. In some cases there was 100% insemination rate. Willett (1953) used a cage measuring 15 x 8 x 5 cm for maintaining G. swynnertoni (5 to 15 flies per cage). He found that the death rates in cages containing 11 - 15 flies were greater in proportion than in less crowded cages although the difference was not statistically significant. He also reported that 10 flies could be kept without causing overcrowding. This fact suggests a relationship between cage size and number of flies. Thus, there should be a

preferred number which could facilitate encounters with the sexes as well as preventing high mortality.

During the course of this investigation, it was noticed that most of the cages where very high insemination rates were recorded contained 8 females and an equal number of males. In Figure 13, it can be seen that flies aged 8 and 10 days performed very well with 100% insemination being achieved (these flies were kept at 8 females per cage during the mating experiments). It was also observed that when the number was increased to between 13 - 15, insemination rates dropped. This effect could be due to crowding. Eight appears to be the ideal number for our standard PVC cages (18 x 8 x 4 cm). Leegwater-van der Linden (1983) reported that 12 females per cage (13.5 x 5 x 8 cm) was maximum at which the performance was not affected.

Large cages have some disadvantages. Feeding was a problem since they could not be strapped onto the ears of rabbits. It became cumbersome as the flies had to be transferred to standard cages for feeding. There could also be a problem of locating a mate if the cage is too big and the flies are few. Probably, increasing the number of flies will help but the problem of feeding will still be there. Dean et al (1968) found that Glossina lived longer and were more reproductive in small cages measuring 8 x 8 x 11 inches than in large cages. Mellanby (1936) also found that large cages did not show any adverse effect on reproduction. There could be a problem of exhaustion in large cages however, as there will be more room for increased activity.

In the laboratory, flies in cages were nearly always found to rest at the end nearest the window and no fly was found resting in the darker parts and none stayed long in the direct rays of the sun (Mellanby, 1936). According to Mellanby, this observation compares well with resting in the sun flecks near the edge of a clearing but seldom in dense forest under a heavy canopy. It is also probable that G. pallidipes would prefer to mate in shaded places because of its more crepuscular habits (Willett, 1953). Though this could be true because of the difficulty of observing G. pallidipes mating in the field, no significant difference in insemination was found between flies kept in the dark and those kept under normal conditions. Dean et al (1968), however, reported generally poor insemination in the dark in G. morsitans. The results presented here also differ from those obtained by Leegwater-van der Linden (1983) who reported that light apparently promotes female and male encounters. It would appear that it is close contact which promotes male and female encounters. This could be observed with G. morsitans in mating tubes when sometimes it is necessary to blow a current of air over the pair or just shake the tubes to bring the pair together. Small cages are therefore advantageous in that close contact can easily be facilitated.

In conclusion, the results show that group-mating was more effective than single for optimum reproductive performance of G. pallidipes. Small cages were advantageous in that feeding could be carried out easily and the sexes were easily brought

together. A mating period of 72 hours resulted in high insemination rates. Whether mating was carried out in continuous darkness or under normal conditions did not matter as high insemination rates could be achieved under both conditions. The normal condition of L D 12:12 for mating tsetse is preferred as no additional effort is required. These factors taken together should improve receptivity and result in high insemination rates in G. pallidipes.

CHAPTER 8

MATING BEHAVIOUR OF MALE G. PALLIDIPES

8.1 INTRODUCTION

In the laboratory, a common characteristic of males of G. pallidipes is their apparent reluctance to mate. Whether this behaviour is characteristic of a particular age group or a common attribute of all males of this species is not clear. Willett (1953) pointed out that a major difficulty in the rearing of successful colonies of G. pallidipes is the attainment of an adequate fertilisation rate among mated females. This could be related to the relative abilities of different age groups to inseminate well. Differences between allopatric populations have been reported by Jaenson (1978a) and Van Etten (1981), though Wall (1989b) did not think that there are gross differences among such populations. There are very few studies on male mating behaviour in G. pallidipes to rely on; therefore, any additional information is of the utmost importance.

In G. pallidipes, Rogers (1972) reported that there was no difference in fertility in females mated by males of different ages especially when the males were over 6 days old. In addition, Rogers (1973a) found that certain males showed more sexual activity than

others and mated up to four times in a period of 5 hours, whereas others did not mate at all. Leegwater-van der Linden and Tiggelman (1984) also found that virgin males inseminated four times in succession and still remained eager to copulate. They also found that experienced and virgin males were equally successful at mating but after three successive matings alternated with periods of rest, the males did not regain their original inseminating capacity. Jaenson (1979a) also studied males that were two days old and found that only 10% copulated normally but by day 10, all the males had copulated. He also found that the MSV of females inseminated by males in different age groups were not significantly different.

Several studies have been carried out on male mating behaviour in other species of Glossina. For example, Nash (1955) found that the virility of young males of G. palpalis under 7 days old was far less than that of older males aged 9 to 33 days. Einyu and Kapaata (1983) also showed that in G. m. centralis pairs became more frequent with increase in age, and coupling was usually immediate. Male Glossina has been reported to mate a number of times in succession. For example, in G. morsitans, Dame and Ford (1968) showed that the mean number of females inseminated per male ranged from 4.9 to 5.6. Jordan (1972b) also found that male G. morsitans and G. austeni never refused a female even after mating a number of times.

Jaenson (1978a) has shown that differences existed in copulation time between two different populations. Van Etten (1981) also found that flies from Nguruman and Mwalewa in Kenya differed from each other

in copulation time, pupal weight, age at which the first larva is produced and duration of interlarval periods. These findings show that differences do exist and these differences whether minor or major, could affect the laboratory rearing of this species. This study was therefore carried out in order to obtain more information on the mating behaviour of males of G. pallidipes in the laboratory with respect to the age at mating and inseminating ability at various ages, with a view to elucidate why this species does not readily mate in the laboratory.

8.2 MATERIALS AND METHODS

8.2.1 PRE-MATING BEHAVIOUR OF G. PALLIDIPES COMPARED WITH G. MORSITANS

Eight males of G. pallidipes at various ages were introduced into cages containing 8 females aged 9-13 days. Individual ages were not observed but instead combined into groups. The pre-mating behaviour of the males was recorded by direct observation for a period of one hour. Observations for each age group were repeated three times with fresh males and females. Males of G. morsitans that were 3 and 7 days old were also introduced into cages containing females of the species. Their pre-mating behaviour was observed as described above.

8.2.2 DEVELOPMENT OF THE ARG

Males of G. pallidipes at various ages ranging 1 - 15 days were dissected and the diameter of the widest section of the ARG was measured. Fifteen flies per age group were dissected. Since the ARG branches into two, the mean of the two branches was taken in order to determine the growth in size of the gland as the fly matured.

8.2.3 INSEMINATING POTENTIAL AT VARIOUS AGES

Males were grouped into various age groups and were then introduced into female cages at ratio of 1:1. After a period of 72 hours, the males were separated from females and the MSVs of mated females were recorded after dissection. In this experiment, it was possible that one male could inseminate more than one female. This could not be avoided. To ensure insemination of females, it was considered desirable to leave the pairs together in the cages for up to 72 hours. However, in Chapter 6, it was reported that females of G. pallidipes did not re-mate and the conclusion was that if this happens in nature then it must be very rare. One can therefore assume that multiple matings are not likely to significantly affect the results.

8.3 RESULTS

8.3.1 PRE-MATING BEHAVIOUR OF G. PALLIDIPES COMPARED WITH THAT OF G. MORSITANS

(a) G.PALLIDIPES

Table 22 describes the pre-mating behaviour of males of different ages. The typical behaviour shown by males attempting to court at the age of 10 and above is as follows: the male jumped on the back of the female, attempted to make hypopygial connection, was rejected, and then flew away. This was repeated with the same or other females during group-mating (when several males were present) until one male finally succeeded. At other times, one or two males mated immediately without going through this 'elaborate courtship' when introduced. Immediate copulations were usually few.

After flying about in the cage for sometime both males and females would suddenly stop and become inactive. Sometimes, a male would start to fly, and this would appear to trigger mass flying of males and females. When the cage was disturbed by tapping or the sudden opening of doors, the flies would start to fly again. Males as a rule did not persist when rejected but the ability of some to persist increased with the age of the male (at older ages a few strongly persisting males were observed). Some males then succeeded in mounting females while others were rejected by females. This behaviour was repeated several times (i.e. alternate periods of passivity and activity).

