STUDIES ON THE BIO-ECOLOGY AND TAXONOMY OF *GLOSSINA* WITH PARTICULAR REFERENCE TO *GLOSSINA FUSCIPES FUSCIPES* (NEWSTEAD)

BY

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Thesis submitted for the
Degree of Master of Science

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ABSTRACT

This thesis reports studies on three aspects of *Glossina* biology:-

a) Studies on the peridomestic behaviour of *Glossina fuscipes fuscipes* in the sleeping sickness epidemic focus of Busoga, Uganda.
b) Application of analysis of cuticular hydrocarbons in the chemotaxonomy of *Glossina* and
c) Studies on the ovulation and late mating of virgin *Glossina*.

A search near homesteads, made over a period of 20 months, revealed tsetse puparia and puparial shells under *Coffea canephora* (coffee), *Musa* sp. (banana) and *Lantana camara* thickets, under house verandahs and, once, inside a hut. Studies on the peridomestic resting sites were carried out both at night and during the day. Flies were caught, marked with fluorescent powders and then released in coffee and banana plantations, *Lantana camara* thicket and adjoining forest habitat and potential resting sites subsequently searched. Leaves, twigs and branches between 0.5 and 1 cm in diameter were found to be preferred resting sites. The resting heights ranged from 0 to 3.66 m. The breeding and resting sites of *G. f. fuscipes* in the peridomestic habitat are described for the first time. The implication of these findings in relation to the transmission and control of the current epidemic of sleeping sickness in Busoga is discussed.

Analyses of the cuticular hydrocarbons of members o the *fusca* group, allopatric populations of *G. pallidipe* different age groups of *G. f. fuscipes* and puparial she of *G. f. fuscipes* and *G. p. gambiensis* were carried out using Gas Liquid Chromatography. Discriminant analysis used to analyse the data obtained. Significant differen in hydrocarbon composition were found between members o the *fusca* group and allopatric populations of *G. pallidipes*. The technique was also able to separate age groups of *G. f. fuscipes* and to identify puparia. T prospects for using this technique as a tool to age tse and to identify puparia found in the field are discusse

Ovulation and late mating have been studied in virg *G. f. fuscipes*, *G. p. gambiensis*, *G. tachinoides* and *G. m. morsitans*. In most cases ovulation took place normally but in some flies eggs were found piled up in uterus and ovary. These eggs were either eventually extruded from the uterus or their egg yolk degenerated the empty chorions were extruded. One fly extruded a premature second instar larva which was well developed, with a tracheal system and segments. Late mated flies d not larviposit and the significance of multiple mating the field is discussed. These studies provide a basis f further investigation of abnormalities in virgin female in relation to parthenogenesis and late mating.

CHAPTER 1

INTRODUCTION

1.1 Biology of the Tsetse Fly

Tsetse flies belong to the genus Glossina. Table 1.1 gives their taxonomic position.

The genus only occurs in Africa between latitudes circa 14° North and 20° South. There are 22 Glossina species, of which 8 are found in Uganda. The species are separated into three groups, now recognized as sub-genera, according to differences in their genital armature. The groups are fusca (sub-genus Austenina), palpalis (sub-genus Nemorpina) and morsitans (sub-genus Glossina s.str.) and all are represented in Uganda (Table 1.2; Map 1.1).

Though tsetse flies are occasionally confused with muscid flies (Family Muscidae), tsetse possess several distinguishing features:

a) a projecting proboscis sheathed by a pair of long maxillary palps.

b) resting tsetse have wings which are folded above the abdomen like a pair of scissors and extend slightly beyond the tip of the abdomen.

c) resting tsetse have splayed legs.

d) the hairs of the arista are branched and

e) the cell between wing vein iv and v is hatchet shaped.

More information on tsetse taxonomy is given in Chapter 4.

Male and female tsetse can be separated by the knob-like hypopygium which is present only in the male.

Females usually copulate 3-5 days after emergence and males mate successfully from 7 days of age (Jordan 1972). One act of mating is sufficient to render the female fertile for life. Sperm are transferred to the female in spermatophores (Pollock 1970) and stored in two spermathecae. Sperm are released by the spermathecae into the spermathecal ducts at the time of ovulation. The mechanisms involved have been described by Roberts (1971)

Tsetse flies are viviparous, producing one larva ever 8-10 days. The free-living larval stage is very short, lasting only a few minutes. The larva burrows into the soil and the third-instar integument becomes a blackish sclerotized shell known as the puparium, in which pupatic and metamorphosis to the adult fly takes place. Reproductive physiology is considered in Chapter 5 in relation to virgin females and late mating.

Most puparia are found a few centimetres below the surface, often under leaf litter. Choice of larvipositio sites by gravid females varies between fly species and with the local vegetation. Pupal sites are discussed in Chapter 2.

Glossina are vectors of the various forms of African trypanosomiasis which are known as sleeping sickness in man and nagana in animals. The diseases are caused by parasitic flagellates of the genus *Trypanosoma*. Two sub-species of *Trypanosoma* (sub-genus *Trypanozoon*) are pathogenic to man: *T. brucei gambiense* causes a chronic form of the disease found mostly in West Africa. *T. bruc rhodesiense* causes the more acute form of the disease

found in East Africa. Animal trypanosomiasis is caused by T. vivax sub-genus Duttonella, T. congolense sub-genus Nannomonas and less frequently T. brucei. Pigs are infected by T. simiae of the sub-genus Nannomonas.

All species of Glossina are susceptible to infection with trypanosomes but because some flies, particularly the forest species, rarely come into contact with man or his domestic animals, they are less economically important. The flies which are considered to be the main vectors of human trypanosomiasis are G. palpalis and G. tachinoides in West Africa and G. f. fuscipes, G. morsitans, G. swynnertoni, and G. pallidipes in East Africa. In Uganda G. f. fuscipes has been largely responsible for the sleeping sickness epidemics (Bruce et al. 1903; Morris 1959, 1962; Ford 1971; Rogers et al. 1972; Kutuza and Okoth 1981).

Glossina transmit trypanosomes from one host to another while taking blood meals. Ingested trypanosomes undergo a cycle of development in tsetse which results in the production of infective metacyclic forms, which can be transmitted to a new host. The life cycle of the sub-genera Trypanozoon, Nannomonas and Duttonella differ in their sites of development in Glossina. The details of their life cycle are given by Hoare (1972), the role of tsetse as vectors of trypanosomes is discussed by Jordan (1976) and the factors which influence trypanosome development in Glossina by Molyneux (1977). Recently evidence of the genetic basis of susceptibility of tsetse to trypanosomes has been provided by Maudlin (1982) and Maudlin et al. (1985).

1.2 Tsetse Distribution in Uganda

There are ten species and sub-species of Glossina in Uganda and their distribution is shown in Map 1.1.

1.2.1 Fusca group

Species of this group are commonly known as the fore: species although G. longipennis is an exception. This species is found in dry savanna woodland, in Northern Karamoja. The other species are found in the forests of Kigezi, Ankole, Toro, Mubende and Bunyoro in Western Uganda. These forests are at medium altitudes and vary between moist evergeen forests of Parinari, moist semi-deciduous forests of Cynometra and Celtis and forest/savanna mosaics. G. brevipalpis is also found in the moist semi-deciduous forests of South Bukedi, South Busoga and adjacent areas of Lake Victoria but it has be reported to be rare (Okoth 1982). This species occurs together with G. pallidipes and G. fuscipes (see below).

1.2.2 Palpalis group

Members of this group are traditionally known as riverine and lacustrine species. G. fuscipes fuscipes, t only representative of the group in Uganda, occupies muc of Eastern and North Western Uganda. In recent years, th fly has become peridomestic (Okoth 1982; 1986; also see Chapter 2). In Eastern Uganda, it occurs together with G. brevipalpis and G. pallidipes while in the Western Region, it occurs together with G. m. morsitans, G. fusc and G. pallidipes.

1.2.3 Morsitans group

Members of this group are known as savanna species. G. pallidipes and G. m. submorsitans occur in grassland savanna and dry thickets of Bunyoro near Butiaba. In East Madi and North Acholi,they are found in Vitex-Phyllanthus-Sapium-Terminalia woodlands. G. m. submorsitans is restricted to parts of Bunyoro in Northern Uganda, infesting Acacia, Combretum, Butyrospermum and palm savannas. A population of G. m. centralis occurs in the grass and Acacia savannas of Isingiro in Ankole and Southern Masaka. In Busoga/Bukedi, G. pallidipes are found in moist thickets.

1.3 Trypanosomiasis in Uganda

1.3.1 The major sleeping sickness epidemics in Uganda are listed in Table 1-3

Glossina f. fuscipes has been associated with the transmission of both chronic and acute forms of sleeping sickness in Uganda and in most areas where the disease is found, it was the only tsetse species present (Bruce et al. 1903; Morris 1959, 1962; Rogers et al. 1972; Kutuza and Okoth 1981). The present outbreak in Busoga is described by Abaru (1985).

1.3.2 Animal trypanosomiasis in Uganda

Accounts of the history of cattle trypanosomiasis in Uganda, caused by T. congolense, have been given by Simmons (1929), Poulton (1938) and Ford (1971). Cattle trypanosomiasis or nagana was first recognised in Western

Uganda in about 1909 when it was introduced by one of the vectors, Glossina morsitans centralis as it spread westwards along the Kagera valley. Detailed summaries of infection by district between 1949-55, as recorded by the Uganda Veterinary Reports, are given by Ford (1971). During those six years mixed populations of Glossina, mainly G. pallidipes, G. fuscipes and G. brevipalpis were found in Buganda and Busoga. In recent years animal trypanosomiasis has been detected in areas where G. f. fuscipes occurred alone and this fly is probably th principal vector at present (Mwambu 1966, 1971; Okuna and Mayende 1981).

1.3.3 Epidemiology of trypanosomiasis

Recent advances in our knowledge of the epidemiology of sleeping sickness have made it apparent that T. brucei rhodesiense may be widely transmitted among wild and domestic animals and that it is changes in man-fly contac that produces local human epidemics (Willett, 1965; Onyango 1969; Lumsden 1974). The 1971 T. b. rhodesiense outbreak at Busesa (Kangwagye 1975) and a similar outbrea in the adjacent counties in 1976 (Kutuza and Okoth 1981) was probably the same disease widely transmitted among th wild animals. Gibson and Gashumba (1983) suggested that the rhodesiense zymodeme which caused the human trypanosomiasis in the 1940s was still present in the 197 outbreak. Evidence to support this theory was obtained in 1959 when Robertson and Grainge (1960) found two workers of the Game Department at the Kabalega (Murchison) National Park infected with T. b. rhodesiense. Further

support came in the 1971 outbreak when a 4.8% T. brucei infection rate in G. f. fuscipes was partly attributed to T. b. rhodesiense. Parasites isolated from G. f. fuscipes involved in the outbreak caused disease in both cattle and human volunteers (Onyango and Mwambu, unpublished). Cattle have been shown to be reservoir hosts for T. b. rhodesiense (Onyango et al. 1966; Gibson and Wellde, 1985) and in Busoga, where most game animals have been eliminated by hunting, cattle are probably the main reservoirs of the disease (Gibson and Gashumba 1983).

It has also been shown in recent years that pigs may act as reservoirs for T. b. gambiense (Gibson et al., 1978, Mehlitz, 1982) and T. b. rhodesiense has been isolated from a domestic pig in the epidemic area of Busoga (Okuna, N.M., personal communication); the biochemical characterisation of this strain needs to be compared in detail with other isolates.

1.4 Trypanosomiasis control in Uganda

The disastrous epidemics of Gambian sleeping sickness in the Albert Nile basin and in Busoga/Bukedi at the beginning of the century had a great impact on the colonial administration. The British Government sent a team of scientists to Entebbe to undertake research into the disease and this led to the formation of the Human Tryanosomiasis Institute at Entebbe in 1925 (Onyango 1971). This Institute joined with similar Institutes in Kenya and Tanganyika (now Tanzania) in 1948 to form the East African Tsetse and Trypanosomiasis Research and

Reclamation Organization (EATTRRO). Studies on reclamation were abandoned in 1956. The organization then became the East African Trypanosomiasis Research Organization (EATRO) with headquarters in Sukulu, Tororo.

Control of trypanosomiasis in Uganda was and is based on information gathered by EATRO staff. Entomology, Protozoology, Biochemistry and Medical and Veterinary Sciences are all involved. Initially control of the vector took two forms and was under the administrative control of the Ministry of Animal Industry and Fisheries:-

a) alteration of tsetse habitat and

b) insecticidal spraying (Wooff 1969).

Alteration of the tsetse habitat involved a) shooting species of mammals on which tsetse fed and b) cutting down all trees and shrubs which provided shelter for the flies Selective application of insecticide to the most favoured resting sites proved to be a most effective and rapid method of controlling tsetse. Further information on tsetse control in Uganda is discussed in Chapter 3 in relation to resting sites of G. f. fuscipes.

Evacuation of populations from epidemic areas to disease free zones often only served to spread the epidemic. Early stage cases carried the parasites with them and because the vectors were widespread transmission amongst the population was often rapid (see Section 1.3.1). A more sensible approach developed in which sleeping sickness surveillance teams and Treatment Centres were set up in endemic areas. This system made possible the early detection of cases and their removal

for treatment. When, after the 1940-3 T. b.rhodesiense epidemic in Busoga and Bukedi (MacKichan 1944) it became clear that cattle could be reservoirs of the disease, block treatment of domestic animals was carried out wherever animal trypanosomiasis was detected. These efforts to break the transmission cycle, coupled with vector control, often brought trypanosomiasis outbreaks under control.

1.5 Aims

This thesis is divided into three parts

1.5.1 The study of the breeding and resting behaviour of Glossina fuscipes fuscipes Newstead.

This tsetse species has been associated with all the outbreaks of trypanosomiasis in Uganda and is probably now the only vector of the disease in Busoga. Although it is described as a peridomestic fly, its ability to breed in peridomestic sites has never been confirmed (Willett 1965; Okoth 1982). Conventional methods of tsetse control have failed to limit this fly's distribution; instead the fly has continued its spread away from water courses (Map 1.1).

1.5.2 The application of a chemotaxonomy by Gas Liquid Chromatography as a tool in taxonomy of tsetse.

Glossina which belong to the same species and sub-species have been reported to have genetic and behavioural differences (Jaenson 1978; Gooding 1982; Langley et al. 1984). Analysis of cuticular hydrocarbons by Gas Liquid Chromatography (GLC) has been used to separate populations of insects which otherwise appear

identical (Lockey 1976; Carlson and Service 1979, 1980). This technique has been used in the present study to identify members of the *fusca* group, to investigate differences in allopatric populations of *G. pallidipes*, investigate age differences in tsetse and to identify tsetse puparia.

1.5.3 Studies on ovulation and late mating in virgin *Glossina*

There is doubt as to whether mating is a necessary prerequisite for ovulation in virgin tsetse (Odhiambo 1971; Dodd 1971; Chaudhury and Dhadiala 1976; Vanderplank 1947; Leegwater-van der Linden 1981, 1982). In view of these conflicting reports regarding ovulation in virgin *Glossina*, the present study was designed to further our knowledge on ovulation in virgin *Glossina fuscipes fuscipes*, *Glossina palpalis gambiensis*, *Glossina tachinoides* and *Glossina morsitans morsitans* and on abnormalities in reproduction due to late mating.

Table 1.1 Taxonomic position of the genus *Glossina*

Phylum	Uniramia
Class	Insecta
Sub-class	Pterygota
Division	Endopterygota (Holometabola)
Order	Diptera
Sub-order	Cyclorrhapha
Family	Glossinidae
Sub-family	Glossininae
Genus	*Glossina*

Table 1.2 *Glossina* species and sub-species in Uganda

1. *Fusca* group (or sub-genus *Austenina*):

    fusca fusca Walker, 1949.

    fusca congolensis Newstead and Evans 1921.

    longipennis Cortis 1895

    brevipalpis Newstead 1910

    nigrofusca hopkinsi van Emden 1944

    fuscipleuris Austen 1911

2. *Palpalis* group (or sub-genus *Nemorhina*):

    fuscipes fuscipes Newstead 1910

3. *Morsitans* group (or sub-genus *Glossina* s. str.):

    pallidipes Austen 1903

    morsitans submorsitans Newstead 1910

    morsitans centralis Machado 1970.

Table 1.3 Major sleeping sickness epidemics in Uganda

Year	Areas affected (See Map 1·1)	Parasite	Associated events	References
1901	Busoga and Bukedi	*T. b. gambiense*	The disease is believed to have been imported into Northern Uganda from West Africa along Ubangi and Welle Rivers by explorers and slave traders. Further movement of the same along the Albert Nile and Victoria Nile to the shores of Lake Victoria introduced the disease in Busoga/Bukedi	Bell, 1909; Morris 1959, 1962; Ford, 1971
1904	West Nile, Madi and areas between Albert Nile and Victoria Nile in	*T. b. gambiense*	As above	Morris 1959, 1962; Ford 1971

Table 1.3 (Contd.)

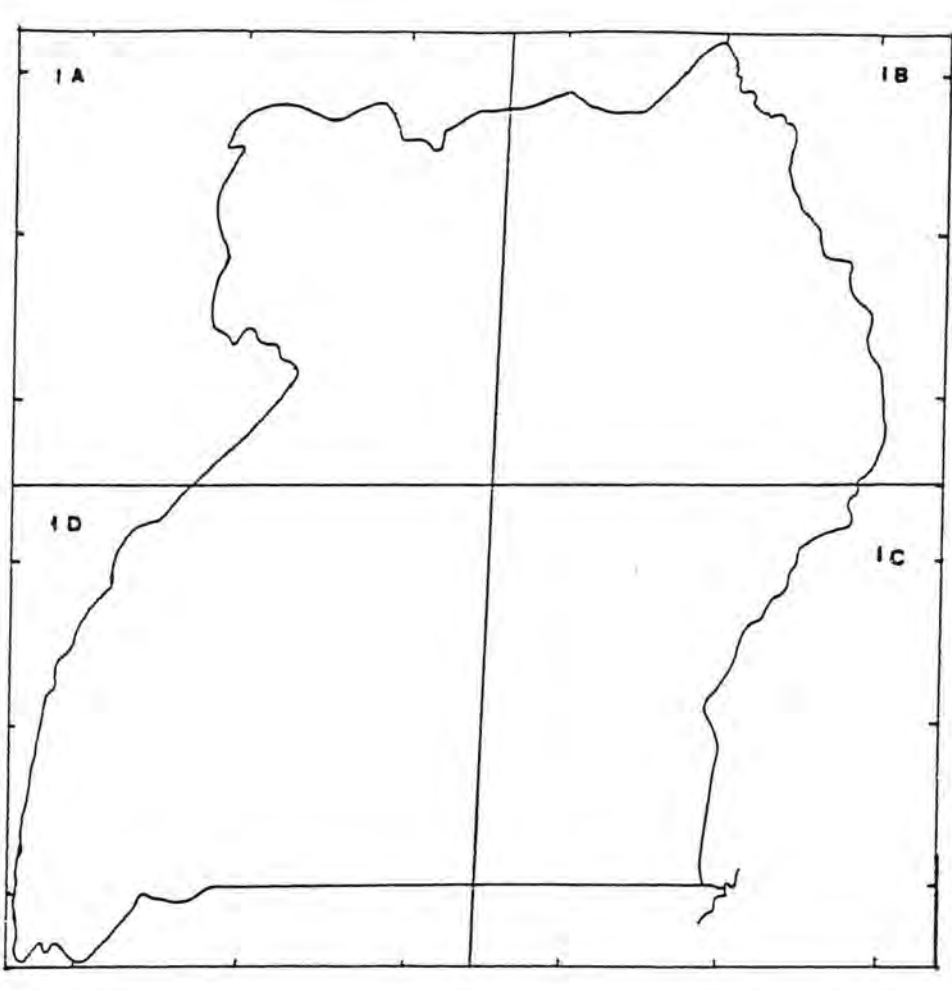
Year	Areas affected (See Map 1·1)	Parasite	Associated events	References
1912	Toro at Katwe Salt Lake on Lake Edward	*T. b. gambiense*	The disease originated from an endemo-epidemic Semliki valley focus in Zaire and was introduced into the area by salt traders.	Morris 1959, 1962; Ford 1971
1927	West Nile and Madi	*T. b. gambiense*	Increased human movement due to increased trade in the area	Morris 1959, 1962; Ford 1971
1932	Busongora in Toro at the slopes of Ruwenzori	*T. b. gambiense*	Resettled population evacuated from Katwe epidemic area	Morris 1960; Ford 1971
1935	Acholi and Lango along Aswa River	*T. b. gambiense*	Evacuation of the population from Madi epidemic area	Morris 1959, 1962; Ford 1971

Table 1.3 (Contd.)