TABLE 22

CHANGE IN MALE PRE-MATING BEHAVIOUR
OF *G. PALLIDIPES* WITH AGE

AGE OF MALE(DAYS)	BEHAVIOUR
2 - 3	No mating attempt within the first 10 minutes and thereafter
4 - 5	No mating attempts within the first 10 minutes and thereafter
6 - 7	No mating attempt within the first 10 minutes but very few weak attempts thereafter
8 - 9	No mating attempts within the first 10 minutes but few weak attempts and occasional strong ones when the cage was shaken
10 - 11	Still generally lethargic but several weak and occasional strong attempts made even when the cages were not shaken
12 - 13	Several strong attempts. Flies were generally active
14 - 15	Many aggressive strikes. Some flies showed strong persistence. The flies were generally active
> 15	Very aggressive. Many will dart around and strike a female and attempt to mount her.

Most males that were younger than 10 days old remained inactive in the cages and usually tried to mount only when the cage was shaken. Occasionally, one or two could be observe *in copula*. In general males were not observed as being keen to mount females.

(b) *G. MORSITANS*

Many males that were 3 days old were observed to strike. However, a few remained passive. Most of the active males moved swiftly. They darted across the cage siezed a female, sometimes in flight and immediately engaged genitalia. This behaviour was best observed in older males. With 7-day-old males, almost 100% of the males siezed any female they could see close by. It was usual to observe about 3 males all trying to mate with a single female. This kind of behaviour was completely absent in male *G. pallidipes*. When I tried to separate some of these males, they held tightly on and could not be easily separated. Their ability to persist was characteristic and sometimes even when rejected they finally succeeded in over-powering the female. They gave up only when rejection by the female was very strong. This happens when the female violently kicked in every direction and rolled over continuously.

8.3.2 DEVELOPMENT OF THE MALE ACCESSORY GLAND(ARG)

The ARG increased in diameter with increase in age (Figure 15). Growth was continued from day 2 to 3, after which it appeared to stop by day 5. Growth was then continued again and ceased at the age of 11 days. This age actually coincides with the age at which the males began to make strong mating strikes (Table 22). At this age, the mean diameter of the ARG was 0.17mm $\pm$ 0.01 SD (Table 23). It can be seen that by the time the males were 10 days old, the ARG diameter had reached 0.16mm; a reasonably mature size seen even in much older flies. However, less than 9 days of age, the ARG diameter appeared to be very small(0.13mm) and high degrees of insemination may not be achieved. The largest ARG diameter recorded was 0.20mm, but it seemed that many flies did not reach this level of development. The most common size was 0.16 - 0.17mm.

8.3.3 INSEMINATING POTENTIAL AT VARIOUS AGES

Table 24 shows that males that were 7 days old and above inseminated well; usually an average MSV of over half was recorded. At the age of 7 days, the MSV was low compared with flies that were over 7 days old. However, an analysis of variance test showed that there was no significant difference between the different age groups ($F = 1.26$, $P>0.05$). Increased willingness to mate reflected high inseminating potential in males; probably males less than 7 days old will not inseminate well because of the size of the ARG at this stage.

FIGURE 15

CHANGE IN DIAMETER OF ACCESSORY REPRODUCTIVE GLAND(ARG)
OF MALE G. PALLIDIPES WITH AGE

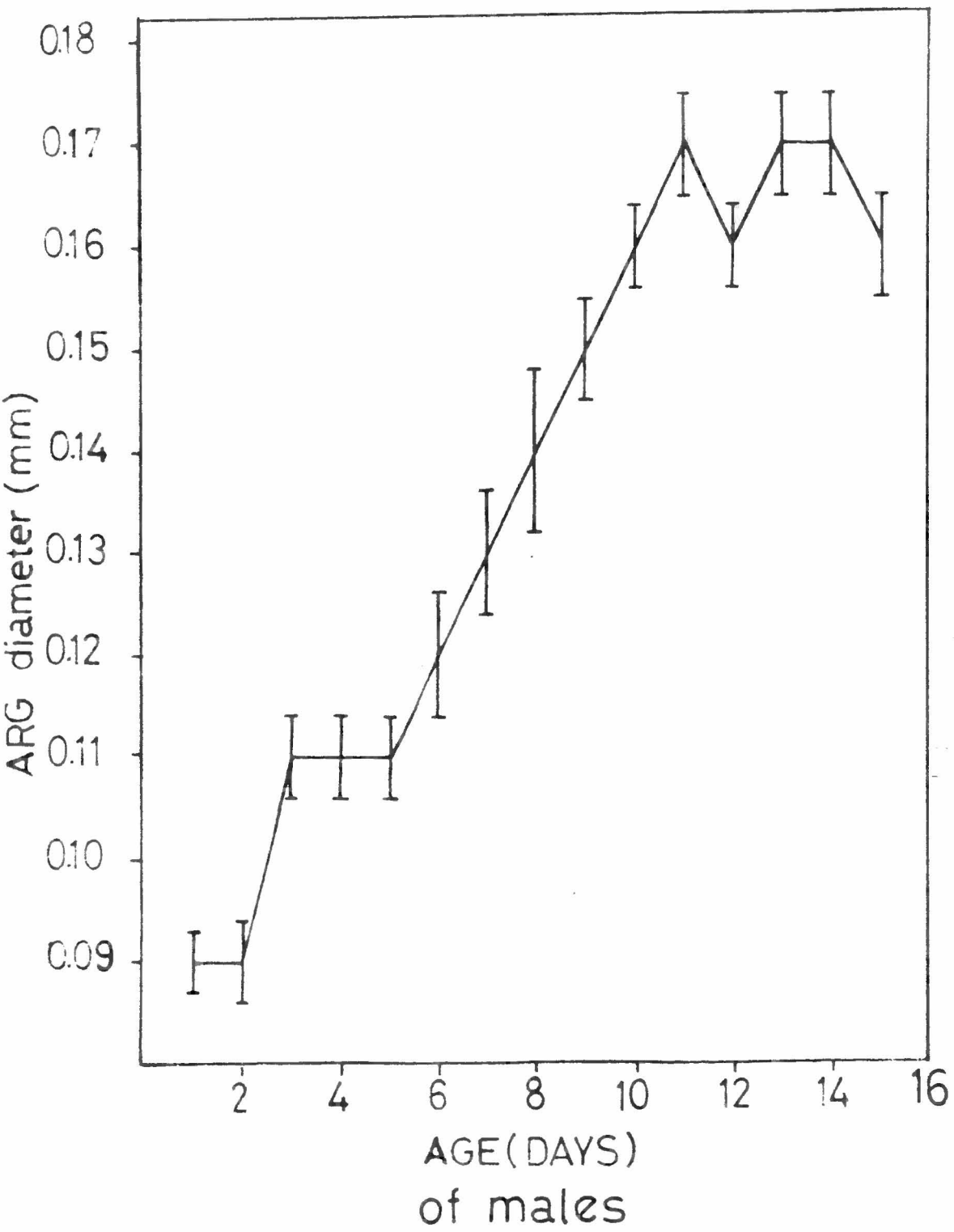

0.18
0.17
0.16
0.15
0.14
0.13
0.12
0.11
0.10
0.09
ARG diameter (mm)
2
4
6
8
10
12
14
16
AGE(DAYS)
of males

TABLE 23

CHANGE IN DIAMETER OF ACCESSORY REPRODUCTIVE GLANDS(ARG) IN VIRGIN MALES

AGE(DAYS) OF MALES	DIAMETER OF ARG(MM) MEAN ± SD
7	0.13 0.02
8	0.14 0.03
9	0.15 0.03
10	0.16 0.02
11	0.17 0.01
12	0.16 0.02
13	0.17 0.02
14	0.17 0.01
15	0.16 0.02

TABLE 24

INSEMINATING ABILITY OF MALE *G. PALLIDIPES*
(MEASURED AS MSV) AT VARIOUS AGES (DAYS)

AGE(DAYS) OF MALES	N	MSV MEAN ± SE
7	10	1.68 0.04
8 - 10	22	1.84 0.02
11 - 13	13	1.94 0.01
14 - 20	36	1.79 0.01

($F = 1.26, P > 0.05$)

N = NUMBER OF MALES DISSECTED

8.4 DISCUSSION

Males of G. pallidipes were less sexually active than males of G. morsitans. Once males of the right age (11 days old) were used for mating, high insemination rates were achieved. However, compared with G. morsitans, the observed behaviour of G. pallidipes did not show that zest or willingness to mate and the persistence so characteristic of G. morsitans. Wall (1988) also found that fundamental differences do exist in the mating activity of the two species. Wall showed that 60% of all interactions initiated by male G. pallidipes ended within the first minute, whereas, in G. morsitans only 11% of interactions did so. In this respect, males of different populations of G. pallidipes appear to show the same behaviour.