Year	Areas affected (See Map 1·1)	Parasite	Associated events	References
1940	Busoga and Bukedi	T. b. rhodesiense	Rwandese seeking employment in the Kakira Sugar Estate on the edge of the Busoga forests introduced the disease	MacKichan 1944
1957	Busoga/Bukedi	T. b. rhodesiense	Increased fishing activity on Lake Victoria	Roberts 1963
1971	Busesa in Busoga	T. b. rhodesiense	Increased fishing activity; unchecked movement of people from endemic areas to disease free zones and settlement in endemic areas	Kangwagye 1975
1976	Luuka and Kigula counties in Busoga	T. b. rhodesiense	As above; G. f. fuscipes associated with coffee plantations and Lantana	Kutuza and Okoth 1981

Map 1.1 has been divided into four to fit the format of the thesis.

Map I.I Tsetse Distribution in Uganda (After Hooff I969, Ford and Katondo I977, Ford I97I, Kangwagye I979, Okoth I982)



Infested areas....................................

G.f.fuscipes (peridomestic)....................................

G.m.morsitans....................................

G.m.centralis ....

G.pallidipes ....

G.f.fuscipes ....

G.fusca ....

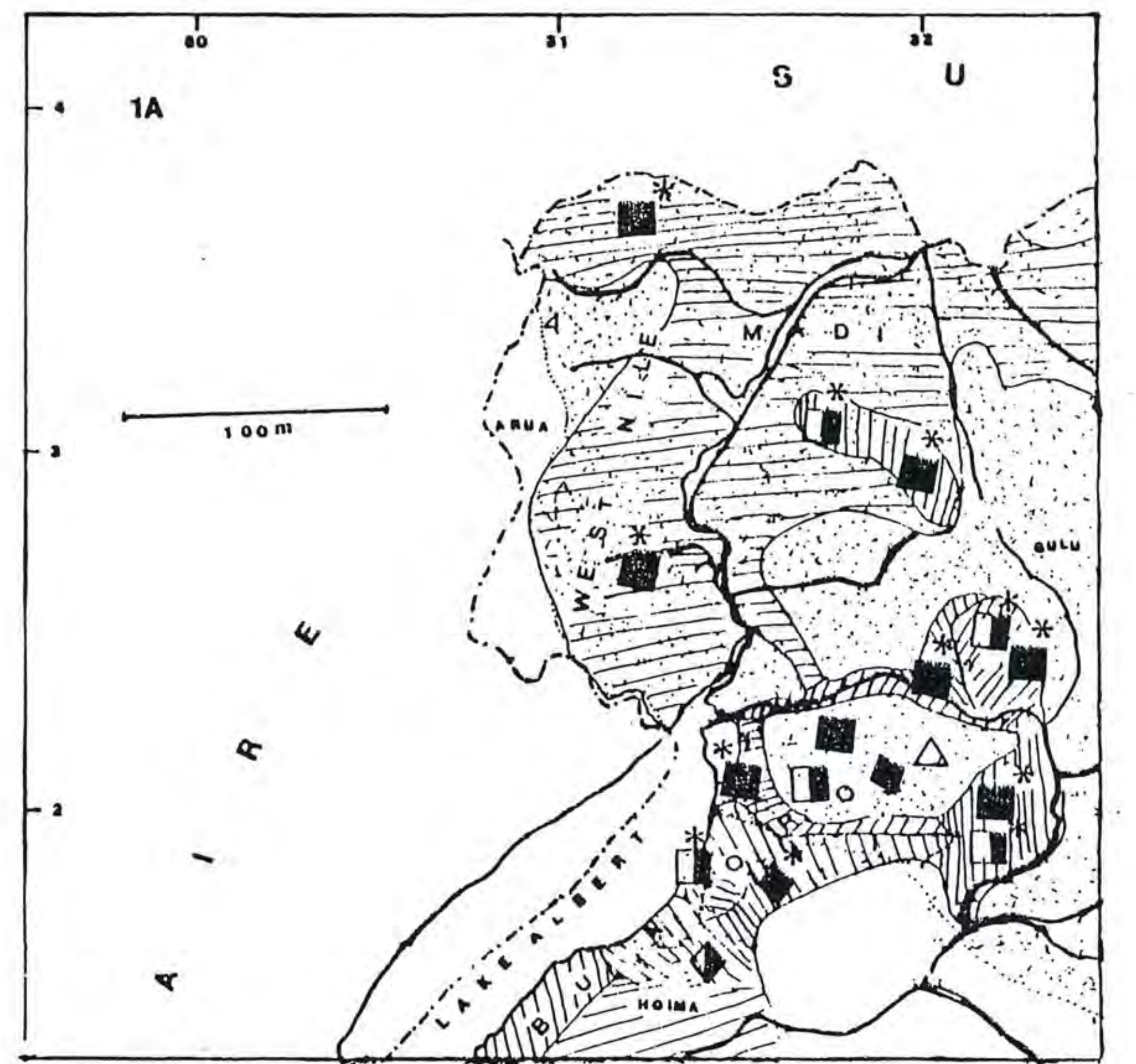
G.fuscipleuris ....

G.brevipalpis ....

G.nigrofusca** ....

G.longipennis ....

Predominant species in shaded area....................*



1A
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32
4
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2
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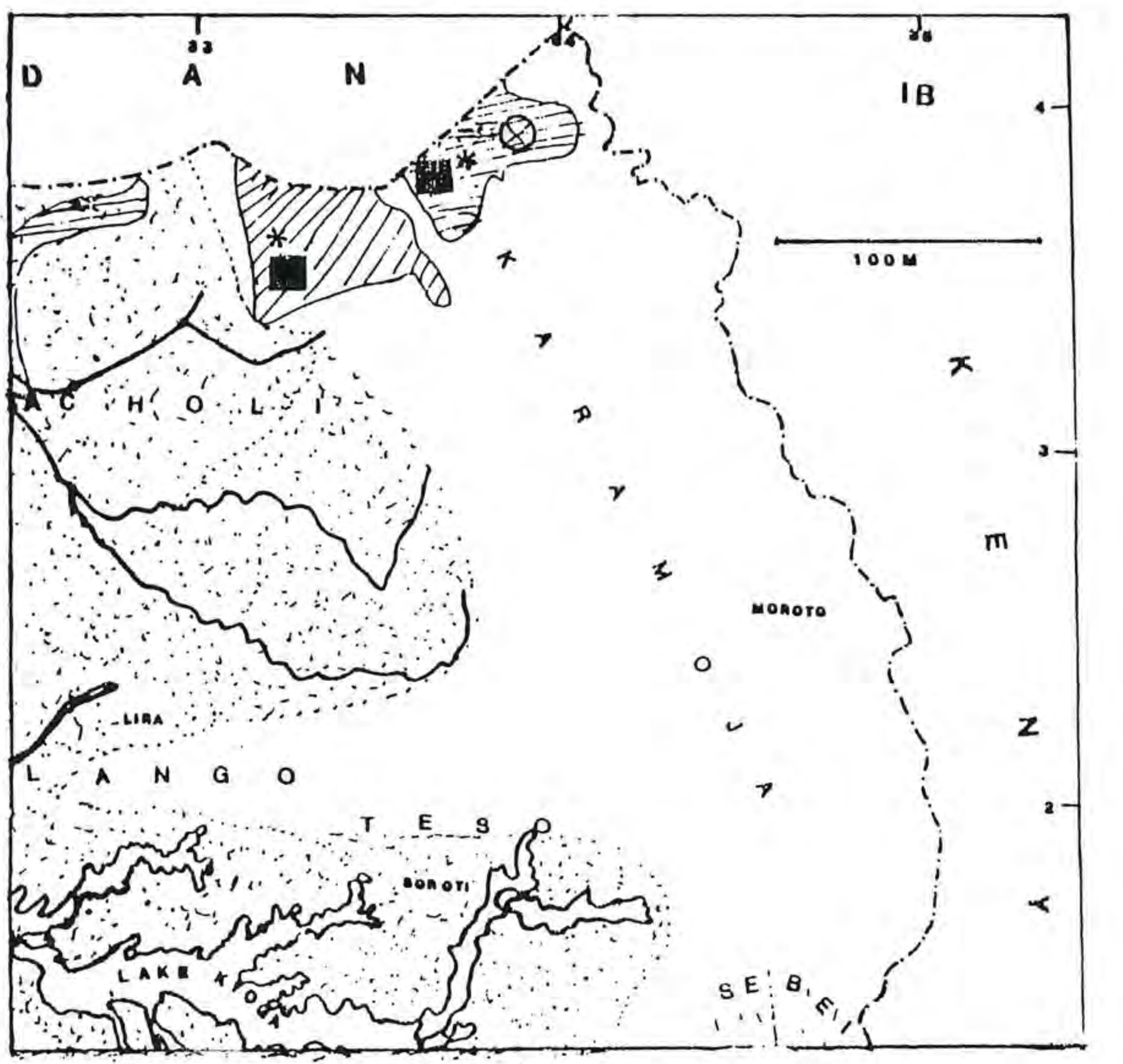
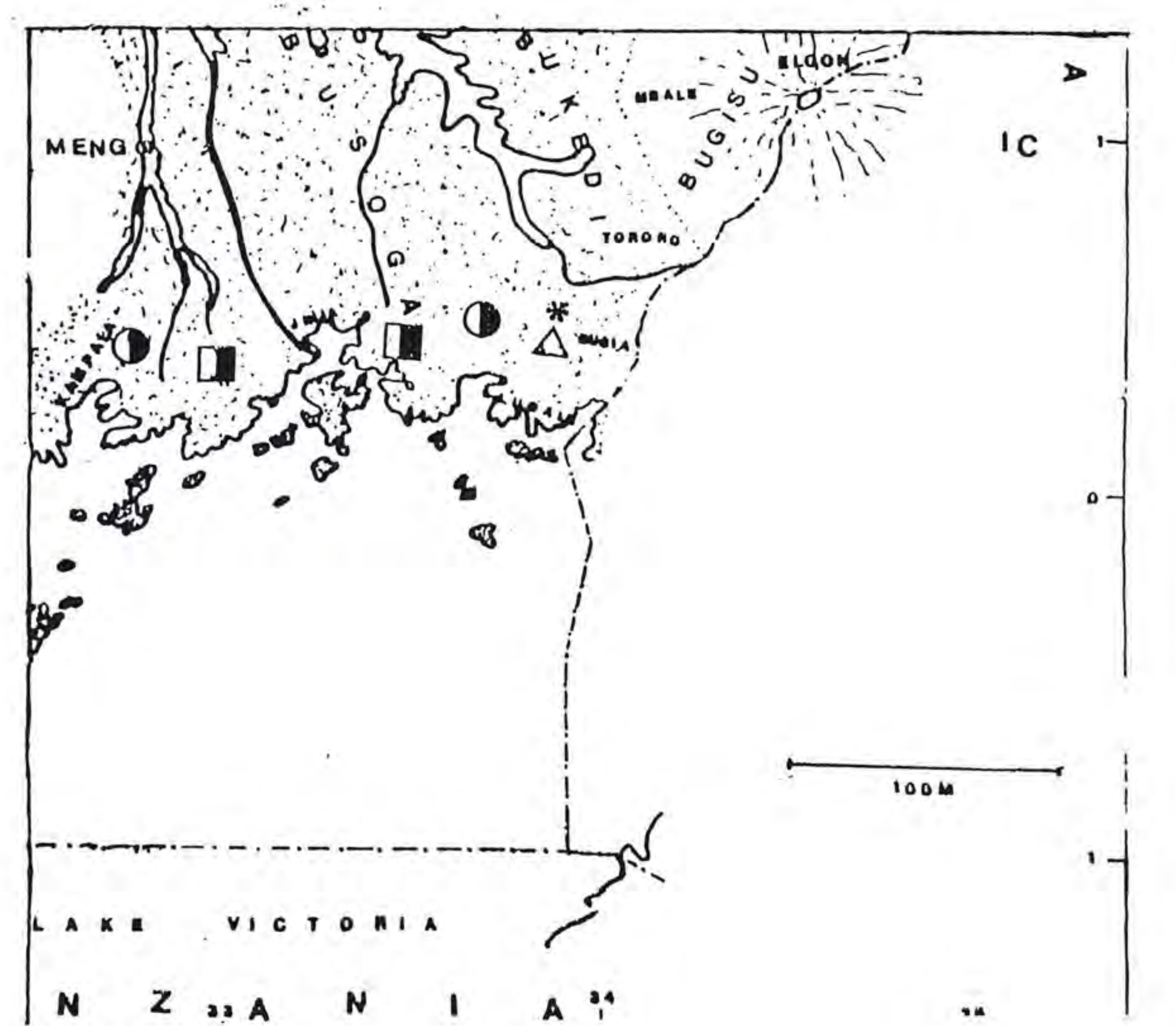
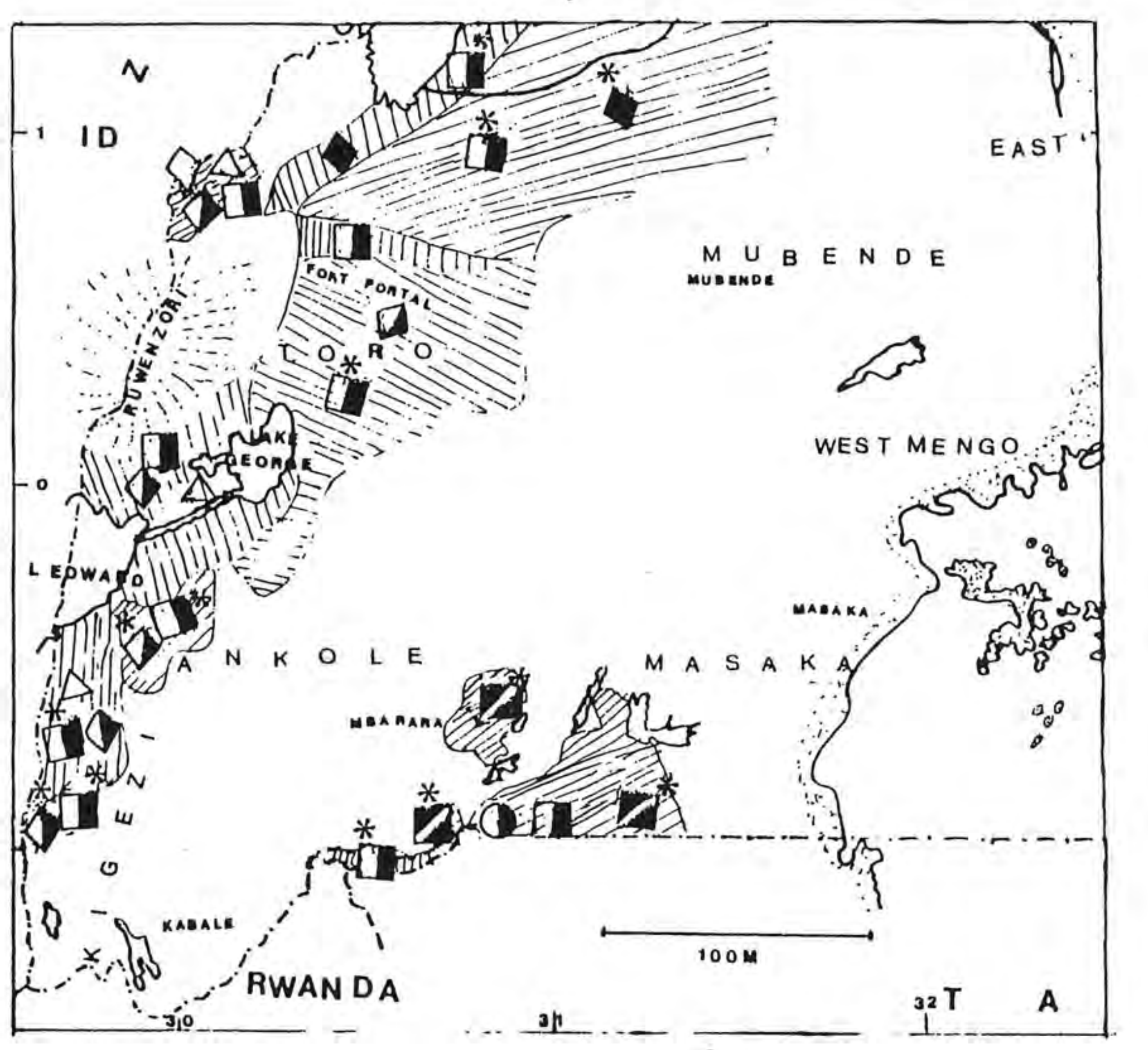
ARUA
GULU
HOIMA
LAKE ALBERT
WEST NILE
MADI
Z A I R E



KARAMOJA
MOROTO
ACHOLI
LANGO
LIRA
TESO
SOROTI
LAKE
SEBEI
100M



MENGO
BUSOGA
BUKEDI
BUGISU
ELGON
MBALE
TORORO
BUSIA
JINJA
LAKE VICTORIA
100M



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CHAPTER 2

PERIDOMESTIC BREEDING SITES OF GLOSSINA FUSCIPES FUSCIPES (NEWSTEAD) IN BUSOGA, UGANDA

2.1 Introduction

The distribution of *Glossina fuscipes fuscipes* in Busoga in the 1960's was given by Wooff (1969, see Map 2.1a). The distribution then was restricted to the northern shores of Lake Victoria and along the Rivers Lumbuye, Kibimba and Malawa and Lake Kyoga but this species can extend its distribution away from its normal riverine and lacustrine habitat. For example, Chorley (1944) noted that *G. fuscipes* could be found near human settlements 12 miles from the shores of Lake Victoria. Other surveys (see Map 1.1) indicate that *G. f. fuscipes* now occupies the whole of Busoga and that it may be breeding far from water courses, in peridomestic situations.

The "traditional" breeding or larviposition sites of tsetse include sites sheltered by fallen logs, oblique tree trunks, beneath rocks, in the mouths of caves, and i cavities and other holes in trees. These sites usually have loose, dry soil. Puparia* may also be found in shade sites scattered over the woodland floor (Potts 1973; Atkinson 1971). There are variations with the different species and vegetation types.

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* The term "puparium" *sensu stricto* refers to the third instar integument but it is traditional in tsetse work to extend the meaning to include the contents of the integument.

Gravid female tsetse choose larviposition sites where the larva is most likely to survive the environmental conditions. The larva is free-living and has both photonegative and thigmotactic responses which direct its movement to dark crevices and soft substrates. The larviposition spot is not usually the puparial site. Some studies on the choice of breeding sites by pregnant female tsetse have been reported by Parker (1956,a)and Okoth (1979). Pioneer studies on the breeding sites of G. palpalis fuscipes (= fuscipes fuscipes) are well documented by Carpenter (1920), Fiske (1920) and Buxton (1955).

In recent years there has been an increasing number of reports on the colonization of particularly peridomestic sites, by members of the palpalis group. In West Africa, G. tachinoides were found associated with pigs and pupae were found around pigsties (Baldry 1964, 1968, 1969). Sékétéli and Kuzoe (1984) found that the peridomestic breeding of G. palpalis s.l. in Ivory Coast was confined to areas with a large pig population. In East Africa, Willett (1965) attributed the sleeping sickness epidemic in Alego, Central Nyanza, Kenya in 1964 to Trypanosoma brucei rhodesiense transmitted by G. f. fuscipes. He found that this fly had colonized Lantana thickets and other vegetation surrounding Luo huts. Although he mentioned that teneral flies and puparia were found under vegetation near homesteads, he did not describe any breeding sites or give numbers of teneral flies and puparia found. Onyango et al. (1964) working in

the same area during the same outbreak, reported that " significance of Lantana in the area was not precisely determined but it is probable that Glossina was breedin in this vegetation type in some areas." In 1971, in Busoga, Uganda, T. b. rhodesiense, transmitted by G. f. fuscipes, occurred for the first time north of th Jinja-Tororo Road. This was outside the endemo-epidemic focus (Rogers et al. 1972). The present epidemiological situation in Busoga has been reported to be similar to that of Alego during the 1960s (Kutuza and Okoth 1981) a has recently been described by Abaru (1985).

The present study was designed to determine if peridomestic breeding of G. f. fuscipes in Busoga was associated with Coffea canephora (Coffee), Musa sp. (banana) and/or Lantana camara plantations. Proof of suc peridomestic breeding would allow a better understanding of the complexity of the epidemiology of sleeping sickne in Busoga. However, to further this understanding, a stu of the environmental conditions was necessary and is als briefly reported here.

2.2 Study Area

2.2.1 Choice of the study area

A study area was chosen which was:-

a) A sleeping sickness epidemic focus other than the old endemo-epidemic focus south of the Jinja-Tororo Road.
b) An area in which plantations of coffee and banana and Lantana camara thickets were abundant. (G. f. fuscipes has been reported to be associated with this vegetation).

c) An area adjoining forest habitat so that it was possible to check whether flies were breeding in the plantations and thickets and that these sites were not merely "feeding grounds".

d) Accessible by a vehicle and within easy reach of the Tsetse Control Camp.

e) Representative of a common Busoga environmental setting.

2.2.2 Wakatanga

Wakatanga was found to meet all of the above conditions. It is situated at approximately $0^{o}40'$ N and 33° 35' E, close to the main Jinja-Tororo Road, 80 km from Tororo (Maps 2.1a and 2.1b). It was the focus for the 1971 *T. b. rhodesiense* epidemic in which *G. f. fuscipes* was the main vector (Rogers *et al.* 1972) (see Table 2.1). It is located in Bugweri county in Iganga District. The area under study is about 12.5 km^2. It has five villages, namely Kiwanyi, Bulunguli, Butakanira, Nakibweru and Kikunyo (Table 2.1). These villages are not topographically different from each other.

2.2.2.1 Physical features

Map 2.1b, shows the two main forests and a seasonal swamp along the western border of the study area. Much of the original forest has been cleared for cultivation (Plate 2.1) and to provide fire-wood and building materials. The forest is being replaced by *Lantana* thickets which now form much of the under storey and extends from the forest to encircle many homesteads (Plate 2.2). Further from the forest, *Lantana* still occurs

in thickets and is used as a living fence, but coffee an banana become predominant. Two sugar plantation schemes exist along the forest edge. The swamp is being altered rice cultivation (Plate 2.3) and the planting of exotic trees (Eucalyptus sp.) which have drained the southern end.

Throughout the study area, trees are planted around gardens and homesteads to provide building materials, shade, fruit and fire-wood (Plate 2.4). The most common trees found around gardens are Mangifera indica (Mango tree), Carica sp. (Pawpaw), Citrus sp. (Orange), Artocarpus sp. (Jack-fruit), Persea sp. (Avocado), Psidi sp. (Guava), Ficus natalensis (Bark-cloth tree), Cassia sp., Delonix regia (Flamboyant) and Chlorophora excelsa (Mvule tree).

Coffee, banana and most of the trees retain some leaves throughout the year and so the area is seldom without shade, even when grass fires pass through during the dry seasons.

2.2.2.2 Climatic conditions

Harley (1965) recognised four seasons in Busoga name Dry season (November-March), Wet season (March-May), Coc dry season (June-August) and Hot dry season (August-October). There are, however, seasonal variatior from year to year especially in the length of each seasc and dry seasons are often broken by rains. In the study area, the mean rainfall per day is about 3.5 mm and dail mean maximum and minimum temperatures are 27°C and 15°C respectively. Mean relative humidity is about 80%. These weather records were taken during the present study.

2.2.2.3 Human population

The population of Wakatanga is 2101; about 168 persons per km^2 (census taken during this study). Human distribution in the area is shown in Table 2.1. The National Census of 1980, revealed that the population density in Busoga varied between 12 and 300 persons per km^2 (Census Uganda Atlas 1980).

2.2.2.4 Wild and domestic animals

Few wild animals were seen in the area, suggesting either that they are rare or inconspicuous. Foot marks of *Tragelaphus scriptus* (bushbuck) were seen in the early mornings along paths leading to water holes. Analysis of tsetse blood meals from the area also revealed the presence of *Potamochoerus porcus* (bush pig), Primates (monkeys) and *Varanus* sp. (monitor lizards).

There was a total of 1386 domestic animals in the area: 731 cattle, 534 goats, 79 sheep, 26 pigs and 16 dogs; an animal density of 111 per km^2. The distribution of domestic animals is shown in Table 2.2.

2.2.2.5 Agricultural practices

A system of land tenure is customary in Busoga. The main food crop is banana but millet, sorghum, maize, cassava, sweet potatoes and groundnuts are also cultivated. Coffee and cotton represent the main cash crops. Coffee and banana plantations are maintained around homesteads. Land may be left fallow for several years as part of a crop rotation system. Such uncultivated land rapidly provides fire-wood and building materials.

2.2.2.6 Cultural practices and beliefs

Many of the Basoga still believe in the traditional religion and its superstitions. They build "god huts" usually in plantations near homesteads (Plate 2.5a, 2.5b) Homes in which deaths due to unknown diseases have occurred are usually abandoned (Plate 2.6).

2.3 Methods

2.3.1 Location of potential larviposition sites

Potential breeding sites were located in different vegetation types. These sites, named sub-areas 1 to 10 a briefly described in Table 2.3 and are shown in Map 2.1b

2.3.2 Artificial breeding sites

Artifical larviposition sites (Plate 2.14) were constructed in selected sub-areas. They had a floor area of 4 x 1.5 m, a back 0.5 m high and a front 1.25 m high. The floor was divided into sectors by means of strings t facilitate systematic puparial search.

2.3.3 Puparial search

The study was carried out for 20 months from March 1983 to January 1985. Sharpened sticks were used to sear for puparia in selected sites and in shelters which were examined at weekly visits. Four searchers were normally assigned to each sub-area and these were rotated weekly.

Initially each group of searchers also carried a biconical trap (Challier and Laveissiére 1973) which the used to trap and catch teneral flies. These were set up the edge of the vegetation (Plate 2.7) or in open groun inside the thicket (Plate 2.8). The presence of teneral

flies indicated that breeding sites were nearby. Trapping was abandoned once teneral flies were caught.

The viability of puparia found was assessed by placing them in glass tubes 2.3 cm x 7.5 cm. These were closed at one end with nylon mesh and placed open end downwards over puparia in the soil. Flies emerged from two pupae under this arrangement but the method was abandoned because the tubes were being removed by children or monkeys. The pupae could have been hatched in the laboratory at Tororo but this was not attempted because of the very different climate there compared with the collection area. However, empty puparial shells were examined and any found with the normal posterior opening or operculum were considered to have hatched a viable fly.

Puparia of other Diptera were collected in order to determine which other flies share the breeding sites with G. f. fuscipes. These were kept in tubes in a room at the field station within the study area.

The presence of chickens or any other possible predators of tsetse puparia were noted.

2.4 Results

Tables 2.3 and 2.4 show details of puparial collections over a period of 20 months. Table 2.5 compares the performance of the artificial larviposition sites with the natural ones in the sub-areas in which they were erected. These results show that 63.5% of the puparia came from the artificial sites.

Plate 2.9 shows the general view of sub-area 2; witl this area, puparia were found under a log (Plate 2.10) under the Lantana thicket (Plate 2.11). Similar larviposition sites were also detected in sub-area 1. Plate 2.12 shows the general view of sub-area 3C; 2 puparia were found under banana plants (Plate 2.13). In the coffee plantation, sub-area 3, puparia were found b in the artificial site and under coffee plants (Plate 2.14). In sub-area 3B, a puparium was found unde hut inhabited by man (Plate 2.15) and in sub-area 9A, 8 puparia were found under a verandah and one inside a housing calves and goats (Plate 2.16). 5 puparia were found under coffee and banana plants in sub-area 9 wher pigs were tethered (Plate 2.17).

No puparia were found in sugar cane plantations (sub-area 8A), forest scrub (sub-area 8B) or grassland (sub-area 4).

Collections tended to be larger during the two dry seasons (Figure 2.1).

All puparial shells found were recorded as having hatched viable flies.

Four different types of dipterous puparia were fou (Plates 2.18-2.21). None of them hatched even though t were kept for several months.

2.5 Discussion

This study has shown for the first time that G. f. fuscipes not only rests in vegetation surroundir homesteads in Busoga, but also that this species breec

there. Okoth (1980, 1982) studied the composition of the *Glossina* population in the Busoga fly-belt and found that increases in human activity had reduced the population of *G. brevipalpis* and *G. pallidipes* to the point where they were rarely found whereas the population of *G. f. fuscipes* had remained constant. *G. f. fuscipes* now breeds and rests in peridomestic situations in Busoga different from the traditional riverine and lacustrine habitats.

This atypical behaviour makes man/fly contact intense even at a very low density. A man-fly-man cycle of infection is thus the most likely cause of human infection in the current sleeping sickness epidemic in Busoga.

There is an increase in peridomestic breeding during the dry season (Figure 2.1). Although there are views that there might be a breeding season for *Glossina*, this has never been confirmed (Potts 1973; Buxton 1955). However, during the dry season, much of the vegetation is burnt in preparation for sowing millet, maize and sorghum. As the natural vegetation burns, flies probably take refuge in and are confined to plantations, particularly coffee and banana. Plate 2.22 shows a fly transect before the fires and Plate 2.23 after the fires. It is suggested that this change of the natural vegetation increases peridomestic breeding. The difficulty of sorting through wet soil during the rainy season is not attributed to this observation since the artificial shelters were rain-proof.

It was found that most breeding took place where coffee, banana, *Lantana* and other vegetation grew under larger trees, (*Ficus*, *Mangifera*, *Cassia*, etc.) which provided suitable micro-climates required by the fly.

These trees, which are abundant in Busoga, provide fruit, fencing, shade, fire-wood and building materials and are thus important in the social life of the Basoga. Leaving land fallow as part of a crop rotation is similarly part of Basoga life. Sub-area 1 (Plate 2.24) was being cleared for cultivation after many years and a neighbouring plot (Plate 2.25) was being left fallow to provide grass for roofing houses; a Lantana thicket is developing in it. This kind of cultivation practice is a continuous process throughout Busoga so that at any one time there are suitable breeding sites for tsetse. Some land owners who work in cities leave their land undeveloped, and this provides further potential breeding sites.

Buxton (1955) discussed sacred groves as potential breeding places of G. palpalis in West Africa. He mentioned that these sacred groves were often near villages "and provide a difficult problem of control owing to their social and religious importance". In Busoga, a similar situation exists. Plate 2.5b shows a sacred grove near the kraal; here the god which protects the cattle lives. At the abandoned home (Plate 2.6), there was evidence that rituals often took place; Plate 2.26 shows a fire place for such rituals. Successful performance of rituals may please the spirits of the ancestors who might allow the reoccupation of the land and Plate 2.27 shows the same land being cultivated. Local people are very reserved about their social and religious beliefs. Due to ethical constraints, these "god huts" and sacred groves

were not searched for puparia; there is no doubt tsetse breed there since artificial shelters built nearby revealed many puparia.

It was observed that chickens spent much time scratching and basking in potential breeding sites (Plate 2.28). It is possible that chickens may eat puparia; they certainly kill puparia by scratching on them and pecking them. Puparia were mixed with millet grains and offered to chickens; some puparia were eaten but most of them were pecked at and crushed. It is therefore suggested that the activities of the chickens contributed to the relatively small number of puparia found. Buxton (1955) points out that guinea-fowl (Numida etc.) and Bush fowl or Francolin (Francolinus etc.) may devour or destroy puparia since they scratch the surface of the ground. However, the work of Simpson (1918) and Carpenter (1912) revealed no evidence that these birds destroyed the puparia.

No insect parasites were observed. However, a puparium (Plate 2.29), though not of a tsetse (see below), was parasitized by an insect. Syntomosphyrum glossinae and ants of the genus Pheidole (Nash 1970; Gruvel 1977 cited Rogers, personal communication) have been reported to parasitize tsetse puparia on the shores of Lake Victoria. H.M. Lloyd was reported by Swynnerton (1936) to have found G. f. fuscipes puparia on the island in Lake Victoria damaged by insect parasites. It is possible that such parasites may be present at Wakatanga.

Puparia of Cyclorrhaphous Diptera resemble those of tsetse flies and may be easily confused by inexperienced field workers, particularly when they are fragmented and/or occur mixed with those of tsetse (see Chapter 4). The only way to correctly identify them is by hatching them and identifying the adult flies. Dr. R.W. Crosskey (personal communication 1985) believes that some of the puparia collected probably belonged to the Tachinidae. The tachinid larvae probably drop from their hosts to pupate in sites shared by breeding tsetse. The relationship between these flies and/or their hosts would enable us to discover if there is any parasitism involved. Smith and Baldry (1969) studied some dipterous puparia resembling, and found among, those of tsetse in Nigeria. None of the puparia they found resemble the ones found in Busoga.

Searching for puparia is a laborious occupation. Though relatively few puparia were found in the selected sites, the total area of similar habitats in Busoga is immense and it seems likely that substantial numbers of G. f. fuscipes are breeding in peridomestic conditions. It can be postulated that when its natural habitats and wild hosts are destoyed, G. f. fuscipes will, like other members of the group adapt itself completely to the peridomestic habitat.

Summary

Puparia and puparials shells of G. f. fuscipes were found under coffee, banana and Lantana thickets, the most common forms of vegetation around homesteads and under verandahs and on one occasion, inside a hut.

Table 2.1 Wakatanga human population by village and sleeping sickness cases from 1971 to 1984

Village	No. above 15 years old		No. below 15 years old		Total		Grand Total	No. infected and treated since 1971		Total infected*
	Male	Female	Male	Female	Male	Female		Male	Female	
KIWANYI	171	179	157	132	328	311	639	9	5	14
BULUNGULI	145	170	161	185	306	355	661	8	6	14
BUTAKANIRA	122	132	147	105	269	237	506	28	8	36
NAKIBWERU	50	57	43	44	93	101	194	4	5	9
KIKUNYO	25	25	23	28	48	53	101	3	0	3
TOTAL	513	563	531	494	1044	1057	2101	52	24	76

* The number infected at least once but does not include deaths due to the disease

Table 2.2 Domestic animals at Wakatanga by village

Village	Cattle	Goats	Sheep	Pigs	Dogs	Total
KIWANYI	161	138	17	12	0	328
BULUNGULI	230	169	49	2	7	457
BUTUKANIRA	225	126	7	12	5	375
NAKIBWERU	45	59	0	0	0	104
KIKUNYO	70	42	6	0	0	122
TOTAL	731	534	79	26	16	1386

Table 2.3 Sub-areas where searches for puparia were made

Area	Vegetation	Size	Shade Trees	Soil	Sites Searched	Puparia & Shells
1	*Lantana camara*	1.5	*Ficus natalensis*	Loose,	Artificial shelter	11
	surrounded by	hectares	*Mangifera indica*	dark,	Fallen log	3
	Musa sp.			litter	Under *Lantana*	5
2	*Lantana* and *Musa*	1.5	*Ficus capensis*	Loose,	Artificial shelter	7
	inter-growing,	hectares	*Mangifera indica*	dark,	Fallen log	1
	surrounded by			litter	Under *Musa*	0
	seasonal crops				Under *Lantana*	6
3	*Coffea canephora*	1.2	*Cassia* sp.	Loose,	Artificial shelter	4
	close by homestead	hectares	*Ficus capensis*	dark,	Under coffee plant	3
				litter		
3A	*Lantana* thicket	0.75	No shade tree	Loose,	Under *Lantana* plant	0
	surrounded by seasonal	hectare		litter		
	crops					

Table 2.3 (Contd.)

Area	Vegetation	Size	Shade Trees	Soil	Sites Searched	Puparia & Shells
3B	*Coffea* and *Musa* intergrowing near homestead	0.5 hectare	*Markhamia platycalyx*	Dark loam	Under *Musa*	0
					under *Coffea*	0
					Under verandah of hut	1
					Under built shade	0
3C	*Musa* plantation near homestead	1 hectare	*Ficus natalensis*	Loose dark	Under *Musa*	2
					Under log	1
4	Thicket in open grassland	20 metres diameter	*Chlorophora excelsa*	Dark	Artificial shelter	0
					Under tree	0
5	Residual forest, undergrowth: *Phoenix* and *Lantana*	Large (see Map 1·1)	Forest trees	Clay, black	Artificial shelter	77
					Under log	5
					Under *Phoenix*	2
					Under *Lantana*	4

Table 2.3 (Contd.)

Area	Vegetation	Size	Shade Trees	Soil	Sites Searched	Puparia & Shells
5	Residual forest, undergrowth: *Phoenix* and *Acacia*; water logged	Large (See Map 1·1)	Forest trees	Clay, black	Artifical shelter	2
					Under trees	29
6A	Forest edge, homestead with coffee and kraal		*Ficus natalensis*	Loam black	Under verandah of human hut	3
					Kraal *Lantana* fence	0
7A	Forest edge, homestead and kraal with *Lantana* hedge		No shade trees	Loam, black	*Lantana* hedge	4
					Under verandah or sheep and calves hut	2
7B	Forest edge, homestead and kraal surrounded by seasonal crops		No shade trees	Loose, black	Under verandah or human hut	1
					Kraal living fence	0

Table 2.3 (Contd.)

Area	Vegetation	Size	Shade Trees	Soil	Sites Searched	Puparia & Shells
8A	*Saccharum officinarum* (sugar cane) plantation	20 hectares	No shade tree	Clay, black	Under *Phoenix*	0
					Under sugar cane	0
8B	Forest scrubland along seasonal swamp		Generally open	Clay, black	Under tree	0
					Under *Lantana*	0
9	*Musa* and *coffea* intergrowing near homesteads with pigs	1.5 hectares	*Ficus natalensis* *Carica* sp. (pawpaw)	Loose, black	Under coffee	3
					Under banana	2
9A	*Coffea* and *Lantana* intergrowing near a homestead with cattle	1.5 hectares	*Ficus natalensis*	Dark, loose	Under verandah of human hut	0
					Under verandah of calves	8
					Inside calves hut	1
					Under *Coffea*	0
					Under *Lantana*	0
10	Forest area dominated by *Lantana*	2 hectares	*Acacia*	Black soil	*Lantana* ground floor	47
					Fallen tree	12
17						246

Table 2.4 Monthly collection of puparia and puparial shells in Busoga.