Males of G. pallidipes do not usually persist when rejected. In some other species of Glossina mating is usually immediate. Nash (1955) reported that the typical behaviour of G. palpalis when added to a mating cage containing females of the right mating age was that pairs appeared on the wall as the males were shaken out of their containers, 'but with such speed that the eye could not easily catch it happening'. The same behaviour was observed by Einyu and Kapaata (1983) who observed that G. m. centralis coupled immediately; the male usually made one attempt. They also found that 96% of 15-day-old males coupled immediately.

Probably, the aggressive behaviour seen in G. m. morsitans, G. m. centralis and G. palpalis explains why these species mate very easily in the laboratory. Sexual vigour of males is affected both by mean level of activity and its variability within strains (Kence and Byrant, 1978). It appears that species which mate easily take a much longer time in copula (about 1 - 3 hours). In Chapter 6, G. pallidipes was shown to take a short period in copula (usually less than 30 minutes). Short duration of copulation appears to have a relationship with less willingness to mate.

In terms of evolutionary advantage, a short duration of copulation seems to take into consideration the time needed for the sexes to come together. It is therefore understandable that since males of G. pallidipes waste time courting, when they do have the opportunity to mate, they only need to spend a much shorter time in copula. This has the advantage of freeing these males so that they could court other females, otherwise this species will be selected against. In this case, most females will have the opportunity of being inseminated before age makes them refractory. The selection of a mate of the correct species is therefore very important.

In female G. morsitans which can mate several times early in life, even if gametes are wasted at a first mating there is a possibility of a second mating. This will ensure high insemination rates among its females whereas this may not be so in G. pallidipes. However, in the laboratory, mating females with males over 10 days old should give high insemination rates and high degrees of insemination.

The onset of 'male appetitiveness' seems to correlate with the growth and development of the ARG. The ARG was observed to increase with age until about day 10 when it stopped (Figure 15). This age coincides with the age when males begin to become more active and court well. Jaenson (1978b) used males that were 10-12 days old but Langley (1989) used males that were 9-12 days old. Though the results obtained in this study showed that there was no significant difference in degree of insemination between ages 9 - 12, lack of sexual appetite could result in lower insemination rates. However, Langley (1989) obtained good results with his G. pallidipes which originated from Lugala in Uganda. This is probably another example of behavioural differences between different populations. On the other hand, the inactivity of his 9-day-old flies could have compensated for by his 11-12-day-old flies. Since multiple mating is a feature of males of this species also (Rogers, 1973a; Leegwater-van der Linden and Tiggelman, 1974), laboratory performance was not affected. Wall (1989b) has however rejected the idea of gross geographic differences in G. pallidipes mating behaviour but accepted the possibility of minor differences.

In conclusion, the results show that as male G. pallidipes aged their libido gradually increased, the maximum appetitiveness was being reached about the age of 14 days. Males apparently need to court females for a certain length of time before the female is ready. The time of sexual appetitiveness was observed to coincide

with maturity of the ARG. At this time high insemination rates can be achieved. These facts have to be observed in order to obtain high insemination rates as well as high degrees of insemination in the laboratory.

CHAPTER 9

GENERAL DISCUSSION

Refractory behaviour was investigated in G. m. morsitans. Sexual receptivity and male 'sexual appetitiveness' were also investigated in G. pallidipes in order to explain their apparent reluctance to mate in the laboratory. Females of many species of insects will not accept courtship advances after successful mating (Matthews and Matthews, 1978). In some species like Aedes aegypti, this refractory behaviour is temporary (Williams and Hagan, 1977), while in others, it lasts for life (Matthews and Matthews, 1978). What causes refractory behaviour in females of Glossina? The causes could be chemical or mechanical, or both. Whether it is only one or both factors responsible for refractory behaviour is not entirely clear for many insects, and this is so for Glossina.

In G. morsitans, receptivity declined after mating (Chapter 4). However, during the first few hours of mating about 60% of mated females remated. Perhaps this period is too short for the chemical or mechanical factor to register completely. Females may remate a number of times early in life, an evolutionary significance to increase the amount of sperm received at a less successful mating or to have another chance of being inseminated because the previous mating was with an abnormal male, or one which had exhausted its sperm due to repeated matings. Jordan (1958) also found that in

G. palpalis the occurrence of re-mating was more frequent among younger flies. Using a phenotype genetic marker Okra, it was possible for Kawooya (1977) to demonstrate that reinsemination could occur in G. m. morsitans when successful remating takes place.

Multiple mating early in life presents several advantages to those species that will lose their receptivity for the rest of their lives since sperm mixing can occur and new genes can also be exchanged. This happens in other dipterans too. For example, Richmond and Ehrman (1974) reported that in Drosophila paulistorum, sperm mixing might occur during remating. According to Davey (1985), if multiple matings occur before oviposition begins, mechanisms which ensure sperm mixing should be favoured. In males, the risk of subfertile mating is outweighed by the advantage of seizing every opportunity for a fertile one (Manning, 1966), whereas in females subfertile mating is disadvantageous as it can lead to refractoriness for the rest of the female's life.

The induction of refractoriness in G. m. morsitans was found to be mechanically activated (Chapter 4). Other factors known to affect receptivity in some species of dipterans, for example, the duration of copulation, degree of insemination or male accessory gland secretion did not show any effect (see Chapter 4). In some insects, for example, the housefly (Musca domestica), male ARG secretions inhibit re-mating by chemical means, mediated through the central nervous system; this is most often characterised by an active display of resistance by the female to a courting male (Leopold, 1976). In other insects, for example, Aedes aegypti, as the bursa

copulatrix fills with seminal fluid, an immediate switch off of receptivity occurs. This is probably due to mechanical stimulation also. However, Gillott and Langley (1981) suggested that both mechanical stimulation and ARG secretion acting together were responsible for the onset of refractoriness in G. m. morsitans. However, the present studies showed that this did not appear to be the case. Infact, it appeared that mechanical stimulation due to intense male jerking at the end of mating was the cause of refractoriness in G. morsitans. When there was no male jerking (that is, there was no sperm transferred though ARG secretion had been transferred) the flies remained receptive.

Further work needs to be done using different species of Glossina in order to confirm that the male ARG is not important in the onset of refractory behaviour. This is important, for example, in G. pallidipes where the present studies (Chapter 6) showed that this species completely lost its receptivity immediately after a first mating. In the German cockroach, Cochran (1979) found that the first mating provided adequate sperm for a female's entire life. However, Leegwater-van der Linden and Tiggleman (1984) reported observing females of the Uganda strain of G. pallidipes re-mating, though this was rare. Maybe the 'switch off' process is very effective in this species and should be further investigated; probably enough time was not given in the present study. This could shed more light on the onset of refractory behaviour in Glossina in general. More work also needs to be done in order to understand how this 'switch off' mechanism actually works; that is whether a chemical is released

or whether it is due only to the stimulation of the terminal setae or other stretch receptors present on the abdomen. Experiments with haemolymph from mated females injected into virgin females did not show that a chemical factor was released into it (Chapter 4); since haemolymph was obtained after decapitation of the mated females, dilution of the material could have taken place as decapitation causes salivary gland tubes, oesophagus and haemocoel to open and release salivary gland secretion, gut content etc. In G. pallidipes, Leegwater-van der Linden (1983) reported that accessory gland secretions did not have any effect on refractoriness. Thus, no receptivity inhibiting substance was found in the male ARG secretions in G. morsitans and it is concluded that male ARG secretions were not important in the onset of refractory behaviour.

The fact that it has been established that early in life multiple matings take place in Glossina sp. raises another pertinent question. Is it necessary for old females of Glossina to re-mate later in life? In Chapter 5, it has been shown that females did not re-mate later in life and therefore this will not affect the SIT. There are two spermathecae in Glossina sp; as is also the case in phlebotomus sp. (Diptera) but some nematoceran and most higher flies have three (Chapman, 1982). For example, in Culex and Tabanids there are three (Wigglesworth, 1972). Whether the number of spermathecae has any relationship to the number of eggs needed to be fertilised is not clear, but it can be easily appreciated why a mosquito such as Culex or Aedes would need to re-mate later in life.