S = puparial shell; p = puparia; * = months in which searches were not made

Sub-Areas		J	F	M	A	M	J	J	A	S	O	N	D	J	F	M	A	M	J	J	A	S	O	N	D	J	TOTAL S	P
							1983										COLLECTIONS			1984						1985		
10	S	*	*	*	*	*	*	*	*	*	2	29				1				1	5	1	*	*	*	8	47	
	P	*	*	*	*	*	*	*	*	*		2		3						1	4		*	*	*	2		12
9A	S	*	*	*	*	*	*	*	*	*		1									1		*	*	*		2	
	P	*	*	*	*	*	*	*	*	*		1		3	3								*	*	*			7
9	S	*	*	*	*	*	*		2														*	*	*		2	
	P	*	*	*	*	*	*		3														*	*	*			3
8B	S	*	*																				*	*	*			
	P	*	*																				*	*	*			
8A	S	*	*																				*	*	*			
	P	*	*																				*	*	*			
7B	S	*	*																				*	*	*			
	P	*	*											1									*	*	*			1
7A	S	*	*						2														*	*	*	1	3	
	P	*	*											1	1								*	*	*	1		3
6A	S	*	*																				*	*	*			
	P	*	*												3								*	*	*			3

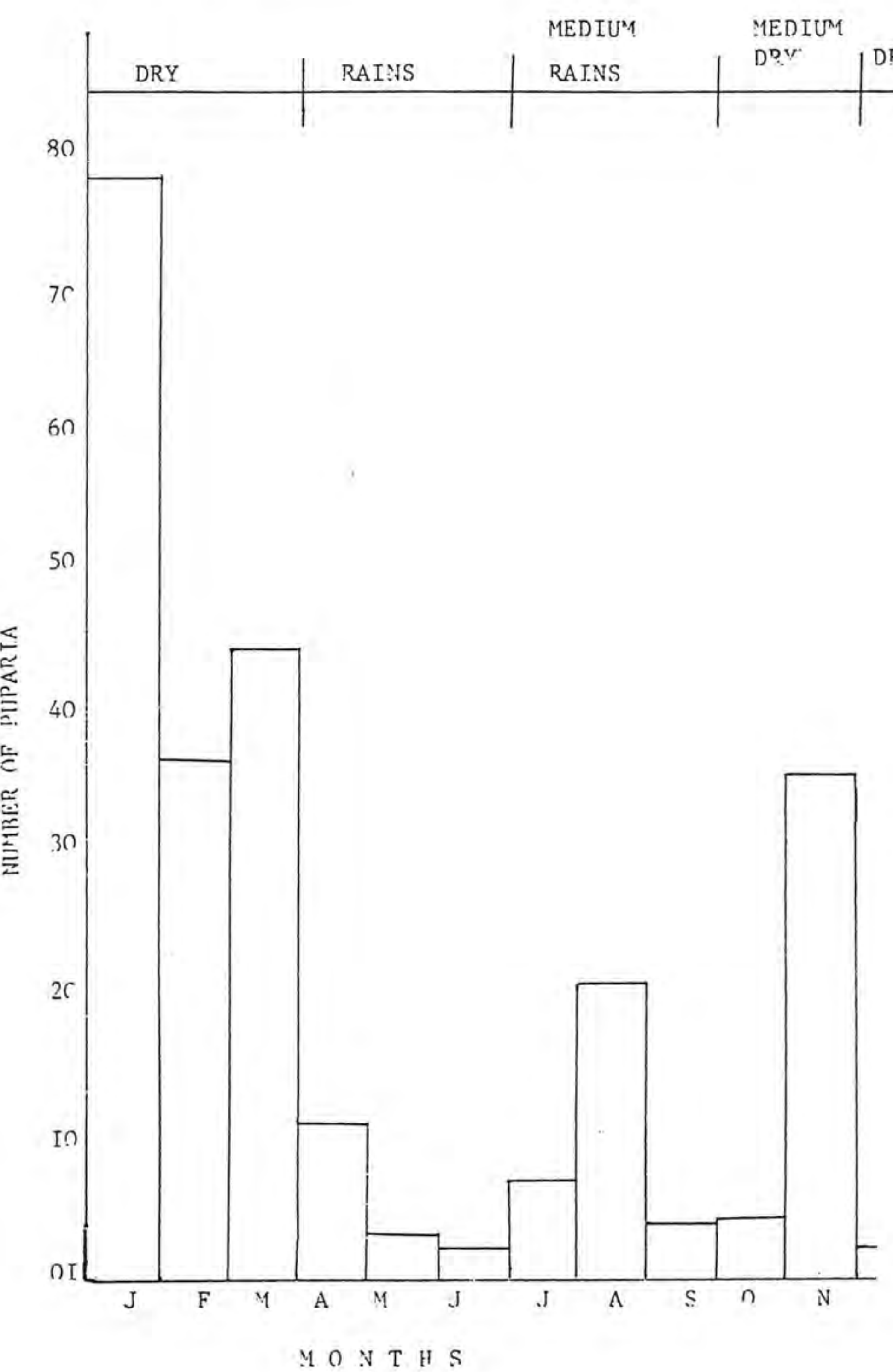
Table 2.4 (Contd.)

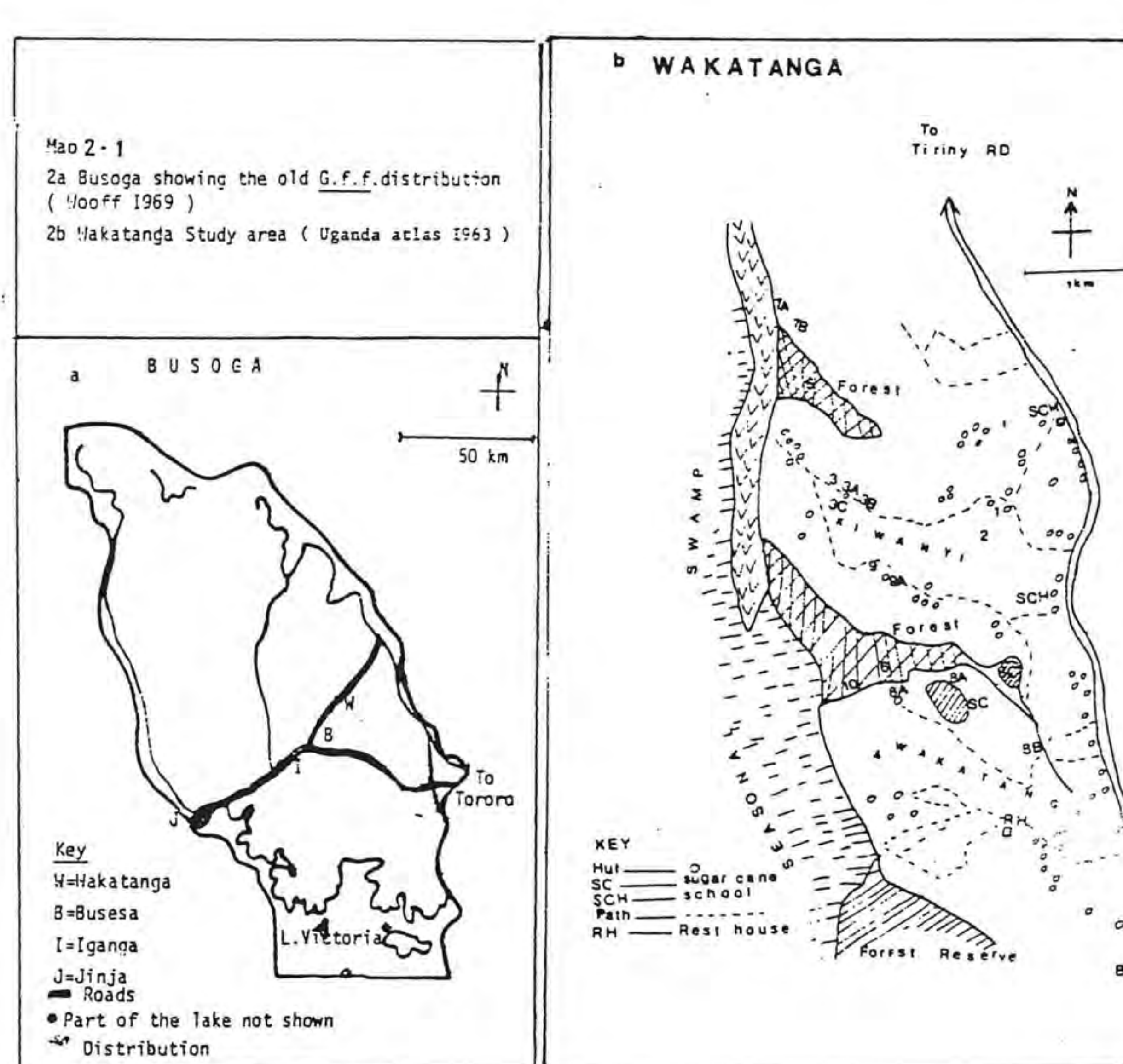
Sub-Areas		COLLECTIONS																									TOTAL	
		J	F	M	A	M	J	J	A	S	O	N	D	J	F	M	A	M	J	J	A	S	O	N	D	J	S	P
							1983												1984							1985		
6	S	*	*	21	2											1							*	*	*	2	26	
	P	*	*	1										2	1								*	*	*	1		5
5	S	*	*	8							1			6	7	3							*	*	*	5	30	
	P	*	*											27	20	5							*	*	*	6		58
4	S	*	*																									
	P	*	*																				*	*	*			
3C	S	*	*	*																			*	*	*			
	P	*	*	*								1		2									*	*	*			3
3B	S	*	*																				*	*	*			
	P	*	*							1													*	*	*			1
3A	S	*	*																				*	*	*			
	P	*	*																									
3	S	*	*									2								1	1		*	*	*		4	
	P	*	*					1						2									*	*	*			3
2	S	*	*		3	1											1			1	1		*	*	*		7	
	P	*	*		2	1				1				1	1	1							*	*	*			7
1	S	*	*		2										1	2	1			1	2		*	*	*		9	
	P	*	*				1						1	5		2				1			*	*	*			10
																											130	116
																											246	

Table 2.5 Comparison of natural and artificial breeding sites in six sub-areas

Sub-area	Artificial shelter (No. puparia)	Natural site (No. puparia)	Total Puparia
1	11	8	19
2	7	7	14
3	4	3	7
4	0	0	0
5	77	11	88
6	2	29	31
Total	101	58	159

Figure 2·1 Effects of seasonal conditions on the peridomesti
breeding at Wakatanga.





Map 2-1
2a Busoga showing the old G.f.f. distribution (Wooff 1969)
2b Wakatanga Study area (Uganda atlas 1963)

Plate 2.1 Part of the forest cleared for cultivation

Plate 2.2 *Lantana* thicket forming much of the forest under storey. Also showing hand-net fly collection

2.I

2.2

Plate 2.3 Rice Scheme

Plate 2.4 Showing trees around gardens and homesteads

2.3

2.4

Plate 2.5a God huts

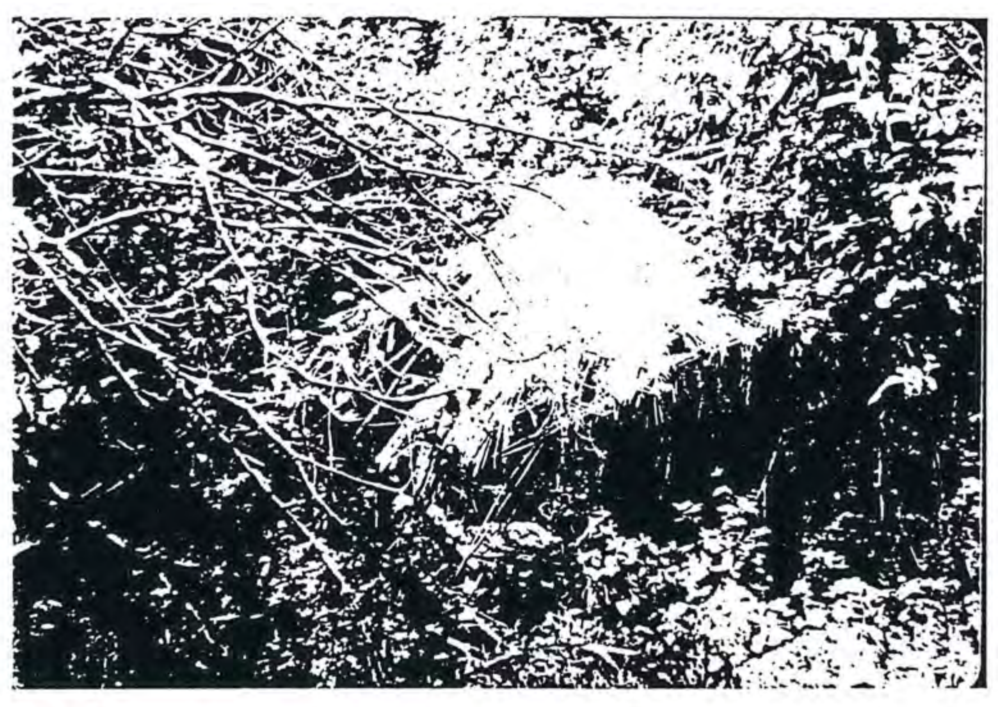
Plate 2.5b God's hut in sacred grove

2.5a

2.5b

Plate 2.6 Abandoned home

Plate 2.7 Trapping Tsetse at the edge of the vegetati



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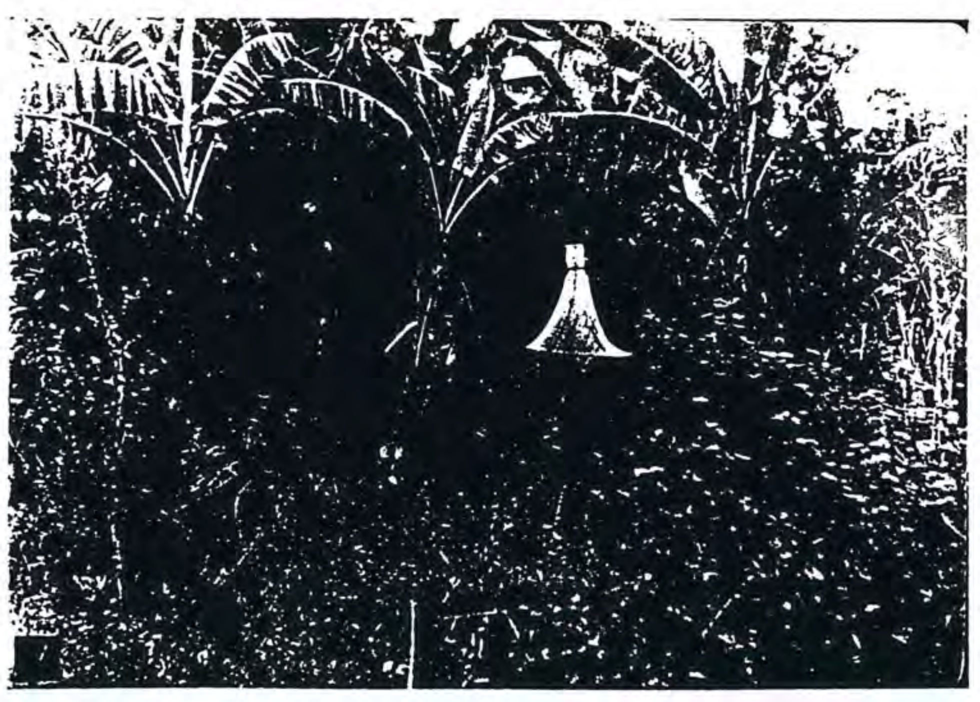


2.7

Plate 2.8 Trapping tsetse inside *Lantana* thicket

Plate 2.9 General view of sub-area 2

2.8

2.9

Plate 2.10 Puparial site under log in Lantana thicket

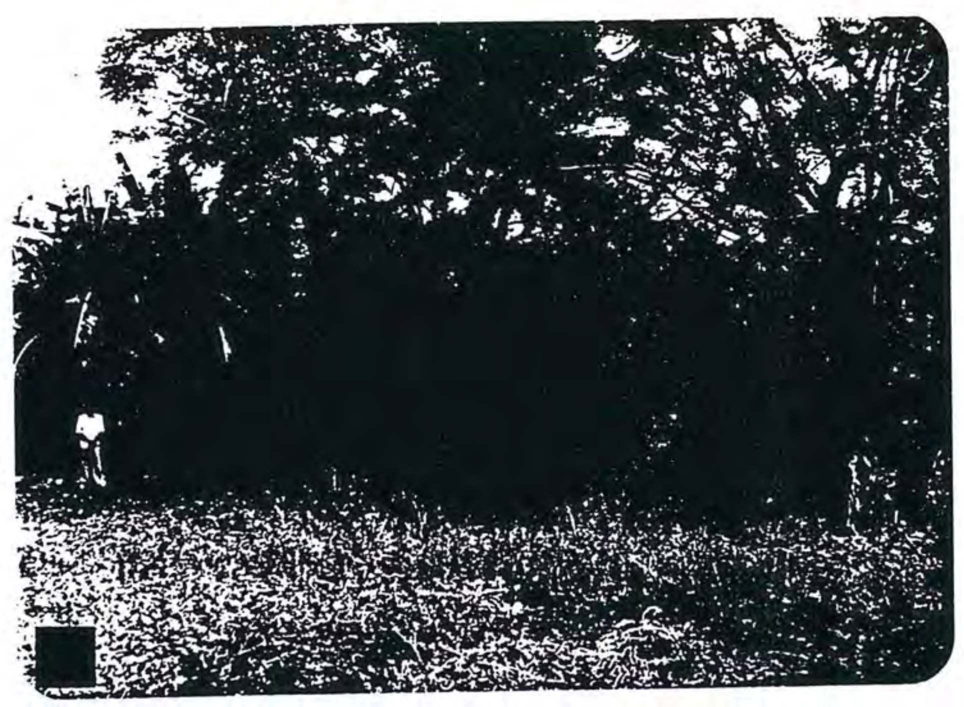
Plate 2.11 Puparial site under Lantana thicket

2.I0

2.II

Plate 2.12 General view of sub-area 3C

Plate 2.13 Puparial site under banana plant



2.I2

2.I3

Plate 2.14 Showing artificial shelter and puparial site under coffee plant

Plate 2.15 Puparial site under a hut inhabited by man

2.I4

2.I5

Plate 2.16 Puparial site under and inside a hut housing calves and goats

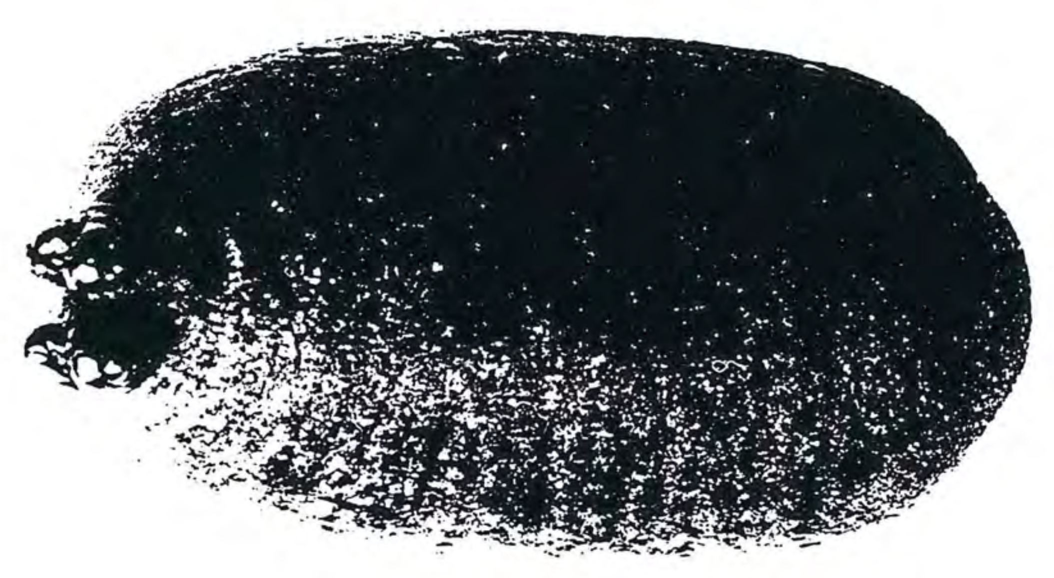
Plate 2.17 Tsetse breeding in coffee and banana plantations where pigs are tethered

2.I6

2.I7

Plate 2.18 Dipterous puparium (Natural size approx. 9.5 mm)

Plate 2.19 Dipterous puparium (Natural size approx. 8.5 mm)

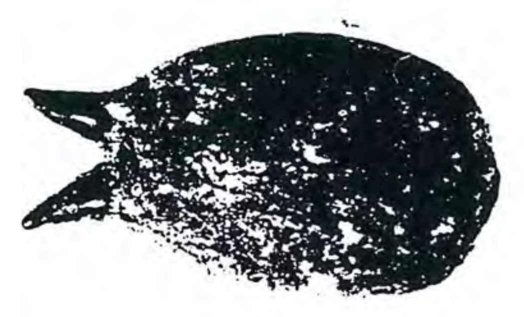


2·18

2·19

Plate 2.20 Dipterous puparium (Natural size approx. 8.5 mm)