In Culex, the production of a large number of eggs needs a good store of sperm for fertilisation, probably this is why Culex has three spermathecae. However, the production of only one larva every 10 days would need considerably less sperm, although fertilisation is usually a wasteful process as a large amount of sperm is released. Does this lead to sperm exhaustion? It has infact been shown that one mating was usually sufficient since the sperm remain alive for a long time because they are probably nursed nursed by secretions from cells that surround the lumen of the spermathecae (Jordan, 1972a and 1973). A second mating is therefore unnecessary later in life. On the other hand, males of G. morsitans can mate a number of times and it appears that only sperm exhaustion would prevent them from inseminating as they did not appear to lose their appetite for females even in their old age (Chapter 5).

Tsetse females of a number of species normally mate within a few days of emergence (Jordan, 1986; Langley, 1977), but G.pallidipes does not appear to readily mate in the laboratory when males and females are introduced together in mating vials or cages. Whether this behaviour is common in the field is not known. However, in the field ovarian abnormalities and insemination failure are rare, and abortion is the only major source of loss of fecundity (Turner and Snow, 1984). Langley (1989) puts it succinctly when he said that, "we have little idea of what constitutes normal mating behaviour in G. pallidipes". Langley and Hall (1984) reported

that in Zimbabwe, few G. pallidipes were seen to interact with decoys, and mating couples were not observed. The reason could be that they either do not readily mate in captivity or have a different mating strategy. The latter seems plausible. For example, Jaenson (1978b) showed that females were most receptive when they were 9 - 13 days old. This behaviour was found to be so in the present investigation, which is probably why Vale (cited by Langley, 1989) found a larger proportion of teneral G. pallidipes females uninseminated in the Zambezi Valley.

In G. pallidipes, the Nguruman strain was found not to be reluctant to mate in the laboratory as has been generally suggested for most strains of this species. This strain of G. pallidipes mated relatively easily when males and females of the right age were given sufficient time together, and other factors like group-mating and sex ratios (see Chapter 7 and 8) were taken into consideration. Under these conditions, over 90% of females could be inseminated. However, it should be noted that males and females of G. pallidipes do not immediately go in copula (as seen in G. m. morsitans) even though they may be of the right age.

The onset of receptivity in female G. pallidipes was found to be related to the maturation of the first egg (Chapter 6). It has been shown in other species of dipterans such as the housefly that mating was related to ovarian development (Adams and Hintz, 1969). According to Langley (1989), late mating could be of survival advantage if adult mortality is an important factor in the regulation of tsetse population numbers early in life. Mating at an old age

could be advantageous though this cannot be applied to G. morsitans which mate early and whose population is also regulated early in life by adult mortality (Langley, 1989). Though we still do not know much about the mating behaviour of G. pallidipes it nevertheless, is certain from the present investigation that G. pallidipes does mate in the laboratory when given the right conditions. Not all species of insects will readily mate even in the wild because an elaborate courtship display may be a pre-requisite. Perhaps, this apparent unwillingness to mate may be due to the fact that the females need to be adequately stimulated before they are pacified. What type of stimulation is required needs to be investigated in G. pallidipes.

Females of the Nguruman strain of G. pallidipes will mate when they are 9 - 13 days old and high insemination rates can be achieved if they are kept with males (about 12 days of age) for a period of about 72 hours. Perhaps, mating in the laboratory should be carried out with females that are slightly younger (7-8-day-old females) so that a cycle is not missed when mated at the time the first egg is already mature. Missing one cycle may seriously affect a colony such as G. pallidipes since the maximum possible reproductive capacity of the mated females will not be realised. At the age of 7 - 8 days, the eggs would be in the developing stage. Probably, this may not be so with some other strains of G. pallidipes, but more work needs to be carried out to determine determine this. According to Obata (1988), "mating refusal" in the ladybird beetle was due to incomplete ovarian development.

Barton-Browne *et al* (1980) also reported that some females of the blowfly, *Lucilia cuprina* whose ovarian development was greater than average failed to mate, whereas some with less than average ovarian development mated. In the field, the flies may not live for very long to realise their full reproductive potential; however, in the laboratory, it is necessary to have them reach the maximum possible reproductive potential especially for a fly like *G. pallidipes* which is well noted for its low reproduction rate in the laboratory (Jaenson, 1978b). Though the females may be less willing to mate at the age of 7 days, keeping them with males for 3 days when they would be 9 days old has been shown to result in high insemination rates.

There are various other factors such as, group-mating or single mating, the lighting conditions in the mating room, the size of the cage and the length of time males and females are kept together, which could also affect the sexual aggressiveness and receptivity of males and females respectively in the laboratory. These factors could cause high or low insemination rates depending on their suitability and this is a reflection of high or low receptivity in females or high sexual appetite in males. The results discussed in Chapter 7 showed that some of these factors like cage size and length of time the flies were kept together were important, while others like the lighting conditions in the insectary were not necessary. The number of flies per cage could also affect receptivity, however, this factor was not examined in detail in the present investigation. The effect of the size of cage was examined, though only two sizes of cages were

compared; this showed that the small cage was more effective than the large cage. Various cage sizes should be compared in order to conclusively determine whether a bigger space is needed by G. pallidipes for mating. However, a small sized cage is usually preferable because it is easy to use for feeding tsetse. A sex ratio of 1:1 was used in most of the present investigation, but it needs to be investigated whether other sex ratios could be used to get the same insemination rates obtained in a 1:1 ratio.

The effect of photoperiod (L D 12:12 and continuous darkness) on receptivity was also examined, though not in great detail. No significant difference was found between flies that had been kept in L D 12:12 or in continuous darkness. Future studies should also examine the effect of different light intensities on sexual receptivity. This is more so because of the crepuscular nature of G. pallidipes, Willett (1953). Van Etten (1982) reported that G. pallidipes from Nguruman, especially males were usually active in late afternoon.

Males of G. pallidipes did not usually persist when courting but preferred to strike, disappear and come back (Chapter 8). Very old males (over 40 days old) were not observed in this study but for mass rearing and in order to fully understand their population dynamics, more experiments with old males would prove of value. It is however certain that males of G. pallidipes though appearing less willing to court usually succeeded in mating if given the opportunity to remain with females for a period of about 72 hours during group-mating. It is suggested that 'male coyness' develops in the case of single matings. When a male is constantly rejected

it may become 'shy' and refuse to strike. However, males may succeed with other females if given the opportunity which is provided by group-mating. Better results will probably be achieved with males from 14 days of age instead of the usual 12-day-old male. The use of 9-day-old males with success by Langley (1979) could be due to different behavioural mechanisms in different populations, or probably, due to the effect of the younger males being outweighed by the 12-day-old males.

One reason among others given for the failure of some laboratory colonies of G. pallidipes is the presence of the tsetse virus. Since this virus may be transmitted from mother to progeny, problems such as reduced insemination rates, fecundity, life-span and sex ratio distortion could result (Jaenson, 1986).

What will probably be needed is to follow the development of mated females in a laboratory rearing facility to see whether they develop infections since Jaenson (1978c and 1986) had reported that virus-like particles occured in hyperplastic salivary glands of field and laboratory bred G. pallidipes, and that infected males were completely sterile, and females showed abnormal growth of the ovarioles. Different strategies would have to be planned and studied to take care of probable abortions in mated females. Experimental evidence of whether failure to obtain viable colonies of G. pallidipes is due to virus infection has not been documented; this is an area for further studies.

For several years the Nguruman strain of *G. pallidipes* has not yielded to successful colonisation. Several workers, especially those at the ICIPE have tried to rear this species unsuccessfully in a small scale for experimental purposes. This is apparently the very first time that a small scale rearing method has yielded reasonable numbers of flies for experimental purposes. The only modification to previous methods which yielded this successful result was the provision of feeding opportunities every day except on Sunday and also patiently making sure that most of the flies in a cage fed. It occasionally took about 30 minutes to feed a cage of flies. It would appear that with this species, a lot of patience and care are necessary for successful results compared with species such as *G. morsitans*. Any laxity will probably lead to the collapse of the colony. The microclimatic conditions in the insectary were also closely monitored so that the temperature never fluctuated widely and was always about 25° ± 1°C; relative humidity varied between 75 - 80%. Very wide fluctuations were avoided at all times. From the results obtained in Chapters 6-8, it is suggested that the Nguruman strain of *G. pallidipes* may not be very difficult to mass produce.

CHAPTER 10

GENERAL CONCLUSIONS

1. Some aspects of the mating behaviour of G. morsitans and G. pallidipes were investigated in the laboratory.

2. The literature was reviewed and relevant studies were extensively quoted.

(a) G. MORSITANS:

3. There was a tendency of young females of G. morsitans to re-mate soon after a first mating usually within a 24-hour period, after which receptivity diminished rapidly.

4. The duration of copulation, degree of insemination of the spermathecae, male accessory gland secretion and haemolymph of mated females did not determine a female's unwillingness to re-mate.

5. Mechanical stimulation caused by vigorous movements during the 'male jerking phase' however caused a significant decline in receptivity.

6. One mating was usually sufficient to provide the complement of sperm needed for larval production throughout a female's life. The sperm complement was usually not exhausted and therefore females did not re-mate later in life to replenish it. The SIT will not be affected.

7. Males did not lose their appetite for females nor did they fail to inseminate at very old ages (failure to inseminate at old ages could only be due to sperm exhaustion caused by repeated matings). However, the SIT will not be affected.

(b) G. PALLIDIPES:

8. In G. pallidipes, females were highly receptive when 9-13 days old and high receptivity coincided with maturity of the first egg.

9. The duration of copulation was short overall, usually it took between 19 and 28 minutes. There was no significant difference between duration of copulation of female flies aged 6 to 18 days.

10. The degree of insemination was high in females aged 6 - 13 but dropped at older ages.

11. Females once mated rarely re-mated immediately.

12. Ovulation occurred in virgin females but was not a common occurrence until when they were very old (after 30 days of age).

13. Generally, ovulation was accelerated by mating and within 24 hours, 60% of females that were over 8 days old had ovulated.

14. Group-mating gave better results than single-mating and very high insemination rates were achieved.

15. High insemination rates were also achieved when pairs were kept together for 72 hours rather than 24 hours.

16. Use of small cages (standard PVC ICIPE Laboratory/holding cages) gave high insemination rates than large cages and had the added advantage of being easy to handle.

17. There was no significant difference between pairs kept in continuous darkness and those kept in a regime of L D 12:12 for 72 hours.

18. Male G. pallidipes were less persistent than those of G. morsitans in courting females; however, aggressiveness and persistence in G. pallidipes increased with age.

19. Generally, male G. pallidipes over 12 days of age were more active and persistent; persistence and aggressiveness were related to the development of the male accessory reproductive gland.

20. There was no significant difference between the degree of insemination in female G. pallidipes mated to males that were 7 - 15 days old.

21. It is expected that in rearing G. pallidipes the careful observation of these findings should lead to a measure of success.

R E F E R E N C E S

REFERENCES

Adams, T S and Hintz, A M (1969). Relationship of ovarian development, and the corpus allatum to mating in the housefly, Musca domestica. J. Insect Physiol.15, 201-215.

Adams, T S and Mulla, M S (1968). Ovarian development, pheromone production, and mating in the eye gnat, Hippolates collusor. J. Insect Physiol.14, 627-635.

Adams, T S and Nelson, D R (1968). Bioassay of crude extracts for the factor that prevents second matings in female Musca domestica. Annals of the Entomological Society of America 61, 113-116.

Azevedo, J F de and Pinhao, R D (1964). The maintenance of a laboratory colony of Glossina morsitans since 1959. Bull. Wld. Hlth Org. 31, 835-841.

Barton-Browne, L, Von Gerwen, A C M and Bartell, R J (1980). Effects of the ingestion of components of a protein-rich diet on the sexual receptivity of female of the Australian sheep blowfly, Lucilia cuprina. Physiological Entomology 5, 1-6.

Barton-Browne, L and Von Gerwen, A C M (1988). The effect of prior housing conditions on the sexual receptivity of females of the blowfly, Lucilia cuprina. Journal of Insect Behaviour 1, 187-196.

Bastock, M and Manning, A (1955). The courtship of Drosophila melanogaster. Journal of Insect Behaviour 8, 85-111.

Brightwell, R and Dransfield, R D (1986). Tsetse population manipulation. In Fourteenth Annual Report, The International Centre of Insect Physiology and Ecology. pp.60-61.

Carlson, D A and Langley, P A (1983). Sex pheromones in Glossina pallidipes. OAU/STRC 17th Meeting ISCTRC, Arusha, Tanzania, 1981, 431-440.

Carlson, D A, Langley, P A and Huyton, P (1978). Sex pheromone of the tsetse fly: isolation, identification, and synthesis of control aphrodisiacs. Science 201, 750-753.

Challier, A (1977). Trapping technology. In Tsetse: the future for biological methods in integrated control (Ed. by M Laird) pp.109-123, IDRC, Ottawa.

Challier, A (1982). The ecology of tsetse (Glossina spp.) (Diptera, Glossinidae): a review (1970-1981). Insect Science and Appl.3, 97-143.

Chambers, D L (1977). Quality control in mass rearing. Ann. Rev. Entomol.22, 289-308.

Chapman, R F (1982). The Insects: Structure and Function. Macmillan, India Ltd.

Charlwood, J D and Jones, M D R (1980). Mating behaviour in the mosquito, Anopheles gambiae s.l. II. Swarming behaviour. Physiological Entomology 5, 315-320.

Chaudhury, M F B (1988). The role of nutritional and hormonal factors on sexual maturation and behaviour in G. pallidipes. In Sixteenth Annual Report, The International Centre of Insect Physiology and Ecology, pp.56-57.

Chaudhury, M F B and Ball, H J (1974). Effect of age and time of day on sex attraction and mating of the face fly, Musca autumnalis. J. Insect Physiol. 20, 2079-2085.

Chaudhury, M F B, Dransfield, R D and Golder, T K (1984). Reproductive Biology of Glossina pallidipes. In Twelfth Annual Report, The International Centre of Insect Physiology and Ecology. pp.43-44.

Cheng, T C (1973). General Parasitology. Academic Press. New York.

Coates, T W and Langley, P A (1982). The causes of mating abstention in male tsetse flies Glossina morsitans. Physiological Entomology 7, 235-242.

Cochran, D G (1979). A genetic determination of insemination frequency and sperm precedence in the german cockroach. Ent. exp. appl. 26, 259-266.

Craig, G B (1967). Mosquitoes: Female monogamy induced by male accessory gland substance. Science 156, 1499-1501.

Curtis, C F (1968a). Some observations on reproduction and the effects of radiation on Glossina austeni. Trans. R. Soc. Trop. Med. Hyg.62, 123-124.

Curtis, C F (1968b). Radiation sterilization and the effect of multiple mating of females in Glossina austeni. J. Insect Physiol.14, 1365-1380.

Curtis, C F and Jordan, A M (1968). Calculations of the productivity of Glossina austeni Newst. maintained on goats and on lop-eared rabbits. Bull. ent. Res.59, 651-658.

Curtis, C F and Jordan, A M (1970). Calculations of the productivity of Glossina austeni Newst. maintained on goats and on lop-eared rabbits. Bull. ent. Res. 59, 651-658.

Dame, D A and Ford, H R (1968). Multiple mating of Glossina morsitans Westw. and its potential effect on the sterile male technique. Bull. ent. Res. 58, 213-219.

Dame, D A and Schmidt, C H (1970). The sterile-male technique against tsetse flies, Glossina spp. Bulletin of the Entomological Society of America 16, 24-30.

Davy, K G (1985). The female reproductive tract. In Comprehensive Insect Physiology Biochemistry and Pharmacology, 1 (edited by G A Kerkut and L I Gilbert), pp.15-36. Pergamon Press, Oxford.

Dean, G.J.W, Paget, J. and Williamson, B.R (1968). Survival and reproduction of Glossina morsitans Westw. in different types of cages exposed to variable and constant climatic conditions. Bull. ent. Res. 58, 773-785.

de Wilde, J and de Loof, A (1973). Reproduction - Endocrine Control. In The Physiology of Insecta (Edited by M Rockstein), 97-157.

Einya, P and Kapaata, R M (1983). Mating behaviour in relation to sexual maturation of male Glossina morsitans centralis (Machado, 1970) (Diptera: Glossinidae). Insect Sci. Application 4, 345-349.

Foster, M A (1976). Male sexual maturation of the tsetse flies Glossina morsitans Westwood and G. austeni Newst. (Dipt., Glossinidae) in relation to blood feeding. Bull. ent. Res. 66, 389-399.