Plate 2.21 Dipterous puparium (Natural size approx. 9.0 mm)

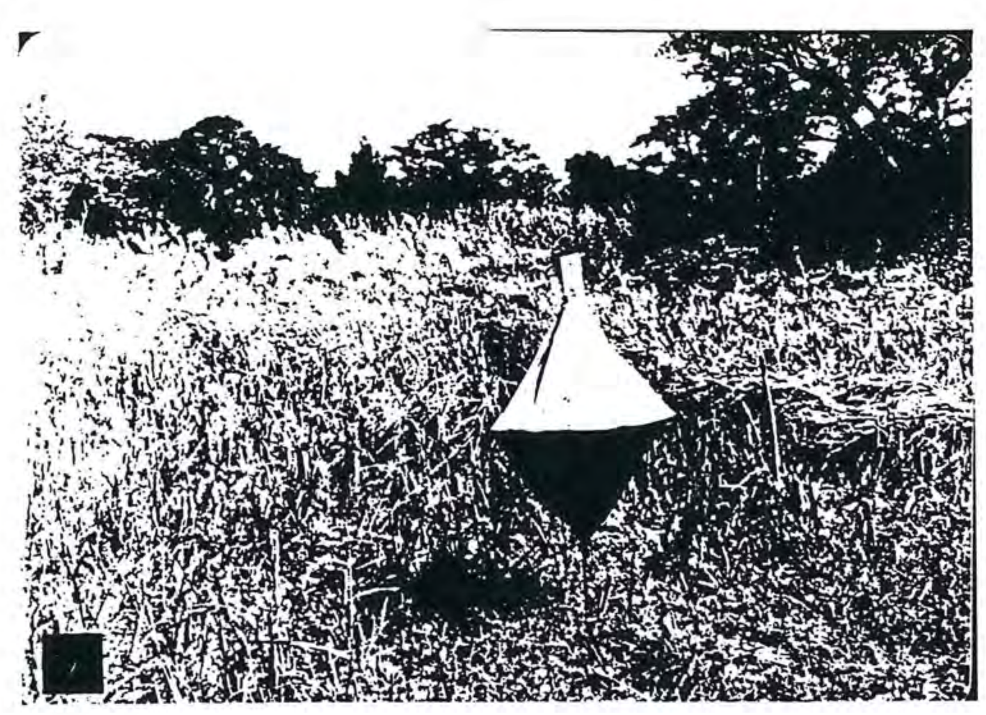


2 · 20

2 · 21

Plate 2.22 Transect fly-round before fires

Plate 2.23 Transect fly-round after fires



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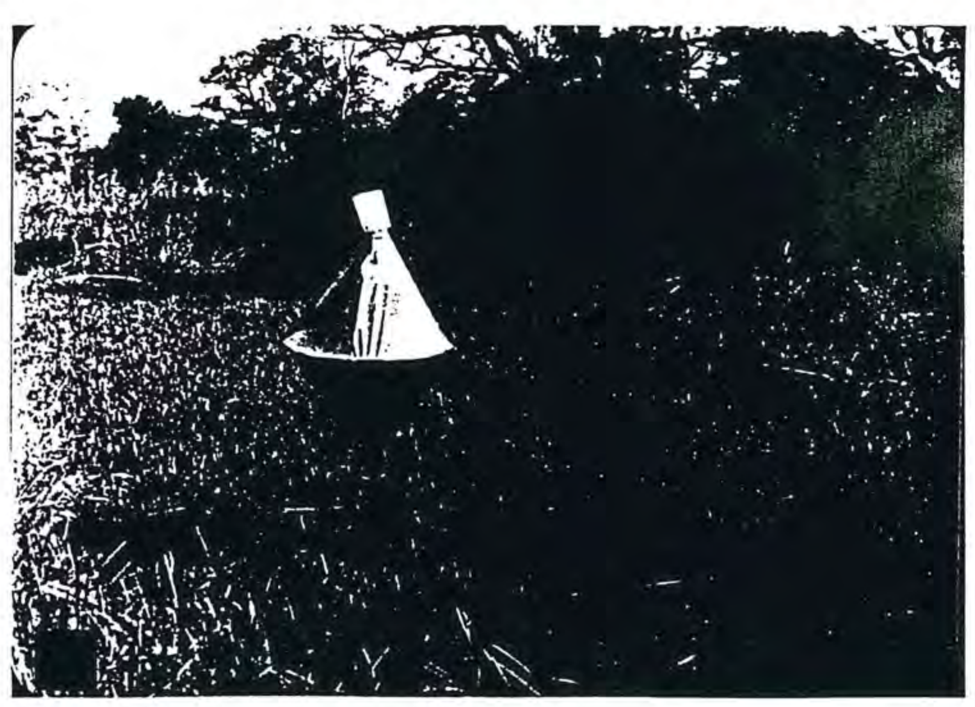


2.23

Plate 2.24 Sub-area 1 being cultivated (cf. Plate 2.11)

Plate 2.25 Land left fallow; showing *Lantana* thicket developing in it

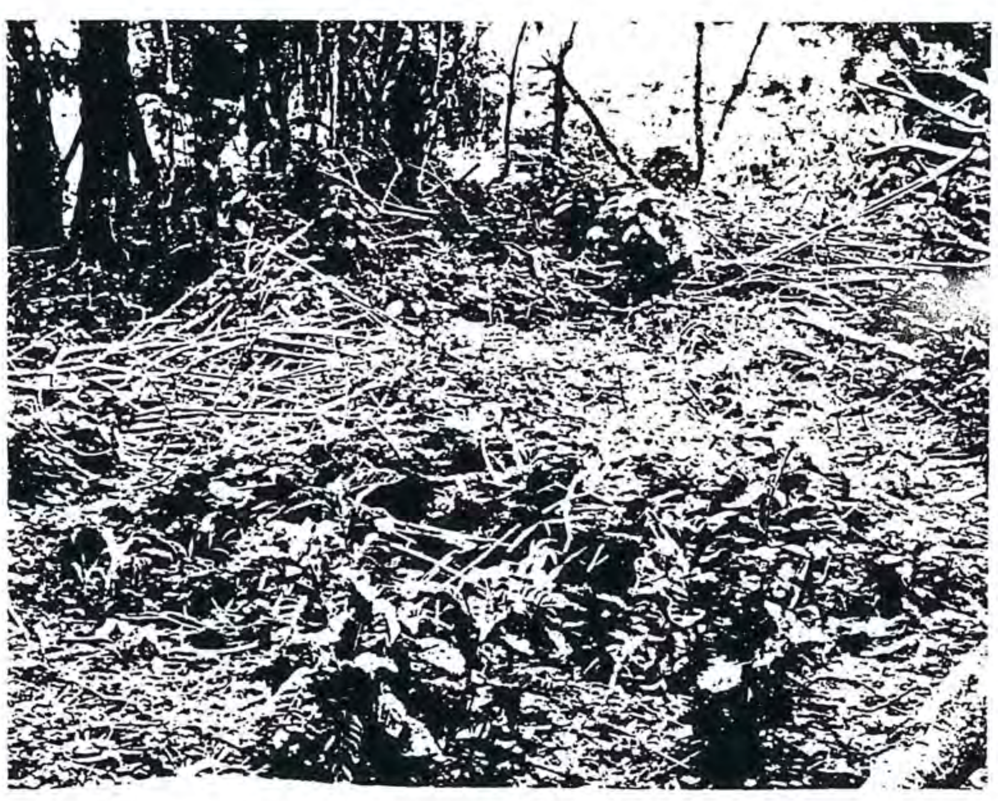
2.24

2.25

Plate 2.26 Fire place for rituals (cf. Plate 2.6)

Plate 2.27 Abandoned home being reoccupied (cf. Plate 2.6)

2.26



2.27

Plate 2.28 Chickens scratching potential tsetse breeding sites

Plate 2.29 Parasitized dipterous puparium

2.28

2.29

CHAPTER 3

A STUDY OF THE RESTING SITES OF GLOSSINA FUSCIPES FUSCIPI (NEWSTEAD) IN RELATION TO LANTANA CAMARA THICKETS AND COFFEE AND BANANA PLANTATIONS IN THE SLEEPING SICKNESS EPIDEMIC FOCUS, BUSOGA, UGANDA

3.1 Introduction

Tsetse flies spend most of their time resting; it is estimated that the daily activity period of *Glossina* is only about 30 minutes long (Bursell 1959). Researchers have located tsetse resting sites and applied residual insecticides to them in order to control the flies. This selective application of insecticides has been an important method of control in Uganda (see Chapter 1).

There have been many reports on the resting behaviou of flies of the *palpalis* group in recent years. Some of them are reviewed below:

3.1.1 *G. p. palpalis*

Abdurrahim (1971) studied the diurnal resting behaviour *G. palpalis* in southern Zaria, Northern Nigeria. He observed that most flies rested on the undersides of the stems and/or branches of climbing plants. They also used roots exposed by erosion and shaded by overhanging river banks. He found over 90% of the resting flies at heights between 0.3 and 2.15 metres. The circumferences of the most favoured resting objects ranged from 2.5 to 5 cm.

In the Congo, Carnevale and Adam (1971) found the favoured resting sites of *G. palpalis* to be dead leaves

(26.3%), twigs (24.0%), liana (15.2%) and green leaves (13.4%). The remaining resting sites, which amounted for less than 10% of the flies each, included branches, tendrils, roots, trunks, plant stems and dead wood. They noted that over 80% of the flies observed rested less than 0.5 m above ground level; females resting higher than males. Of the flies found resting on leaves, 40% were on vertical leaves, 25% on horizontal, 11.6% on oblique leaves and 22% on leaves lying on the ground.

The night resting sites of G. p. palpalis were studied by Scholtz et al. (1976) in a riverine swamp at Bara, northern Nigeria. 72% of the flies observed rested on leaves and 20% on twigs and creepers. The resting heights ranged from 0 to 18 m. Females rested slightly higher than males.

Onyiah (1979) observed 126 resting flies (mostly G. palpalis) at Egbe, Nigeria and found that most of them were on stems betwen 0.5 cm and 4 cm in diameter at heights below 3.0 m. Favourite resting spots were undersides of tree branches.

3.1.2 G. p .gambiensis

Challier (1973) working in Upper Volta found 70-75% of the resting G. p. gambiensis on the green leaves of small plants, 9% on the green leaves of other types of plants, 6% on dead leaves and the rest on the other plant parts excluding the trunks. The majority of flies rested less than 30 cm from the ground.

Okiwelu (1981) studied the distribution of resting G. p. gambiensis in a gallery forest in the Northern

Guinea Savanna in Mali. He found more than 80% of the flies resting on fallen logs. They showed no significant preference for the top or side of the logs. The second preferred site-type were the boles of trees; about 90% c flies were found in boles below 1.0 m and none was founc above 2.0 m.

3.1.3 G. tachinoides

Laveissière et al. (1981) found that, during the day the choice of resting site by G. tachinoides depended partly on temperature and relative humidity. They observ that "the resting site is selected according to the interaction of ecoclimatic, ecidioclimatic and biotic factors: when temperature is high, negative reaction to light leads G. tachinoides to select dark supports, clos to the ground."

Küpper and Koch (1983) studied night resting sites c G. tachinoides in Western Burkina Faso. They found that during the rainy season 66.2% and in the dry season 59.l of the flies preferred the upper surfaces of leaves. The mean resting heights were 0.74 and 1.57 m for females ar males respectively.

3.1.4 G. f. fuscipes

Glasgow (1963) reported that most G. f. fuscipes res at heights between 0 and 1.5 m on twigs, leaves and low branches.

Van Vegten (1969) found that in the isolated Nebolo: Hill forest in Samia, Bukedi, Uganda, G. f. fuscipes preferred twigs and branches 1.5 to 2.7 m from the grour as its resting sites.

These reports indicate that there are differences in resting behaviour within the palpalis group, even among different sub-species of the group and in different vegetation types.

Previous studies on the resting behaviour of these flies have concentrated on the riverine habitats but some members of the palpalis group have been reported to be peridomestic (Willett, 1965; Baldry 1980; Okoth 1982). An understanding of the resting sites of these flies in peridomestic habitats is necessary before their control, using residual insecticide application, can be efficiently carried out.

The resting behaviour of G. f. fuscipes, the only species involved in the transmission of the present T. b. rhodesiense sleeping sickness epidemic in Busoga (Kutuza and Okoth 1981) is reported here in relation to the vegetation most frequently found near homesteads, i.e. the adjoining forest and Lantana camara thickets and coffee and banana plantations. The significance of the results in relation to vector control in the sleeping sickness focus in Busoga, Uganda, is discussed.

3.2 Materials and Methods

3.2.1 Study area

Wakatanga, the study area, has been described (see Chapter 2). Resting site studies were carried out in sub-areas 1 (Lantana thicket), 3 (Coffee plantation), 3C (Banana plantation) and 6 (adjoining forest) (Map 2.1b).

3.2.2 Night resting site studies

Glossina fuscipes fuscipes were caught using biconica traps (Challier and Laveissiére 1973) along a transect fl round (Plate 2.22) and by hand-nets (Plate 2.2) within th Wakatanga area. The flies were kept in a cool place unti late afternoon when a sample was fed on a goat (Plate 3.1). The flies were separated into four groups - fed males, unfed males, fed females and unfed females. T allow flies from each group to be recognised in the fiel each group was dusted with a different colour of fluorescent powder. Flies were kept in cages and dusted using blowers at a distance of about 30 cm (Plate 3.2).

Flies were released in the study area at dusk. The search for resting flies began one or two hours after sunset and was maintained until all likely sites had bee examined, usually for one to two hours (Plate 3.3). A generator powered ultra-violet lamp was used to detect flies. The number of flies released and the number detected were noted and records were made of the sex, hunger stage, resting height, and posture (head up, dow or horizontal) for each fly and the nature of the suppo (leaf, twig, stem, etc.), its size, colour and botanica name and the surface used by the fly (upper , under or side). A whirling hygrometer and an ordinary thermomete were used to measure relative humidity and temperature the start and at the end of each study period. As far a possible resting heights were measured in situ with graduated sticks but when vegetation was not penetrabl estimate was made by comparing the fly's height with o

of the sticks. The diameter of twigs and stems used as resting sites was measured with a calibrated V-shaped device made from galvanised iron (Figure 3.1). An ordinary torch was used to check that resting flies and not simply patches of fluorescent powder were being recorded. It was also used to take thermometer and other readings. A human recorder was positioned with a hurricane lamp about five metres from the observation team.

3.2.3 Day resting site studies

Tsetse flies for the daylight studies were treated in the same way as for the night study. They were released into the study area at dusk to prevent too much dispersal before they rested. Search for resting flies began at 6.00 a.m. the following day until 6.00 p.m. local time. The study in a Lantana thicket is shown in Plate 3.4 and in banana plantation in Plate 3.5. Flies seen stationary for more than 5 minutes were considered to be resting and details of the fly and resting site were recorded as in the night studies. Resting flies were rarely disturbed by slight movement and this allows for site measurement to be taken (Plate 3.6). Wild unmarked flies seen resting were also recorded. Climatic data were recorded hourly and each time a fly was seen throughout the study period.

In both night and day studies, one vegetation type was studied at a time. Initially the studies were planned to be carried out monthly for one year in each vegetation type. However, the time taken to repair the ultra-violet lamp after it malfunctioned made this impossible.

3.3 Statistical Analysis

Logarithmic transformations ($Log_{10}(x + 1)$) were made of resting heights where necessary to compensate for skewness and to stabilize variance before the heights we analysed. Analysis of variance was used to determine the differences, if any, between fly resting heights recorde in day and night observations and the effects of sex or nutritional status of the fly and vegetation types on these heights. 'Multiple classification analysis' was us to supplement analysis of variance which only indicates that one factor in the category is significantly differe from the overall mean. The first column of figures in th multiple classification analysis table gives the number observations, the second, the deviation of the category mean from the overall mean and the third gives this deviation after adjustments for the effects of other factors have been made. The term Eta and Beta represent regression coefficients. Multiple R squared is the proportion of the total variation in resting height accounted for by the main effects and Multiple R is the correlation between resting heights and all the main effects.

3.4 Results

The sex (Table 3.1) and nutritional status (Table 3. of the fly and vegetation types have a significant effec on the fly's resting heights. For example, male flies re low at night (Mean height 0.87 m) and higher during the

day (mean height 1.85 m) in banana plantations (Tables 3.3 and 3.4). Tables 3.5 and 3.6 give results of multiple classification analysis.

Results of one-way analysis of variance show that the differences in resting heights between males and females during the day and night within each vegetation type were not significant (Table 3.7-3.14). However, during night hours, the nutritional status of the flies did have a significant effect on resting heights in coffee (Table 3.15) and banana (Table 3.16) but not in Lantana thickets (Table 3.17) nor forest (Table 3.18). During daylight hours, the nutritional status of the flies had no significant effect on their resting heights in any vegetation type (Tables 3.19-3.22).

Resting positions are shown in Table 3.23 for all the vegetation types. Leaves were preferred resting sites in coffee (Plate 3.7) during the day and night. Plates 3.8 and 3.9 show resting flies in banana plantations. Twigs were preferred sites at night in Lantana (Plate 3.10) and forest (Plate 3.11) but during the day the highest percentage of flies rested on branches 0.5-1 cm thick (Plate 3.12).

Figure 3.2 shows the surface and orientation of the resting support used by the fly and the posture of the fly in the different vegetation types in daylight and at night.

3.5 Discussion

The results show that female flies rest higher than males. Carnevale and Adam (1971) reported the same behaviour for G. palpalis in the Congo. However, the opposite behaviour with males resting higher than female: was observed in G. tachinoides (Kupper and Koch 1983). Table 3.24 shows a comparison of resting behaviour of members of the palpalis group in relation to the type of resting site, its circumference and height above the ground. Because differences in the resting behaviour of G. palpalis group flies exist, it seems essential to stu each fly in its habitat before selective ground spraying of resting sites is carried out.

In daylight most flies were found on the undersides resting surfaces whereas at night (Figure 3.2), except i thick vegetation such as banana, more flies rested on upper surfaces. During the day, sloping supports were generally used and at night vertical supports were preferred especially in banana and forest.

Burnett (1970) mentioned that selective spraying of resting sites was first used against G. f. fuscipes and then G. p. palpalis and G. tachinoides. He stated that " the places where this was done, the habitats of these species were essentially linear and restricted." However G. f. fuscipes distribution in Busoga is no longer "line and restricted" (Map 1.1 and Map 2.1) and control is therefore more difficult. A second difficulty is that while ground application of insecticide to the resting

sites of tsetse in _Lantana_ thickets and forest is possible, for environmental reasons, coffee and banana plantations cannot be sprayed.

Following the 1971 sleeping sickness outbreak at Busesa, Kangwagye (1975) successfully reduced man/fly contact by selective spraying and thus brought the epidemic to a halt. More recently this low level of man/fly contact became too expensive to maintain.

Vector control in Busoga requires a combination of integrated methods involving both the Tsetse Control Department and the local community. Tsetse control by clearing forests, elimination of fly hosts or by massive use of insecticides (see Chapter 1) are all unnecessarily detrimental to the African environment. Other, less destructive methods, of which trapping is an example, offer greater scope for control with conservation. _Lantana camara_, originally used as an ornamental plant and a living fence, has now replaced most of the once common shrubs and has become an important source of firewood in some places (see also Chapter 2). In an environmental setting such as that of Busoga, it is suggested that selected ground spraying of resting sites could be carried out within the forests and tsetse fly density could then be kept at a low density by trapping on a community self-help basis (Okoth 1985, 1986).

Summary

Studies on the peridomestic resting sites of _G. f. fuscipes_ were carried out at night and during the

day in coffee and banana plantations, *Lantana camara* thicket and adjoining forest habitat. Leaves, twigs and branches between 0.5 and 1 cm in diameter were the preferred resting sites of *G. f. fuscipes*. The resting heights ranged from 0 to 3.66 metres. The results are discussed in relation to tsetse control in the Busoga sleeping sickness epidemic area.

Table 3.1 Analysis of variance showing the effects of night and day, sex and vegetation types in relation to resting heights

Source of Variation	Sum of squares	DF	Mean sqaure	F	Signif. of F
Main Effects	2.556	5	0.511	15.750	<0.001
Night x Day	0.765	1	0.765	23.572	<0.001
Male x Female	0.236	1	0.236	7.258	0.007
Vegetation type	1.447	3	0.482	14.866	<0.001
2-Way-Interactions	1.180	7	0.169	5.193	<0.001
Night/day x male/ female	0.073	1	0.073	2.242	0.135
Night day x vegetation type	1.099	3	0.366	11.292	<0.001
Male/female x vegetation type	0.025	3	0.008	0.254	0.858
3-Way-Interactions	0.232	3	0.077	2.383	0.068
Night/day x Male/ female x vegetation	0.232	3	0.077	2.383	0.068
Explained	3.968	15	0.265	8.150	<0.001
Residual	19.701	607	0.032		
Total	23.669	622	0.038		

Table 3.2 Analysis of variance showing the effects of night and day, nutritional status of the flies and vegetation types in relation to resting heights

Source of Variation	Sum of squares	DF	Mean sqaure	F	Signif. of F
Main Effects	2.497	5	0.499	15.293	<0.001
Night x Day	0.950	1	0.950	29.084	<0.001
Fed x Hungry	0.162	1	0.162	4.950	0.026
Vegetation type	1.483	3	0.494	15.138	<0.001
2-Way-Interactions	1.234	7	0.176	5.398	<0.001
Night/day x Fed/ Hungry	0.006	1	0.006	0.169	0.681
Night day x vegetation type	1.029	3	0.343	10.509	<0.001
Fed/Hungry x vegetation type	0.175	3	0.058	1.785	0.149
3-Way-Interactions	0.093	3	0.031	0.951	0.416
Night/day x Fed/ Hungry x vegetation type	0.093	3	0.031	0.951	0.416
Explained	3.824	15	0.255	7.807	<0.001
Residual	19.854	608	0.033		
Total	23.678	623	0.038		

Table 3.3 Mean fly resting heights (metres) by sex at night and day

	Vegetation type	Sex of fly	No. of flies	Mean height	Min.	Max.	95% conf. interval
NIGHT	Coffee	♂	54	1.48	0.00	2.44	1.33–1.63
		♀	34	1.68	0.90	3.66	1.46–1.90
	Banana	♂	21	0.87	0.00	2.44	0.54–1.20
		♀	22	1.28	0.15	3.66	0.80–1.76
	Lantana	♂	108	1.09	0.00	3.05	0.99–1.19
		♀	58	1.18	0.08	2.44	1.04–1.31
	Forest	♂	16	0.94	0.00	2.44	0.55–1.34
		♀	4	1.68	1.22	2.44	0.84–2.52
DAY	Coffee	♂	36	1.68	0.30	3.66	1.39–1.97
		♀	16	1.80	0.91	3.35	1.36–2.23
	Banana	♂	22	1.85	0.53	3.66	1.57–2.12
		♀	23	1.88	0.76	3.35	1.60–2.16
	Lantana	♂	46	1.07	0.30	2.44	0.96–1.19
		♀	39	1.25	0.30	2.44	1.07–1.42
	Forest	♂	82	1.42	0.23	3.05	1.30–1.54
		♀	46	1.46	0.00	3.05	1.26–1.66

Table 3.4 Mean fly resting heights (metres) by hunger stage (HS) at night and day. F = Fed. H = Hungry

	Vegetation	HS	Count	Mean	Min.	Max.	95% conf. interval
NIGHT	Coffee	F	42	1.51	0.00	2.44	1.34-1.68
		H	12	1.37	0.46	2.13	1.03-1.71
	Banana	F	18	0.89	0.00	2.44	0.51-1.27
		H	3	0.76	0.46	1.22	0.24-1.76
	Lantana	F	89	1.11	0.00	3.05	0.99-1.23
		H	19	0.99	0.15	1.52	0.81-1.16
	Forest	F	12	0.88	0.00	2.44	0.37-1.38
		H	4	1.14	0.30	1.52	0.22-2.06
DAY	Coffee	F	11	2.23	0.61	3.66	1.60-2.86
		H	21	1.46	0.30	3.05	1.12-1.79
	Banana	F	14	2.08	1.23	3.66	1.74-2.14
		H	8	1.44	0.53	1.98	1.03-1.85
	Lantana	F	29	1.07	0.30	2.44	0.91-1.22
		H	17	1.08	0.39	1.83	0.88-1.29
	Forest	F	36	1.45	0.23	3.05	1.25-1.66
		H	46	1.39	0.30	2.13	1.25-1.53

Table 3.5 Multiple classification analysis, fly sex (Deviation from grand mean).

Variable + Category	No.	Unadjusted Dev'n	Unadjusted Eta	Adjusted for Independents Dev'n	Adjusted for Independents Beta
Time					
Night	315	-0.04		-0.04	
Day	308	0.04		0.04	
			0.19		0.20
Sex					
Male	385	-0.02		-0.02	
Female	238	0.03		0.02	
			0.10		0.10
Vegetation					
Coffee	140	0.07		0.08	
Banana	86	0.01		0.00	
Lantana	249	-0.05		-0.04	
Forest	148	0.01		-0.01	
			0.25		0.25
Multiple R Squared					0.108
Multiple R					0.329

Table 3.6 Multiple classification analysis, hunger stage (Deviation from grand mean).

Variable + Category	No.	Unadjusted Dev'n	Unadjusted Eta	Adjusted for Independence Dev'n	Adjusted for Independence Beta
Time					
Night	315	-0.04		-0.04	
Day	309	0.04		0.05	
			0.19		0.23
Hunger Stage					
Fed	432	0.00		0.01	
Hungry	192	-0.00		-0.03	
			0.02		0.09
Vegetation					
Coffee	139	0.07		0.09	
Banana	86	0.01		0.01	
Lantana	251	-0.05		-0.04	
Forest	148	0.01		-0.02	
			0.25		0.25
Multiple R Squared					0.105
Multiple R					0.325

Table 3.7 One-way analysis of variance showing the effects of fly sex on resting heights during the day in coffee plantations

Source of Variation	D.F.	Sum of squares	Mean square	F ratio	Signif. of F
Between sexes	1	1.5149	1.5149	0.199	0.6574
Within sexes	50	380.4176	7.6084		
Total	51	381.9324			

Table 3.8 One-way analysis of variance showing the effects of fly sex on resting heights during the day in banana plantations

Source of Variation	D.F.	Sum of squares	Mean square	F ratio	Signif. of F
Between sexes	1	0.1270	0.1270	0.029	0.8653
Within sexes	43	187.4424	4.3591		
Total	44	187.5694			

Table 3.9 One-way analysis of variance showing the effects of fly sex on resting heights during the day in _Lantana_ thicket

Source of Variation	D.F.	Sum of squares	Mean square	F ratio	Signif. of F
Between sexes	1	[illegible]	[illegible]	[illegible]	[illegible]
Within sexes	83	194.9139	2.3484		
Total	84	201.7232			

Table 3.10 One-way analysis of variance showing the effects of fly sex on resting heights during the day in forest

Source of Variation	D.F.	Sum of squares	Mean square	F ratio	Signif. of F
Between sexes	1	0.5611	0.5611	0.153	0.6961
Within sexes	126	461.3672	3.6616		
Total	127	461.9282			

Table 3.11 One-way analysis of variance showing the effects of fly sex on resting heights during the night in coffee plantations

Source of Variation	D.F.	Sum of squares	Mean square	F ratio	Signif. of F
Between sexes	1	8.7650	8.7650	2.469	0.1198
Within sexes	86	305.3144	3.5502		
Total	87	314.0793			

Table 3.12 One-way analysis of variance showing the effects of fly sex on resting heights during the night in banana plantations

Source of Variation	D.F.	Sum of squares	Mean square	F ratio	Signif. of F
Between sexes	1	18.4725	18.4725	2.235	0.1429
Within sexes	39	322.2712	8.2634		
Total	40	340.7437			

Table 3.13 One-way analysis of variance showing the effects of fly sex on resting heights during the night in Lantana thicket

Source of Variation	D.F.	Sum of squares	Mean square	F ratio	Signif. of F
Between sexes	1	2.8901	2.8901	0.940	0.3338
Within sexes	164	504.4904	3.0762		
Total	165	507.3805			

Table 3.14 One-way analysis of variance showing the effects of fly sex on resting heights during the night in forest

Source of Variation	D.F.	Sum of squares	Mean square	F ratio	Signif. of F
Between sexes	1	18.5281	18.5281	3.452	0.0796
Within sexes	18	96.6093	5.3672		
Total	19	115.1375			

Table 3.15 One-way analysis of variance showing the effects of nutritional status of the flies on resting heights during the night in coffee plantations

Source of Variation	D.F.	Sum of squares	Mean square	F ratio	Signif. of F
Between hunger stages	1	46.7330	46.7330	6.578	0.0156
Within hunger stages	30	213.1244	7.1041		
Total	31	259.8573			

Table 3.16 One-way analysis of variance showing the effects of nutritional status of the flies on resting heights during the night in banana plantations

Source of Variation	D.F.	Sum of squares	Mean square	F ratio	Signif. of F
Between hunger stages	1	22.5082	22.5082	6.901	0.0162
Within hunger stages	20	65.2332	3.2617		
Total	21	87.7415			

Table 3.17 One-way analysis of variance showing the effects of nutritional status of the flies on resting heights during the night in *Lantana* thicket

Source of Variation	D.F.	Sum of squares	Mean square	F ratio	Signif. of F
Between hunger stages	1	0.0371	0.0371	0.021	0.8841
Within hunger stages	44	75.9411	1.7259		
Total	45	75.9782			

Table 3.18 One-way analysis of variance showing the effects of nutritional status of the flies on resting heights during the night in forest

Source of Variation	D.F.	Sum of squares	Mean square	F ratio	Signif. of F
Between hunger stages	1	0.7544	0.7544	0.248	0.6202
Within hunger stages	80	243.8256	3.0478		
Total	81	244.5800			

Table 3.19 One-way analysis of variance showing the effects of nutritional status of the flies on resting heights during the day in coffee plantations

Source of Variation	D.F.	Sum of squares	Mean square	F ratio	Signif. of F
Between hunger stages	1	1.9102	1.9102	0.588	0.4466
Within hunger stages	52	168.9045	3.2482		
Total	53	170.8146			

Table 3.20 One-way analysis of variance showing the effects of nutritional status of the flies on resting heights during the day in banana plantations

Source of Variation	D.F.	Sum of squares	Mean square	F ratio	Signif. of F
Between hunger stages	1	0.4465	0.4465	0.077	0.7844
Within hunger stages	19	110.1249	5.7960		
Total	20	110.5714			

Table 3.21 One-way analysis of variance showing the effects of nutritional status of the flies on resting heights during the day in Lantana thicket

Source of Variation	D.F.	Sum of squares	Mean square	F ratio	Signif. of F
Between hunger stages	1	2.3295	2.3295	0.744	0.3904
Within hunger stages	106	332.0228	3.1323		
Total	107	334.3523			

Table 3.22 One-way analysis of variance showing the effects of nutritional status of the flies on resting heights during the day in forest

Source of Variation	D.F.	Sum of squares	Mean square	F ratio	Signif. of F
Between hunger stages	1	2.2969	2.2969	0.377	0.5491
Within hunger stages	14	85.3125	6.0937		
Total	15	87.6093			

Table 3.23 Percentage of flies found in different sites (No. of observations in brackets)

Site \ Vegetation type	NIGHT				DAY			
	Coffee	Banana	Lantana	Forest	Coffee	Banana	Lantana	Forest
Leaf*	76.9 (90)	87.8 (36)	11.8 (22)	25.5 (12)	64.1 (41)	80.4 (37)	10.8 (11)	17.5 (24)
Twig	19.7 (23)	0.0	69.9(130)	61.7 (29)	17.2 (11)	0.0	27.5 (28)	21.9 (30)
Branch:								
0.5-1 cm	0.0	0.0	14.0 (26)	8.5 (4)	6.3 (4)	4.3 (2)	37.3 (38)	32.1 (44)
1 -1.5 cm	1.7 (2)	0.0	3.8 (7)	2.1 (1)	1.6 (1)	2.2 (1)	10.8 (11)	5.8 (8)
1.5-2 cm	0.9 (1)	0.0	0.5 (1)	2.1 (1)	7.8 (5)	4.3 (2)	12.7 (13)	13.9 (19)
2	0.9 (1)	12.2 (5)	0.0	0.0	3.1 (2)	8.7 (4)	1.0 (1)	8.8 (12)
Total	100.0(117)	100.0 (41)	100.0(186)	100.0 (47)	100.0 (64)	100.0 (46)	100.0(102)	100.0(137)

* or banana fibre

Table 3.24 A comparison of resting behaviour of some members of the palpalis group

Species	Country	Favoured sites in order of preference	Circumferences of resting object	Height above ground	References
G. palpalis	Egbe, Nigeria	Branches	0.5–4 cm	3.0 m	Onyiah (1979)
	Bara, Nigeria	Leaves Twigs	– –	0–18 m	Scholtz et al. (1976)
	Zaria, Nigeria	Stems and/or branches	2.5–5 cm	0.3–2.15 m	Abdurrahim (1971)
	Congo	Leaves Twigs	–	0–50 cm	Carnevale and Adam (1971)
G. tachinoides	Western Burkina Faso	Leaves	–	0.76–1.57 m	Kupper and Koch (1983)
G. p. gambiensis	Burkina Faso	Leaves	–	30 cm	Challier (1973)
	Mali	Logs Boles	– –	1.0 m	Okiwelu (1981)
G. f. fuscipes	Bukedi, Uganda	Twigs, Branches	–	1.5–2.7 m	Van Vegten (1969)
	Busoga, Uganda	Leaves, Twigs, Branches	0.5–1 cm	0–3.66 m	Present study

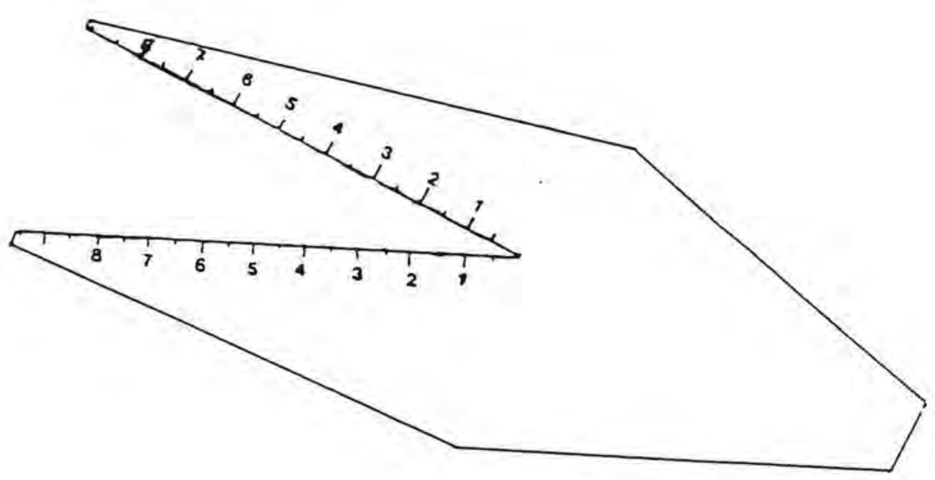


Figure 3.I V-shaped Twig-stem thickness measure
(see also Plate 3.6)

Fig.3·2 Resting surfaces, postures, and support orientation.

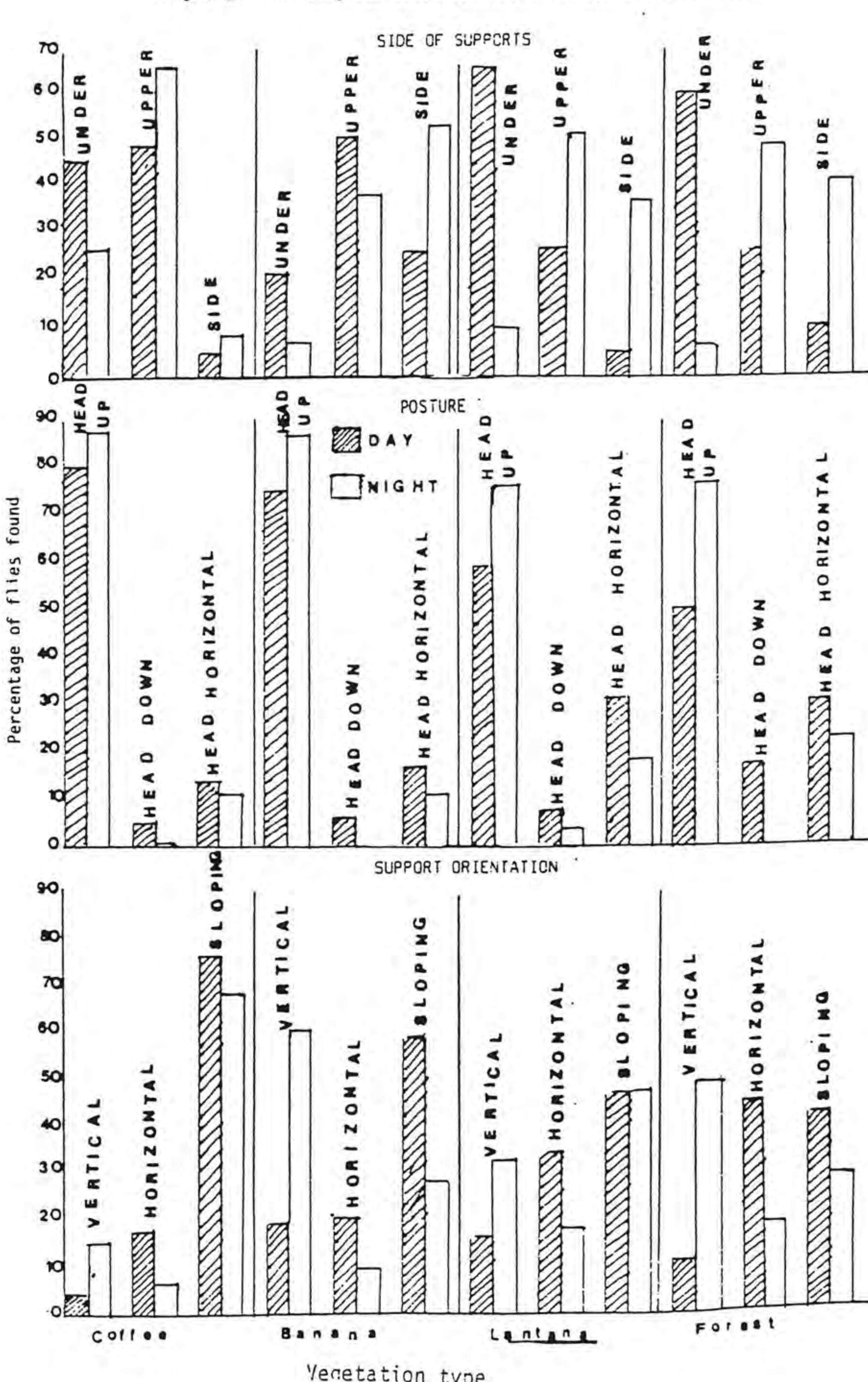


Plate 3.1 Feeding a sample of tsetse flies prior to release for resting site studies

Plate 3.2 Dusting tsetse flies with fluorescent powder

3.I

3.2

Plate 3.3 A search for night resting flies in coffee plantation

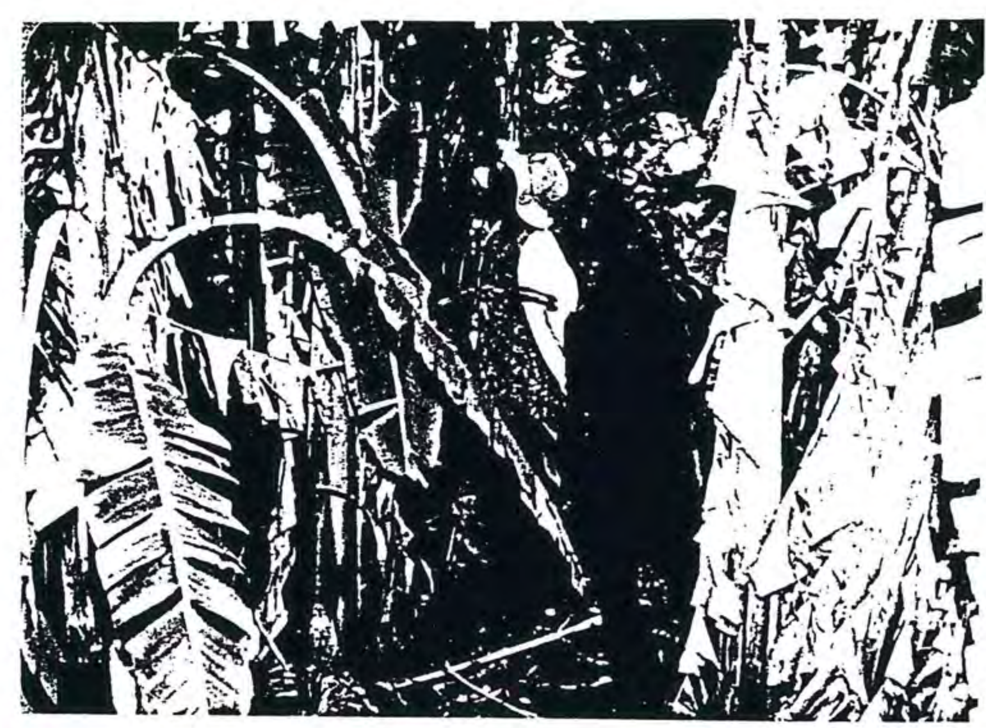
Plate 3.4 A search for day resting flies in *Lantana* thicket

3·3

3·4

Plate 3.5 A search for day resting flies in banana plantation

Plate 3.6 Measuring the resting site diameter



3.5

3.6

Plate 3.7 A fly resting on a leaf in coffee plantation at night

Plate 3.8 A fly resting on a green banana leaf at night

3.7

3.8

Plate 3.9 A fly resting on a dead banana leaf at night

Plate 3.10 A fly resting on a twig in *Lantana* thicket at night

3.9

3.10

CHAPTER 4

CHEMICAL TAXONOMY OF GLOSSINA

4.1 Introduction

The genus *Glossina* was originally placed in the family Muscidae in either the sub-family Stomoxyinae or Glossininae (Newstead *et al.*, 1924; Imms, 1957). However, because of uncertain affinities of this genus with other members of the Muscidae, other authorities decided to place it in a monogeneric family, the Glossinidae (Brues *et al.*, 1954; Haeselbarth *et al.*, 1966). After studying the unique wing venation, the location of the abdominal spiracles, and the details of sperm storage and transfer, Pollock (1971) concluded that *Glossina* should not be regarded as a muscid.