Gillott, C and Langley, P A (1981). The control of receptivity and ovulation in the tsetse fly, Glossina morsitans. Physiological Entomology 6, 269-281.

Huyton, P M and Langley, P A (1982). Copulatory behaviour of the tsetse flies Glossina morsitans and G. austeni. Physiological Entomology 7, 167-174.

Huyton, P M, Langley, P A, Carlson, D A and Coates, T M (1980a). The role of sex pheromones in initiation of copulatory behaviour by male tsetse flies, Glossina morsitans morsitans. Physiological Entomology 5, 243-252.

Huyton, P M, Langley, P A, Carlson, D A and Schwarz, M (1980b). Specificity of contact sex pheromones in tsetse flies, Glossina spp. Physiological Entomology 5, 253-264.

Jaenson, T G T (1978a). Mating behaviour of Glossina pallidipes Austen (Diptera, Glossinidae): Genetic differences in copulation time between allopatric populations. Ent. exp. appl. 24, 101-108.

Jaenson, T G T (1978b). Reproductive biology of the tsetse Glossina pallidipes Austen (Diptera, Glossinidae) with special reference to mating behaviour. Ph.D Thesis University of Upsala.

Jaenson, T G T (1978c). Virus like rods associated with salivary gland hyperplasia in tsetse, Glossina pallidipes. Trans. R. Soc. trop. Med. Hyg. 72, 234-238.

Jaenson, T G T (1979a). Mating behaviour of males of Glossina pallidipes Austen (Diptera: Glossinidae). Bull.ent. Res. 69, 573-588.

Jaenson, T G T (1979b). Mating behaviour of Glossina pallidipes (Diptera, Glossinidae): Duration of copulation, insemination and fecundity. Ent. exp. appl. 26, 1-12.

Jaenson, T G T (1980). Mating behaviour of females of Glossina pallidipes Austen (Diptera: Glossinidae). Bull. ent. Res. 70, 49-60.

Jaenson, T G T (1986). Sex ratio distortion and reduced life span of Glossina pallidipes infected with the virus causing salivary gland hyperplasia. Ent. exp. appl. 41, 265-271.

Jordan, A M (1958). The mating behaviour of females of Glossina palpalis (R-D) in captivity. Bull. ent. Res. 49, 35-43.

Jordan, A M (1972a). Extracellular ducts within the cells of the spermatheca of tsetse flies (Glossina spp.). Dipt., Glossinidae. Bull. ent. Res. 61, 669-672.

Jordan, A M (1972b). Inseminating potential of male Glossina austeni Newst. and G. morsitans morsitans Westw. (Dipt., Glossinidae). Bull. ent. Res. 62, 319-325.

Jordan, A M (1973). Observations on the spermatheca of tsetse flies. Trans. R. Soc. trop. Med. Hyg. 67, 298.

Jordan, A M (1985). Tsetse eradication plans for Southern Africa. Parasitology Today 1, 121-123.

Jordan, A M (1986). Trypanosomiasis Control and African Rural Development. Longman, London.

Jordan, A M and Curtis, C F (1968a). Productivity of Glossina austeni Newst. maintained on lop-eared rabbits. Bull. ent. Res.58, 399-410.

Jordan, A M and Curtis, C F (1968b). The performance of Glossina austeni when fed on lop-eared rabbits and goats. Trans. R. Soc. trop. Med. Hyg. 62, 123-124.

Kangwagye, T N (1971). Observations on Glossina fuscipes Newstead, G. fuscipleuris Austen and G. pallidipes Austen in Western Uganda. In International Scientific Council for Trypanosomiasis Research, Thirteenth Meeting, Lagos, 1971. pp.187-191. Organisation of African Unity/Scientific and Technical Research Commission (Publication No. 105).

Kawooya, J (1977). Demonstration of multiple insemination in females of Glossina morsitans Westwood, using a phenotype genetic marker Ocra. Z. angew. Ent. 84, 321-327.

Kence, A and Byrant, E H (1978). A model of mating behaviour in flies. The American Naturalist 112, 1047-1062.

Langley, P A (1974). Sex recognition in tsetse flies. In Actes du colloque sur les moyens de Lutte contre les Trypanosomes et leurs vecteurs, Paris 12-15 Mars 1974. Revue elev. med. vet. Pays trop. Suppl., 93-95.

Langley, P A (1977). Physiology of tsetse flies (Glossina spp.) (Diptera: Glossinidae): a review. Bull. ent. Res. 67, 523-574.

Langley, P A (1989). Laboratory colonisation of the tsetse fly Glossina pallidipes Austen (Diptera: Glossinidae) using an in vitro feeding method. Bull. ent. Res. 79, 429-435.

Langley, P A, Coates, T W and Carlson, D A (1982). Sex recognition pheromone in the tsetse fly Glossina pallidipes Austen Experentia 38, 473-474.

Langley, P A and Hall, M J R (1984). Aspects of the regulation of mating behaviour in tsetse. Insect Sci. Applic. 5, 351-355.

Langley, P A, Pimley, R W and Carlson D A (1975). Sex recognition pheromone in tsetse fly Glossina morsitans. Nature 254, 51-52.

Lapin, L (1980). Statistics: Meaning and Method. Harcourt Brace Jovanovich, New York.

Leegwater-van der Linden, M E (1980). Recent advances in the rearing of Glossina pallidipes Austen. In Isotope and Radiation Research on Animal Diseases and their vectors, International Atomic Energy Agency, Vienna. pp.23-33.

Leegwater-van der Linden, M E (1981). The effect of a four-day-a-week feeding regimen versus daily feeding on the reproduction of Glossina pallidipes. Ent. exp. appl. 29, 169-176.

Leegwater-van der Linden, M E (1982). Receptivity, Ovulation and larviproduction in Glossina pallidipes, related to mating at various ages. Ent. exp. appl. 32, 91-98.

Leegwater-van der Linden, M E (1983). The rearing of the tsetse fly Glossina pallidipes Austen. Ph.D thesis, University of Amsterdam.

Leegwater-van der Linden, M E and Tiggelman, E P M (1984). Multiple mating and inseminating potential of Glossina pallidipes. Ent. exp. appl. 35, 283-294.

Leopold, R A (1976). The role of male accessory glands in insect reproduction. Ann Rev. Entomol.21, 199-221.

Lumsden, W H R and Saunders, D S (1966). Tsetse flies. In Insect colonization and mass production (edited by C N Smith), pp.153-174. Academic Press Inc. (London) Ltd.

Madhubunyi, L C (1988). Performance of the tsetse, Glossina morsitans morsitans reared under various feeding regimens in Zambia. Ent. exp. appl. 48, 3-8.

Manning, A (1966). Sexual behaviour. In Symposia of the Royal Entomological Society of London No.3 (edited by P T Haskell), pp.59-68.

Manning, A (1967). The control of sexual receptivity in female Drosophila. Anim. Behav. 15, 239-250.

Matthews, R W and Matthews, J R (1978). Insect Behaviour. John Wiley, New York.

Mellanby, H (1937). Experimental work on reproduction in the tsetse fly Glossina palpalis. Parasitology 29, 131-141.

Mellanby, H and Mellanby, K (1937). Rearing tsetse flies in captivity. Proc. R. Entomol. Soc. London Ser. A 12, 1-3.

Mellanby, K (1936). Experimental work with the tsetse fly, Glossina palpalis, in Uganda. Bull. ent. Res. 27, 611-633.

Morrison, P E, Venkatesh, K and Thompson, B (1982). The role of male accessory gland substance on female reproduction with some observations of spermatogenesis in the stable fly. J. Insect Physiol.28, 607-614.

Mulder, J (1989). Control of the tsetse fly in Africa and the environment. The courier 11, 11-12.

Nash, T A M (1955). The fertilisation of Glossina palpalis in captivity. Bull. ent. Res. 46, 357-368.

Nash, T A M (1968). The rearing of tsetse flies (Glossina spp) at Langford, near Bristol. Trans. R. Soc. trop. Med. Hyg.62, 122-123.

Nash, T A M and Jordan, A M (1976). Tsetse flies. In The Ufaw Handbook on the care and management of laboratory animals pp.570 - 580. Churchill Livingstone Press, London.

Nash, T A M, Jordan, A M and Boyle, J A (1966a). A promising method for rearing Glossina austeni (Newst.) on a small scale based on the use of rabbits' ears for feeding. Trans. R. Soc. trop. Med. Hyg. 60, 183-188.