The three sub-genera of *Glossina* have been mentioned in Chapter 1. Although differences between these sub-genera have been confirmed by studies on karyotypes of some species of *Glossina* (Itard, 1966, 1970, 1971; Hulley, 1968; Baldry, 1970; Maudlin, 1970) the arrangement of species within these groups appears unsatisfactory and there are still a number of problems to be solved. Males of the three sub-species of *G. morsitans*, namely *G. m. submorsitans*, *G. m. morsitans* and *G. m. centralis* can be identified by minor features of their male genitalia but the genital differences between the females are smaller and they are therefore harder to separate. Gooding (1982) gives a phenogram based on enzyme

electrophoresis which casts doubt on *G. morsitans'* sub-species status. He found that *G. pallidipes* was genetically closer to *G. m. morsitans* than to either *G. m. centralis* or *G. m. submorsitans*. He therefore suggested that these sub-species should be regarded as independent species, though this idea has yet to be adopted.

In the *palpalis* group, *G. fuscipes* was once thought t be a sub-species of *G. palpalis*, but it is now recognized as an independent species (Jordan, 1977). *G. f. fuscipes* and *G. palpalis* complexes are supposed to have three and two sub-species respectively. These sub-species (as in *G. morsitans*) can be separated from each other by minor features in their genitalia.

Members of the *fusca* group have not been extensively studied because they are of less significance as vectors of trypanosomiasis. The group comprises species and sub-species which have morphologically similar features; separation is sometimes possible only on geographical grounds.

Besides morphological differences, there are also behavioural differences and differences in vectorial capacity between *Glossina* species and sub-species. For example differences in reproductive rate were found to exist between *G. m. morsitans* from Zimbabwe and Tanzania differences in copulation time were found between *G. pallidipes* from Zimbabwe and from Uganda (Jaenson, 1978) and the same species was reported to have genetic and behavioural differences by Langley *et al.* (1984).

G. brevipalpis from Lugala, Uganda, has proved difficult to colonize (Okoth, unpublished) while the same species from Kibwezi in Kenya has been successfully colonized (S.K. Moloo, personal communication 1985).

Recently, advanced biochemical techniques have been applied to studies on the taxonomy of Glossina. Analysis of the cuticular hydrocarbons of insects using Gas Liquid Chromatography may help to solve taxonomic questions posed by closely related species and sub-species and by allopatric populations.

The structure, general chemical composition and function of insect cuticle has been extensively described (Imms 1957; Wigglesworth 1976; Lockey 1974). The cuticular wax of adult insects, which overlies the cuticle, contains complex mixtures of hydrocarbons (Jackson and Blomquist, 1976). The composition of these hydrocarbon mixtures varies with insect species and analyses of them can therefore be used to identify insects. Such analysis was used by Lockey (1976) to show that Locusta, Schistocerca and Periplaneta could be separated on the basis of their cuticular hydrocarbons. Lockey (1978) showed, by chromatographic analyses, that hydrocarbons of closely related species of beetles were similar qualitatively and quantitatively. Following these preliminary reports, other researchers embarked on the evaluation of cuticular hydrocarbon analysis for separating vector species. Analysis of cuticular hydrocarbons using GLC was used to identify Anopheles gambiae complex species, namely An. gambiae s. str. and

An. arabiensis, both vectors of malaria, by Carlson and Service (1979, 1980). Similar studies were carried out by Carlson and Walsh (1981) and Phillips et al. (1985) to separate the Simulium damnosum species complex and Ryan et al. (1986) to separate the sandflies, Psychodopygus wellcomei and P. complexus. Carlson (1981) was able to identify members of the palpalis and morsitans groups of Glossina using this technique.

The present study was designed to explore the possible differences in cuticular hydrocarbons within the members of the fusca group and to discover if the hydrocarbon composition changed with the age of the fly. Such a discovery would be of great use as some of the current methods of ageing tsetse are inaccurate and have limitations. Differences in allopatric populations of G. pallidipes were also examined. Finally puparial hydrocarbons were analysed in order to determine if the technique could be used to identify tsetse puparia found in the field as they are difficult to identify by morphology, particularly where several species of the same group occur together.

4.2 Materials and Method

Adult tsetse flies for the analyses were obtained from various sources (Table 4.1) and stored in a dessicator. Puparia of G. p. gambiensis and G. f. fuscipes, both members of the palpalis group, were used to study puparial cuticular components. These puparia were hatched in individual tubes in an incubator at 24°C and 80% R.H. in order to determine their sexes.

Cuticular components were extracted from dry fly samples by immersing them individually in a 1-ml glass vial of n-hexane (spectrophotometric grade). The crude lipids, which include fatty acids and polar substances, were injected into the Gas Liquid Chromatograph.

The GLC used was a Hewlett Packard 5790A, equipped with an on-column injector and a flame ionization detector. The on-column injector is suitable for analysing crude extracts which have very low concentrations. The column was a 2 m CPsil5 capillary column with i.d. of 0.32 mm and film thickness of 0.15 µm. Helium was the carrier gas used at a flow rate of 1 ml/min, while hydrogen and air formed the flame and were used at flow rates of 40 and 240 ml/min respectively. The detector temperature was 340°C but the injector was at room temperature, as this is "cold" on-column injection.

Data was automatically recorded by a Hewlett Packard 3390A integrator connected to the GLC. The scale of chromatographic profiles was controlled by functions of keys on the integrator. These plot parameters include ZERO which plots the baseline position, ATTENUATION (ATT 2↑) plots the height scale, CHART SPEED (CHT SP) controls paper advance speed, PEAK-WIDTH (PK WD) controls selectivity in distinguishing peaks from noise or each other by filtering data, THRESHOLD (THRSH) rejects noise for integration and AR REJ (AR) rejects peaks with less value than the value set for the AR REJ.

Hydrocarbons in a sample are separated on the column and each has a unique retention time. The integrator prints the retention time and an integrated area of all the peaks which is then used for statistical analysis. T types of peaks produced depend on the conditions in the GLC, integrator parameters chosen and the concentrations of the extracts. These conditions need to be determined before any analysis begins.

4.2.1 Standardization of method

Before the study was carried out, preliminary investigations were necessary to standardise experimenta procedures and conditions suitable for analysis of cuticular hydrocarbons in tsetse fies.

4.2.1.1 Tsetse fly samples

G. f. fuscipes (Table 4.1) were bred at Salford for these investigations. Whole flies, legs, wings and puparial shells were used to determine their suitabilit for chemotaxonomy using GLC.

4.2.1.2 Extraction time

Whole fly samples were individually immersed in 0.4 0.2 ml of n-hexane in glass vials. Legs and wings were immersed in 50 µl, puparial shells in 0.2 ml of n-hexan Crude lipids were extracted from female and male fly samples for either 10 seconds, 30 seconds, 1 minute, 10 minutes, 30 minutes, 1 hour, 6 hours or 16 hours. Th extracts were concentrated by evaporating the solvent t dryness. The extract was re-suspended in 2 µl of n-hexa for 1 minute before injecting 1 µl into the column. Oth

samples of similar extracts were not concentrated by evaporation; 1 µl was injected into the GLC.

4.2.1.3 Gas Liquid Chromatograph conditions

Flow rates of gases mentioned above were maintained. Oven temperature conditions were programmed to give ranges of 75°-310°C, 100°-310°C and 120°-310°C. The initial temperature was run for 2 minutes followed by a 7°C/min rise.

4.2.1.4 The integrator conditions

The sensitivity of the integrator was changed by altering the attenuation and threshold. Two combinations were used (Table 4.2).

4.2.2 External standard

Hydrocarbon standards (C_9-C_{38}) were diluted with hexane to give 100 ppm and chromatographed using the same conditions described above. 5 replicas were made to ensure consistency. These peaks were then used for qualitative analysis by comparing them directly with the unknown peaks of the fly samples and by calculating the Kovat Index (Kovat 1965).

4.2.3 Internal standard

For quantitative analysis of the cuticular hydrocarbons, an internal standard, that is a known amount of a particular hydrocarbon, which is incorporated in the fly's crude extract and whose peak area can be compared to the rest of the unknown peaks of the fly's crude lipids, was used. Hydrocarbons $C_{15}$and C_{17} at 200 ppm were tested for use as internal standards using identical conditions to the above.

4.2.4 Results of the experimental procedures and conditions

In all the fly samples used, the number of peaks increased with the length of extraction time. Whole fly samples gave overload peak concentrations of at least 1000 ppm from 30 min extraction onwards at attenuation 2 and threshold 4. The best results were found with the non-concentrated 10 min extract (Figure 4.1). Good chromatographic profiles were produced with concentrated 10 min extracts for the wings and puparial shells (Figures 4.2 and 4.3). 6-hour extraction produced the bes results for the legs (Figure 4.4).

By comparing the standards (C_9-C_{38}) directly with the unknown peaks of the fly samples, it was found that the hydrocarbon peaks appear at temperatures above 200°C. n-Pentadecane (C_{15}) whose retention time was 1.40 min, came after the solvent peak at 0.20 min and separated wel from the rest of the peaks.

4.2.5 Final choice of procedures and conditions

The following procedures and conditions were adapted for the present study:

(i) Only whole flies were used. These were immersed in glass vials containing 0.2 ml of 25 ppm n-Pentadecane (as an internal standard) in n-hexan for 10 minutes. 1 μl of the whole extract was then injected into the GLC.

(ii) Cuticular components of puparia were extracted in 0.2 ml of n-hexane for 10 minutes and then concentrated by evaporating the solvent. The extract was re-suspended with 2 μl of n-Pentadecane at 200 ppm for 1 minute before injecting 1 μl into the GLC.

(iii) The column oven temperature was programmed to give 120°C for 2 min followed by a rise to 310°C at 7°C/min. Other conditions of the GLC remained as described above.

(iv) Integrator parameters used were:

ZERO	=	0, 0.0
ATT 2↑	=	2
CHT SP	=	1.0
PK WD	=	0.04
THRSH	=	4
AR REJ	=	0

4.3 Statistical Analysis

All chromatographic peaks may be used in the statistical analysis. However, it was noted that there was a great variability with regards to fatty acid peaks which usually appeared before 11.00 min. In some samples fatty acids gave overload concentrations of at least 1000 ppm and in others they appeared to be completely absent (see chromatograms). Similarly peaks which appear after 25.00 min, were not consistent and they also varied from an overload concentration to dilutions that could not be

detected by the GLC. Peaks with retention times between 11.00 min and 25.00 min were found to be consistent. Two further problems must be borne in mind: a) A small sample may not be representative of the parent population, b) with a large number of variables, chance differences may be found between groups. This bias is eliminated by a jack-knifing procedure.

To limit peaks to those produced consistently, only those with retention times between 14.00 and 25.00 min in fusca and 11.00 min and 25.00 min in G. pallidipes and G. fuscipes age groups were used. In studies involving puparial shells, all chromatogram peaks were used as fewe peaks were produced. Sex differences were not examined du to the limited number of fly samples.

Chromatogram peaks with similar retention time in eac species group were allocated a number beginning with the internal standard peak (Peak 1). Tentative qualitative identification of the cuticular hydrocarbons was possible by direct comparison of the retention time of the unknown peaks with those of the known hydrocarbon standards. A correlation between the retention time and known carbon number (Figure 4.5) was drawn. The Kovat retention index (Kovat, 1965) was then determined by linear interpolation and in this way the carbon number of the unknown sample was determined. The Kovat index (KI) indicates whether th carbon is branched or not. The KI for n-alkenes/n-alkane is defined as 100 times the number of carbon atoms.

Closely related species possess similar hydrocarbons

and qualitative differences alone may not be sufficient to allow discrimination between groups. In addition to qualitative differences, there are also quantitative differences which also help to distinguish groups. Discriminant analysis was used to find differences between species groups. In this analysis, the contribution of each hydrocarbon to discrimination between groups is assessed and each hydrocarbon peak is included in the analysis by a step-wise variable selection process. The value of the discriminant function coefficient for each nominated peak indicates its relative importance. The number of discriminant functions is always one less than the number of groups involved. Discriminant functions are sums of products of peak weightings and areas. Eigenvalues indicate the significance of each function (variate). Discriminant function scores are used as rectangular coordinates for plotting scattergrams. A scattergram is a diagramatic way of indicating how similar or different the individual groups are, the more similar the groups the closer they appear on the diagram. Bias due to small sample size compared to the number of variables, which may be found in the discriminant analysis, is finally removed by classifying each individual using discriminant function formed from all the data other than its own. This is known as jack-knifing.

Discriminant analysis has been described by Morrison (1976) and Kendall (1983) and has recently been applied to identify sibling species of Simulium damnosum complex (Phillips et al., 1985), Psychodopygus wellcomei,

P. complexus (Ryan et al., 1986) and Anopheles culicifacies (Milligan et al., 1986).

4.4 Results

4.4.1 Fusca group (sub-genus Austenina)

A total of 118 flies were used (30 G. fuscipleuris, 20 G. medicorum, 34 G. f. congolensis, 14 G. f. fusca and 20 G. brevipalpis). Chromatograms showing typical example by sex and species are shown in Figures 4.6-4.15. By inspection of the profiles some qualitative differences can be seen: small differences between sexes and larger ones between species.

A total of 65 peaks were numbered and 41 of them were selected for the discriminant analysis. Their discriminar function coefficients are shown in Table 4.3. Four discriminant functions were used in separating the specie (Table 4.4). By inspection of their scores (Tables 4.3 and 4.4) based on the sum of products of hydrocarbon weightings and transformed peak areas, peaks that were most important in separating the species can be found (Table 4.5). The significance of the discriminant functi for all the species groups is shown in Table 4.6 and Table 4.7 shows the classification (jack-knifed) results A scattergram of the species is shown in Figure 4.16.

4.4.2 G. pallidipes (sub-genus Glossina s. str.)

75 flies were used (19 from Apach, Lango, 22 from Lugala, Busoga, 17 from Budongo, Bunyoro, all in Uganda and 17 from Zimbabwe). Examples of their chromatogram peaks are shown in Figures 4.17-4.24.

41 peaks were numbered and 26 were selected for the analysis (Table 4.8). Three discriminant functions were used (Table 4.9), their significance in the analysis is shown in Table 4.10. The most important peaks which were used to separate the allopatric populations are shown in Table 4.11 and the jack-knifed classification results are in Table 4.12. A scattergram of the groups is shown in Figure 4.25.

4.4.3 G. fuscipes (sub-genus Nemorhina) - age determination

18 30-day old and 18 1-day old adults and 11 puparial shells were used in this study. Typical chromatograms from these samples are shown in Figures 4.26-4.31.

A total of 40 peaks were numbered for the analysis but only 13 were utilised (Table 4.13). Two discriminant functions were used (Table 4.14) and their significance in the analysis is shown in Table 4.15. Peaks that contributed most in separating the groups are shown in Table 4.16 and the jack-knifed classification in Table 4.17. The scattergram of the groups is shown in Figure 4.32.

4.4.4 G. f. fuscipes and G. p. gambiensis puparial shells (sub-genus Nemorhina)

13 puparial shells each of G. f. fuscipes and G. p .gambiensis were analysed and typical profiles of the chromatograms are shown in Figures 4.33-4.36. Of a total of 41 peaks, 18 were selected for the discriminant analysis (Table 4.18). One discriminant function was used (Table 4.19) and its significance is shown in Table 4.20.

The most important peaks which separated the groups are shown in Table 4.21. The jack-knifed classification results are shown in Table 4.22. A histogram showing the percentage of individuals against discriminant function scores is shown in Figure 4.37.

4.5 Discussion

This is the first time that analysis of cuticular hydrocarbons from members of the _fusca_ group has been attempted. The work of Carlson (1981) on _Glossina_ posed technical problems:- in the treatment of fly samples and data analysis. He obtained crude extracts from different quantities of pooled flies which were given different treatments in terms of solvent (either ether or hexane), and extraction time ("rinsed . . . at least three times", soaked with syringe or sub-merged overnight). This method of approach added new variables to the fly sample extract which were not dealt with in the analysis. In addition, the quantity of hydrocarbons extracted will depend on the length of time used. The quantitative differences reporte may not therefore be reliable. No two individuals are exactly the same. There are variations in composition of cuticular hydrocarbons within species and these variation need to be assessed and compared within the variability between species. Hence the need for individual treatment of fly samples and the use of discriminant analysis. Discriminant analysis also allows all peaks to be considered.

These results (Table 4.7) predict an 80% chance of an member of the _fusca_ group being classified correctly usin

this technique. Table 4.5 shows the peaks which were used to separate members of this group. Peak 12 (KI 2681) has been most commonly used, appearing in G. f. congolensis, G. f. fusca and G. brevipalpis. All three peaks (KI 2553, 2681 and 3056) used in separating G. f. congolensis also appeared in G. f. fusca but the latter also possessed unique carbon numbers with KI values of 2518, 3283, 3310, 3380 and 3422. The closer the groups, the more likely they are to have hydrocarbons with similar KI values.
G. f. congolensis and G. f. fusca are sub-species of G. fusca so the similarity in peaks was expected.
Table 4.7 and the scattergram in Figure 4.17 all indicate a closer relationship between G. f. congolensis and G. f. fusca than between any other members of the group; G. fuscipleuris being very far from the rest of the group. The classification in Table 4.7 predicts a 73.5% and 85.7% chance of correct identification of G. f .congolensis and G. f. fusca respectively. This suggests that either this technique is very sensitive or in fact, that the two sub-species should be considered as independent species. The position of some of the members of fusca group is still uncertain. Jordan (1977) discusses some of the problems. Techniques such as hydrocarbon analysis may supply the answers. It would be of interest to study all the 14 species and sub-species of fusca group and to try to plot a phenogram based on the composition of cuticular hydrocarbons.