Nash, T A M, Jordan, A M and Boyle, J A (1966b). The large-scale rearing of Glossina austeni (Newst.) in the laboratory III - confirmation of the value of pregnant goats as hosts. Ann. trop. med. parasit. 60, 469-481.

Nash, T A M, Jordan, A M and Boyle, J A (1968). The large-scale rearing of Glossina austeni Newst. in the laboratory IV - The final technique. Ann. trop. med. parasit., 62, 336-341.

Nash, T A M and Kernaghan, R J (1965). A high proportion of early deaths among uninseminated females of Glossina austeni Newst. Bull. ent. Res. 56, 65-66.

Obata, S (1988). Mating refusal and its significance in females of the ladybird beetle, Harmonia axyridis. Physiological Entomology 13, 193-199.

Ochieng, R S, Otieno, L H and Banda, H K (1987). Performance of the tsetse fly Glossina pallidipes reared under simple laboratory conditions. Ent. exp. appl. 45, 265-270.

Odhiambo, T R (1968). Influence of age and feeding on the success of mating in a tsetse fly species. Nature 219, 962-963.

Odhiambo, T R (1971). The regulation of ovulation in the tsetse fly, Glossina pallidipes Austen. J. exp. Zool.177, 447-454.

Owaga, M L A (1981). Ecological studies and laboratory rearing of the tsetse species Glossina longipennis Corti in Kenya. Insect Sci. Application 2, 197-200.

Parker, R E (1979). Introductory statistics for Biology - (second edition). Edward Arnold, London.

Peters, T M and Barbosa, P (1979). Influence of population density on size, fecundity and developmental rate of insects in culture. Ann. Rev. Entomol.22, 431-450.

Phelps, R J and Vale, G A (1978). Studies on populations of Glossina morsitans morsitans and G. pallidipes (Diptera: Glossinidae) in Rhodesia. J. appl. Ent. 15, 743-760.

Pinhao, R C and Gracio, A J S (1973). The degree of spermathecal impregnation and the number of matings in Glossina austeni. An Inst. Hyg. Med. Trop. Lisbon 1, 103-106.

Pollock, J N (1970). Sperm transfer by spermatophores in Glossina austeni Newstead. Nature 225, 1063-1064.

Pollock, J N (1974). Male accessory secretions, their use and replenishment in Glossina (Diptera, Glossinidae). Bull. ent. Res. 64, 533-539.

Potts, W H (1970). Systematics and identification of Glossina. In The African Trypanosomiases. (Edited by H W Mulligan), pp.243-272. George Allen and Unwin Ltd., London.

Richmond, R C and Ehrman, L (1974). The incidence of repeated mating in the superspecies, Drosophila panlistorum. Experentia 30, 489-490.

Riemann, J G, Moen, D J and Thorson, B J (1967). Female monogamy and its control in houseflies. Insect Physiol. 13, 407-418.

Riemann, J G and Thorson, B J (1969). Effect of male accessory material on oviposition and mating by female houseflies. Annals of the Entomological Society of America 62, 828-834.

Rogers, A (1972). Studies on the mating of Glossina pallidipes Austen 1: the age of mating. Annals of Tropical Medicine and Parasitology 66; 515-523.

Rogers, A (1973a). Multiple mating by male Glossina pallidipes in the laboratory. Trans. R. Soc. trop. Med. Hyg. 67, 298-299.

Rogers, A (1973b). Duration of mating in Glossina pallidipes. Trans. R. Soc. trop. med. Hyg.67, 441-442.

Rogers, A (1973c). A method for gauging the frequency of multiple mating of female Glossina pallidipes in the field. Trans. R. Soc. trop. med. Hyg. 67, 299.

Rogers, A and Kinyanjui, E N F (1972). The maintenance of a colony of Glossina pallidipes Austen. Annals of Tropical Medicine and Parasitology 66, 267-280.

Romoser, W S (1973). The Science of Entomology. Macmillan Publishing Co., Inc. New York.

Samaranayaka-Ramasamy, M (1981). Influence of feeding and hormonal factors on sexual maturation in male Glossina morsitans morsitans Westw. (Diptera: Glossinidae). Insect Sci Application 1, 273-280.

Saunders, D S (1961). Studies on ovarian development in tsetse flies (Glossina, Diptera). Parasitology 51, 545-564.

Saunders, D S and Dodd, C W (1972). Mating, insemination and ovulation in the tsetse fly. Glossina morsitans. J. Insect Physiol. 18, 187-198.

Schlein, Y, Galun, R and Ben-Eliahu, M N (1981a). Abstinons. Male produced deterrents of mating in flies. J.chem. ecol.7, 285-291.

Schlein, Y, Galun, R and Ben-Eliahu, M N (1981b). Receptors of sex pheromones and abstinons in Musca domestica and Glossina morsitans. Journal of Chemical Ecology 7, 291-303.

Service, M W (1980). A Guide to Medical Entomology. Macmillan Press Ltd., London.

Smith, J M (1956). Fertility, mating behaviour and sexual selection in Drosophila subobscura. J. Genet. 54, 261-279.

Snow, W F (1980). Tsetse ecology on the Kenya Coast. In Eighth Annual Report, The International Centre of Insect Physiology and Ecology - 1979, pp.107-108.

Sohal, R S (1985). Aging in insects. Comparative Insect Physiology, Biochemistry and Pharmacology 10, 595-630.

Spielberger, U, Na'isa, B K, Koch, K, Manno, A, Skidmore, P R and Coutts, H H (1979). Field trials with the synthetic pyrethroids permethrin, cypermethrin and decamethrin against Glossina (Diptera: Glossinidae) in Nigeria. Bull. ent. Res. 69, 667-689.

Spieth, H T (1974). Courtship behaviour in Drosophila. Ann. Rev. Entomol. 19, 385-406.

Takken, W, Oladunmade, M A, Dengwat, L, Feldmann, H U, Onah, J A, Tenabe, S O and Hamann, H J (1986). The eradication of Glossina palpalis palpalis (Robineau-Desvoidy) (Diptera: Glossinidae) using traps, insecticide-impregnated targets and the sterile insect technique in central Nigeria. Bull. ent. Res. 76, 275-286.

Tobe, S S and Langley, P A (1978). Reproductive physiology of Glossina. Ann. Rev. Entomol. 23, 283-307.

Tschinkel, W R (1985). Behaviour and Physiology. In Fundamentals of Insect Physiology (Edited by M S Blum), pp.391-435. John Wiley and Sons, New York.

Turner, D A and Snow, W F (1984). Reproductive abnormality and loss in natural populations of Glossina pallidipes Austen (Diptera: Glossinidae) in Kenya. Bull. ent. Res. 74, 299-309.

Van Der Vloedt, A M V, Baldry, D A T, Politzar, H, Kalzwer, H and Cuisance, D (1980). Experimental helicopter applications of decamethrin followed by release of sterile males for the control of riverine vectors of trypanosomiasis in Upper Volta - Insect Sci Application 1, 105-112.

Van Etten J (1981). A comparison of the performance of laboratory colonies of Glossina pallidipes Austen from two allopatric populations in Kenya. Insect Sci. Application 1, 177-183.

Van Etten J (1982). Comparative studies on the diurnal activity pattern in two field and laboratory populations of Glossina pallidipes. Ent. exp. appl. 32, 38-45.

Von Borstel, R C (1960). Population control by release of irradiated males. Science 131, 878-882.

Wall, R (1988). Analysis of the mating activity of male tsetse flies Glossina m. morsitans and G. pallidipes in the laboratory. Physiological Entomology 13, 103-110.

Wall, R (1989a). The roles of vision and olfaction in mate location by males of the tsetse fly Glossina morsitans morsitans. Medical and veterinary Entomology 3, 147-152.

Wall, R (1989b). Ovulation, insemination and mating in the tsetse fly Glossina pallidipes. Physiological Entomology 14, 475-484.

WHO Expert Committee (1986). Epidemiology and Control of African Trypanosomiasis. Technical Report Series 739, World Health Organization, Geneva.

Wigglesworth, V B (1972). The Principles of Insect Physiology. Chapman and Hall, London.

Willett, K C (1953). The laboratory maintenance of Glossina. Parasitology 43, 110-130.

Williams, R W and Berger, A (1980). The relation of female polygamy to gonotrophic activity in the rock strain of Aedes aegypti. Mosquito News 40, 597-604.

Williams, R W and Hagan, N K B (1977). Efficiency of a single insemination in preventing a second in the rock strain of the mosquito, Aedes aegypti. J. Insect Physiol. 23, 1205-1207.

Young, A D M and Downe, A E R (1982). Renewal of sexual receptivity in mated female mosquitoes, Aedes aegypti. Physiological Entomology 7, 467-471.

Young, A D M and Downe, A E R (1983). The influence of mating on sexual receptivity and oviposition in the mosquito, Culex tarsalis. Physiological Entomology 8, 213-217.

Young, A D M and Downe, A E R (1987). Male accessory gland substances and the control of sexual receptivity in female Culex tarsalis. Physiological Entomology 12, 233-239.

A P P E N D I X

APPENDIX 1

REPLENISHMENT OF MALE ACCESSORY REPRODUCTIVE GLAND (ARG) IN *G. MORSITANS* AFTER MATING

Time (Hours)	ARG Diameter (mm)		
	Mean	±	SD
Unmated (control)	0.17		0.02
0	0.12		0.01
4	0.13		0.02
24	0.14		0.02
48	0.16		0.01
72	0.17		0.02
96	0.17		0.01

APPENDIX 2

EFFECT OF DURATION OF COPULATION ON RECEPTIVITY

No. of times females were willing to mate	Duration of Copulation (Hours) 1 -2	2 - 3	3 - 4	>4
Once	17(53.1%)	23(62.2%)	23(67.6%)	9(69.2%)
Twice	15(46.9%)	14(37.8%)	11(32.4%)	4(30.8%)
Total No of flies	32	37	34	13

APPENDIX 3

EFFECT OF DURATION OF COPULATION ON RECEPTIVITY

(FEMALES WERE GIVEN THE OPPORTUNITY TO MATE FOR 50, 60 AND 80 MINUTES BEFORE SEPARATION)

Duration of Copulation (Minutes)	No. of females and percentage willing to mate as indicated			
	Once	%	Twice	%
50	6	14	38	86
60	2	9	20	91
80	1	5	20	95

APPENDIX 4

EFFECT OF A SECOND MATING ON THE DEGREE OF INSEMINATION

(REMATING WAS WITH 7-10-DAY OLD MALES)

MSV	No. of females inseminated when mated once	%	No. of females inseminated when mated twice (immediately after the first)	%	No. of females inseminated when mated twice (24 hours after)	%
I	3	3	2	3	0	0
II	0	0	3	5	0	0
III	3	3	12	18	0	0
IV	3	3	15	23	0	0
V	61	63	27	41	11	34
VI	27	28	7	11	21	66
	97		66		32	

APPENDIX 5

EFFECT OF SECOND MATING ON THE
DEGREE OF INSEMINATION

(3-DAY OLD ♀♀ WERE MATED WITH 3-DAY OLD ♂♂)
(TWICE MATED FEMALES WERE REMATED WITH 7-10 DAY OLD MALES)

MSV	No. of females inseminated when mated once	%	No. of females inseminated when mated twice	%
I	18	38	1	4
II	1	2	0	0
III	12	25	2	8
IV	12	25	0	0
V	3	6	3	13
VI	2	4	18	75
	48		24	

APPENDIX 6

EFFECT OF DEGREE OF INSEMINATION
ON RECEPTIVITY

(5-10 DAY OLD MALES WERE MATED WITH
3-DAY OLD FEMALES)

MSV	No. of females willing to mate once	%	No. of females willing to mate twice	%
I	0	0	1	2
II	0	0	0	0
III	3	4	3	5
IV	1	1	1	2
V	63	85	49	84
VI	7	10	4	7
	74		58	

APPENDIX 7

OVARIAN DEVELOPMENT OF OVARIOLE A_1 IN FEMALE
G. PALLIDIPES

AGE(DAYS)	LENGTH OF OVARIOLE(MM) MEAN	±	SE
1	0.52		0.01
2	0.56		0.03
3	0.62		0.06
4	0.74		0.04
5	0.98		0.06
6	1.21		0.10
7	1.47		0.07
8	1.29		0.11
9	1.52		0.07
10	1.62		0.02
11	1.66		0.03
12	1.63		0.02
13	1.64		0.02
14	1.62		0.02
15	1.68		0.03

n = 15 for each age

APPENDIX 8

OVARIAN DEVELOPMENT OF OVARIOLE C_1 IN FEMALE *G. PALLIDIPES*

AGE(DAYS)	LENGTH OF OVARIOLE(MM) MEAN ±	SE
1	0.21	0.06
2	0.23	0.02
3	0.25	0.01
4	0.23	0.01
5	0.28	0.01
6	0.32	0.01
7	0.38	0.01
8	0.36	0.01
9	0.43	0.02
10	0.49	0.01
11	0.53	0.03
12	0.57	0.03
13	0.62	0.03
14	0.70	0.10
15	0.69	0.05

n = 15 for each age group

APPENDIX 9

DEVELOPMENT OF ACCESSORY REPRODUCTIVE GLAND(ARG)
IN MALE G. PALLIDIPES

AGE(DAYS)	DIAMETER OF ARG (MM)		
	MEAN	±	SE
1	0.09		0.003
2	0.09		0.004
3	0.11		0.004
4	0.11		0.004
5	0.11		0.004
6	0.12		0.006
7	0.13		0.006
8	0.14		0.008
9	0.15		0.005
10	0.16		0.004
11	0.17		0.005
12	0.16		0.004
13	0.17		0.005
14	0.17		0.005
15	0.16		0.005

n = 15 for each age group

APPENDIX 10

REPLENISHMENT OF MALE ACCESSORY
REPRODUCTIVE GLAND (ARG) IN *G. MORSITANS*

TABLE OF ANALYSIS OF VARIANCE

S.V	df	SS	MS	F	
Diameter of ARG	6	0.02717	0.004528	10.97	**
Error	63	0.01518	0.000413		
Total	69	0.04235			

** Significant at the 0.01 probability level.

APPENDIX 11

ANOVA TABLE FOR THE INSEMINATING ABILITY OF MALES OF *G. MORSITANS* AT VARIOUS AGES

SV	df	SS	MS	F
Age groups	6	1.36	0.2267	2.72 *
Error	154	12.82	0.0832	
Total	160	14.18		

(F = 2.72, P<0.05)

* Significant at the 0.05 probability level.

APPENDIX 12

ANOVA OF MEAN DURATION OF COPULATION OF
G. PALLIDIPES AT VARIOUS AGES

SV	df	SS	MS	F	
Age classes	2	47.522	23.761	0.52	NS
Error	52	2359.460	45.374		
Total	54	2406.982			

(F = 0.52, P>0.05)

NS Not significant

APPENDIX 13

ANOVA TABLE FOR THE INSEMINATING ABILITY OF MALE G. PALLIDIPES AT VARIOUS AGES

SV	df	SS	MS	F	
Age groups	3	0.441	0.147	1.26	NS
Error	77	8.904	0.116		
Total	80	9.345			

(F = 1.26, P>0.05)

NS Not significant

APPENDIX 14

Formula for the d-test

$$d = \frac{\bar{x}_1 - \bar{x}_2}{\left(\frac{S_1^2}{n_1} + \frac{S_1^2}{n_2}\right)}$$

d = standardised normal deviate

(R E Parker (1979)

APPENDIX 15

Formula for χ^2 using Yates Correction
for 2 x 2 contigency table with df = 1

	A	A	Total
B	a	b	(a + b)
B	c	d	(c + d)
Total	(a + c)	(b + d)	(a + b + c + d) = n

$$\chi^2 = \frac{n(ad-bc)^2}{(a+c)(c+d)(a+b)(c+d)}$$

With Yates correction for continuity:

$$\chi^2 \text{ corr.} = \frac{n(|ad-bc|-1/2n)^2}{(a+c)(b+d)(a+b)(c+d)}$$

(After Parker, 1979)

APPENDIX 16

Multiple range test (after Parker, 1979)

$$Q \sqrt{\frac{s^2}{n}}$$

Q = obtained from tables

Having arranged the treatment means in order of magnitude, we test the difference between the largest and smallest against the shortest significant ranges (SSR) for k treatments.

APPENDIX 17

SPEARMAN RANK CORRELATION COEFFICIENT
(AFTER LAPIN, 1980)

$$r_s = 1 - \frac{6 \sum (x - y)^2}{n(n^2 - 1)}$$