Differences in allopatric populations of G. pallidipes from Apach in Lango, northern Uganda, Budongo in Bunyoro,

western Uganda, Lugala in Busoga, eastern Uganda and from Zimbabwe were observed in the study. Vegetation in Bunyor and Busoga was described in Chapter 1. Apach G. pallidipe came from Kamdin on the Victoria Nile and this population extends northwards into Acholi (Map 1.1). In Bunyoro isolated populations exist from the Victoria Nile to Lake Albert (Wooff, W.R., personal communication).

It is most unlikely that the Budongo population near Lake Albert had any physical link with the Apach one. Wha is even more interesting is that there is only a 5.26% chance of a G. pallidipes from Apach being attributed to Budongo by the technique of analysis (Table 4.12). G. pallidipes from Budongo was considerably different fr the species caught elsewhere (Figure 4.26). In view of t fact that we are dealing with geographically separated populations of the same species, 64% correct identifications of a fly to a particular location would regarded as quite high (Table 4.12). This technique shou tell us how closely related are any two or more groups o populations. This observation is further evidence that there are differences in allopatric populations in G. pallidipes (Jaenson, 1978; Langley et al., 1984).

Carlson et al. (1984) attempted to isolate and identify contact sex pheromones in G. pallidipes. They identified the compound as 13,23-dimethylpentatriacontar and when synthesized, it was able to increase male response with the increasing doses. They also reported that this compound was always present in wild-caught females from a wide geographical range. There is need t

show if there are any quantitative differences in this compound in allopatric populations of G. pallidipes, especially populations already shown to be different by analysis of cuticular hdyrocarbons. Such a proof may explain the differences in copulation time observed by Jaenson (1978) between G. pallidipes from Zimbabwe and Uganda. It is emphasized, however, that such a study requires individual treatment of fly samples.

Perhaps the most interesting uses of this technique are to age tsetse and to identify puparia. The results (Table 4.17) show that there is a 72% chance of G. f. fuscipes being aged correctly. It would appear that the amount of hydrocarbons increases with age but it seems likely that at some age this amount stabilises. Further tests are required using large fly samples; examination of flies from 1 day old to 100 days old (at 10 day intervals) with at least 100 flies per group should provide much more information and probably increase the chances of correct ageing. At present the most widely used methods of ageing tsetse are by examination of the female reproductive system (see also Chapter 5) and by wingfray. The ovarian method has limitations in that it can only be used for females and, accurately, for age groups up to 80 days, while wingfray technique is usually used for males;- the judgement of the frays is subjective. Recently a new method of ageing tsetse in which the build-up over time of pteridines in the head capsules is used was reported by Lehane and Mail (1985). This technique and that of the present study may offer greater prospects as tools to age

tsetse since they can be used on both sexes.

In the existing keys for identifying puparia of Glossina (Potts, 1973) minor features are used. Posterior lobes of puparia are among the principal structures used in the identification of puparia. Phrases in the key such as "key-hole more rounded" or "key-hole shallower" are vague and subjective. With this technique, otherwise unidentifiable fragments of puparia can be used. In the field such fragments could give a clue as to where tsetse breed but are easily confused with fragments from pupae o other Diptera which are often found in the same breeding sites (see Chapter 2). The probability of identifying puparia correctly (Table 4.22) is 76% which is probably higher than if morphological characters are used.

There are no means of sexing puparia on morphological grounds. Large numbers of puparial shells can sometimes b found in breeding spots. If these puparial shells can be sexed by this technique, our knowledge of sex ratio in tsetse population studies would be advanced.

Little is known about how insects synthesise cuticula hydrocarbons. Hadley (1977) found that the summer form of the desert beetle (Eleodes armata) exhibited higher quantities of hydrocarbons and a higher percentage of lon chain components than did the winter form. He found littl dietary effect on the epicuticular hydrocarbon composition. Baker et al. (1978) observed that tricalcium phosphate (a food additive in stored grain) altered the ratio of cuticular hydrocarbons when it was incorporated in the diet of the red flour beetle (Tribolium castaneum)

Cuticular hydrocarbons are probably by-products of a genetically controlled metabolic process involving a number of factors of which climate and diet probably are but a few. These limitations on hydrocarbon synthesis may restrict insect distribution; it is known that insects require hydrocarbons as protection against dessication and for sexual attraction. A clearer understanding of hydrocarbon synthesis may allow us to manipulate it and so control insect pests.

Certain modifications are needed to maximise the potential use of cuticular hydrocarbon analysis for improving insect taxonomy:

1. The methodology should be standardized. This includes both:
   a) The chromatographic equipment and
   b) The individual treatment of fly samples.
2. A standard statistical analysis should be employed. Discriminant analysis which deals with samples with many variables, is recommended.
3. There is a need for a central data bank so that new samples can be quickly compared and immediately classified. In view of the variations within species, such data banks should contain random samples of known species taken from all over the infested areas.

In conclusion, the analysis of cuticular hydrocarbons using GLC can separate members of the fusca group and can detect differences between allopatric populations of G. pallidipes. The technique may prove a useful tool for ageing tsetse flies and for identifying tsetse puparia.

Table 4.1 Details of the species of tsetse used in hydrocarbon analysis

Species	Source/Method of Collection	Original Locality/ Country	Year of Collecti
G. f. fuscipleuris	wild, pinned	Toro/Ankole, Uganda	1970/72
G. f. congolensis	wild, pinned	Toro/Ankole, Uganda	1970/72
G. f. fusca	wild, pinned	Budongo, Uganda	1978
G. brevipalpis	Laboratory ILRAD* (received pupae)	Kibwezi, Kenya	1985
G. medicorum	wild,dry	Ivory Coast	1985
G. fuscipes	Laboratory, IEMVT** established 1968	Central African Republic	1985
G. p. gambiensis	Laboratory, IEMVT established 1972	Upper Volta	1985

Table 4.1 (Contd)

Species	Source/Method of Collection	Original Locality/ Country	Year of Collection
G. pallidipes	wild, pinned	Apach, Uganda	1984
	wild, pinned	Budongo, Uganda	1973
	Laboratory UTRO*** established 1966	Lugala, Uganda	1985
	wild, dry	Zimbabwe	1985

* International Laboratory for Research on Animal Diseases (Nairobi, Kenya)

** Institute d'Elevage et Médecine Vétérinaire des Pays Tropicaux (Paris, France)

*** Uganda Trypanosomiasis Research Organization (Tororo, Uganda)

Table 4.2 Integrator parameters tested

Function	Abbreviation	Combination 1	Combina 2
Baseline Position	ZERO	0, 0.0	0, 0.
Scale Height (Attenuation)	ATT 2↑	1	2
Paper Advance Speed	CHT SP	1.0	1.
Peak Width	PK WD	0.04	0.
Threshold Level	THRSH	3	4
Peak Rejection Level	AR REJ	0	0

Table 4.3 Fusca group standardised discriminant function coefficients

Peak	KI	FUNC 1	FUNC 2	FUNC 3	FUNC 4
2	2480	-0.24098	0.30866	0.48843	0.67285
4	2518	-0.16924	-0.00355	0.09386	1.07429
5	2553	1.15594	-1.11346	-0.44102	2.09597
6	2576	-0.09179	0.05059	-0.11543	0.30295
7	2584	-0.07918	0.80145	-0.21619	-0.69333
8	2594	0.59541	0.91336	0.29602	0.39676
10	2617	0.45503	-1.94735	0.55250	-0.61142
11	2650	-0.91526	0.23446	0.10388	-1.76494
12	2681	1.00353	-0.93649	0.64464	-0.04000
13	2717	0.23839	2.36720	-0.15122	0.98729
14	2744	0.84569	0.75363	0.05097	-0.30146
15	2753	-0.92209	0.80189	-1.48160	-0.88242
16	2772	0.05134	0.36490	-0.02042	0.09433
17	2784	-0.56459	-1.05892	0.86605	0.62103
18	2802	-0.29190	-0.32371	0.20031	0.56477
25	2920	-0.04362	1.99519	-0.67029	-0.51800
26	2954	0.79891	0.42414	-0.04074	-0.15469
27	2971	0.51018	1.16300	-0.70431	0.90375
28	2983	-0.04408	-0.26497	-0.51858	0.46505
30	3007	-0.94001	0.23449	0.45959	-0.58615
31	3016	0.52958	0.77062	0.28696	0.74422
33	3056	1.67728	-1.06446	0.59926	-0.38408
34	3075	-0.47920	0.43364	-0.09869	0.15842
35	3087	0.51642	-0.66708	0.87376	-0.34984

Table 4.3 (Contd)

Peak	KI	FUNC 1	FUNC 2	FUNC 3	FUNC 4
40	3176	0.01021	0.05205	-0.22944	-0.57680
45	3237	-1.90279	-2.60439	-0.27340	-0.98724
46	3263	-0.17746	0.84063	-0.28833	0.23900
47	3283	0.09192	-0.43682	-0.02534	1.43543
48	3310	0.80183	-0.36264	0.43706	1.72281
49	3327	-1.01448	-0.68337	0.70775	-0.69003
50	3349	-0.78228	-0.52156	1.12573	-0.78990
52	3360	0.11121	-1.03486	-1.36158	1.11399
53	3407	-0.35258	-0.79635	0.21637	0.46593
54	3422	0.76493	-0.52133	0.12454	1.44586
55	3436	-0.11188	0.94133	-0.36548	0.90114
57	3480	-0.01228	2.50213	-0.74774	-1.41453
58	3510	-0.30538	-0.17091	0.04795	-1.09233
60	3549	0.90833	1.12716	0.53099	-0.49150
62	3581	-0.60444	-0.37093	-0.10018	-0.46972
63	3604	0.85809	0.60426	0.15236	0.04173
64	3624	-0.27806	-0.38816	-0.03416	-1.07004

Table 4.4 *Fusca* group Discriminant functions evaluated at group means (group centroids)

SPECIES	FUNC 1	FUNC 2	FUNC 3	FUNC 4
G. fuscipleuris	-2.16495	-2.36825	-2.20808	0.09335
G. medicorum	-2.26945	5.32865	-0.23866	0.62449
G. f. congolensis	2.67738	0.34839	0.10724	-1.96970
G. f. fusca	4.83483	-0.57366	-0.01021	3.33542
G. brevipalpis	-2.41904	-1.96698	3.37562	0.24918

Table 4.5 The most important peaks used in separating members of the *Fusca* group

Species	Peaks	Kovat Index
G. fuscipleuris	16	2772
	63	3604
G. medicorum	13	2717
	25	2920
	27	2971
	57	3480
G. f. congolensis	5	2553
	12	2681
	33	3056
G. f. fusca	4	2518
	5	2553
	12	2681
	33	3056
	47	3283
	48	3310
	52	3383
	54	3422
G. brevipalpis	2	2482
	11	2650
	12	2681
	34	3075
	49	3327
	50	3349
	60	3549

Table 4.6 Significance of discriminant function (*fusca* group)

	Function			
	1	2	3	4
Eigenvalue	8.24457	7.27666	3.32475	2.6280
% Variance	38.39	33.89	15.48	12.2
Chi-square	666.51	457.45	258.79	121.1
Degree of Freedom	164	120	78	3
Significance Level	<0.0001	<0.0001	<0.0001	<0.000

Table 4.7 Classification results of fusca group (Jack-knifed)

TOTAL = 118

PERCENT CORRECT = 80.5

PERCENT INCORRECT = 19.5

Species	N	G. fuscipleuris	G. medicorum	G. f. congolensis	G. f. fusca	G. brevipalpis
G. fuscipleuris	30	26 (86.67%)	1 (3.33%)	1 (3.33%)	1 (3.33%)	1 (3.33%)
G. medicorum	20	2 (10.00%)	17 (85.00%)	1 (5.00%)	0 (0.00%)	0 (0.00%)
G. f. congolensis	34	3 (8.82%)	1 (2.94%)	25 (73.53%)	4 (11.76%)	1 (2.94%)
G. f. fusca	14	0 (0.00%)	0 (0.00%)	2 (14.29%)	12 (85.71%)	0 (0.00%)
G. brevipalpis	20	3 (15.00%)	1 (5.00%)	0 (0.00%)	1 (5.00%)	15 (75.00%)

Table 4.8 <u>G. pallidipes</u> group standardised discriminant function coefficients

Peak	KI	FUNC 1	FUNC 2	FUNC 3
2	2311	-0.52420	-0.79939	-0.56297
5	2470	0.12648	0.87001	0.90390
6	2490	0.41390	0.50558	0.34264
7	2501	0.52074	0.43923	0.03639
8	2513	-0.67197	0.78394	0.92506
9	2550	-0.57796	-1.26655	-0.59951
11	2610	0.45356	-0.27772	-0.01069
12	2649	-0.22660	-0.50161	-0.94342
13	2676	0.44544	0.37233	-0.09419
18	2808	-0.34557	0.30563	0.19481
19	2879	-0.22348	-0.61625	-1.16113
21	2949	0.48455	-1.59019	0.46418
22	2981	0.15109	1.47250	-0.04705
23	2989	-0.80956	0.28536	0.53103
24	3008	0.60459	0.51089	0.16135
25	3050	-0.03142	0.88348	-0.20017
26	3084	0.97309	0.65961	1.55242
28	3118	-1.35699	0.73426	-0.26333
32	3252	0.46560	-0.10719	0.47257
33	3282	-0.80562	0.15119	-0.07935
34	3323	0.84318	-0.09028	-0.26623
36	3381	-0.30346	-0.77896	-0.31694
37	3454	-0.05566	-0.15476	0.30539
39	3553	-0.70320	0.04899	0.02232
40	3584	1.04272	-0.24576	-1.19927
41	3608	0.28774	0.32983	0.48604

Table 4.9 *G. pallidipes* group Discriminant functions evaluated at group means (group centroids)

LOCALITY	FUNC 1	FUNC 2	FUNC 3
Apach	0.16530	-2.72239	1.00542
Lugala	1.45771	-0.19979	-1.75263
Budongo	-4.62807	1.05233	-0.02706
Zimbabwe	2.55688	2.24889	1.17146

Table 4.10 Significance of discriminannt function (*G. pallidipes* group)

	Function 1	2	3
Eigenvalue	7.35959	3.47180	1.55107
% Variance	59.44	28.04	12.53
Chi-square	268.90	143.62	55.254
Degree of Freedom	78	50	24
Significance Level	<0.0001	<0.0001	0.0003

Table 4.11 The most important peaks used in separating allopatric *G. pallidipes* populations

Locality	Peaks	Kovat Index
Apach, Lango	5	2470
	8	2513
	26	3084
Lugala, Busoga	26	3084
	34	3323
	40	3584
Budongo, Bunyoro	23	2989
	28	3118
	33	3283
	39	3553
Zimbabwe	5	2470
	8	2513
	23	2989
	41	3608

Table 4.12 Classification results for allopatric G. pallidipes (jack-knifed)

TOTAL = 75

PERCENT CORRECT = 64

PERCENT INCORRECT = 36

Locality	No.	Apach	Lugala	Budongo	Zimbabwe
Apach	19	11 (57.89%)	4 (21.05%)	1 (5.26%)	3 (15.79%)
Lugala	22	5 (22.73%)	14 (63.64%)	1 (4.55%)	2 (9.09%)
Budongo	17	2 (11.76%)	1 (5.88%)	14 (82.35%)	0 (0.00%)
Zimbabwe	17	1 (5.88%)	5 (29.41%)	2 (11.76%)	9 (52.94%)

Table 4.13 G. f. fuscipes age groups standardised discriminant function coefficients

Peak	K1	FUNC 1	FUNC 2
8	2727	0.83028	0.37112
10	2774	0.74325	-0.39309
12	2812	-0.67651	0.31547
15	2916	1.18977	0.57656
16	2936	-1.50460	0.10716
19	3006	0.05542	1.16941
23	3118	-0.71284	-1.02726
24	3182	-0.74536	0.55372
31	3359	1.48178	-0.42280
32	3384	-0.75429	0.59987
33	3407	-0.50377	-0.18649
35	3458	0.06076	1.04864
38	3560	0.60120	-0.76484

Table 4.14 G. f. fuscipes group discriminant functions evaluated at group means (group centroids)

AGE GROUP	FUNC 1	FUNC 2
Puparia	2.22731	0.65856
1 day old	-2.18112	0.72029
30 days old	-0.07558	-2.25631

Table 4.15 Significance of discriminant function

(*G. f. fuscipes* age group)

	Function 1	Function 2
Eigenvalue	3.97705	1.6624
% Variance	70.52	29.4
Chi-square	98.195	-37.21
Degree of Freedom	26	1
Significance Level	< 0.0001	0.000

Table 4.16 The most important peaks used in separating age groups of *G. f. fuscipes*

Age groups	Peaks	Kovat Index
Puparia	15	2916
	31	3359
1 day old	12	2812
	16	2936
	23	3118
	24	3182
	32	3384
30 days old	23	3118
	31	3359
	38	3560

Table 4.17 Classification results of <u>G. f. fuscipes</u> age group (jack-knifed)

TOTAL = 47

PERCENT CORRECT = 72.3

PERCENT INCORRECT = 27.7

Age Group	No.	Pupae	1 day old	30 days old
Pupae	16	9 (56.25%)	4 (25.00%)	3 (18.75%)
1 day old	20	2 (10.00%)	15 (75.00%)	3 (15.00%)
30 days old	11	0 (0.00%)	1 (9.09%)	10 (90.91%)

Table 4.17 Classification results of *G. f. fuscipes* age group (jack-knifed)

TOTAL = 47

PERCENT CORRECT = 72.3

PERCENT INCORRECT = 27.7

Age Group	No.	Pupae	1 day old	30 days old
Pupae	16	9 (56.25%)	4 (25.00%)	3 (18.75%)
1 day old	20	2 (10.00%)	15 (75.00%)	3 (15.00%)
30 days old	11	0 (0.00%)	1 (9.09%)	10 (90.91%)

Table 4.18 Puparial shells of G. f. fuscipes and G. p. gambiensis standardised discriminant function coefficients

Peak	KI	FUNC 1
2	1941	17.36350
3	2038	-2.50255
4	2133	30.26029
5	2235	-23.05971
7	2436	4.27782
8	2537	2.34100
11	2731	-6.65753
17	2877	27.15979
18	2901	5.98443
20	2976	-7.91099
21	3002	-6.34321
22	3040	-8.68593
26	3168	4.47059
27	3207	11.16788
33	3425	-11.21610
34	3477	-9.59758
37	3610	13.70351
40	3805	-13.08378

Table -.19 Puparial shells of *G. f. fuscipes* and *G. p. gambiensis* discriminant functions evaluated at group means (group centroids)

PUPAE	FUNC 1
G. f. fuscipes	21.41453
G. p. gambiensis	-21.41453

Table 4.20 Significance of discriminant function

(G. f. fuscipes and G. p. gambiensis puparial sh

	Function 1
Eigenvalue	496.79736
% Variance	100.00
Chi-square	93.153
Degree of Freedom	18
Significance Level	< 0.0001

Table 4.21 The most important peaks used in separating puparial shells of G. f. fuscipes and G. p. gambiensis

Age groups	Peaks	Kovat Index
G. f. fuscipes	2	1941
	4	2133
	17	2877
	27	3207
	37	3610
G. p .gambiensis	5	2235
	22	3040
	33	3425
	34	3477
	40	3805

Table 4.22 Classification results of puparial shells (jack-kni

TOTAL	=	26
PERCENT CORRECT	=	76.9
PERCENT INCORRECT	=	23.1

Pupae	No.	*G. f. fuscipes*	*G. p. gambiensis*
G. f. fuscipes	13	8 (61.54%)	5 (38.46%)
G. p. gambiensis	13	1 (7.69%)	12 (92.31%)

Figure 4.I

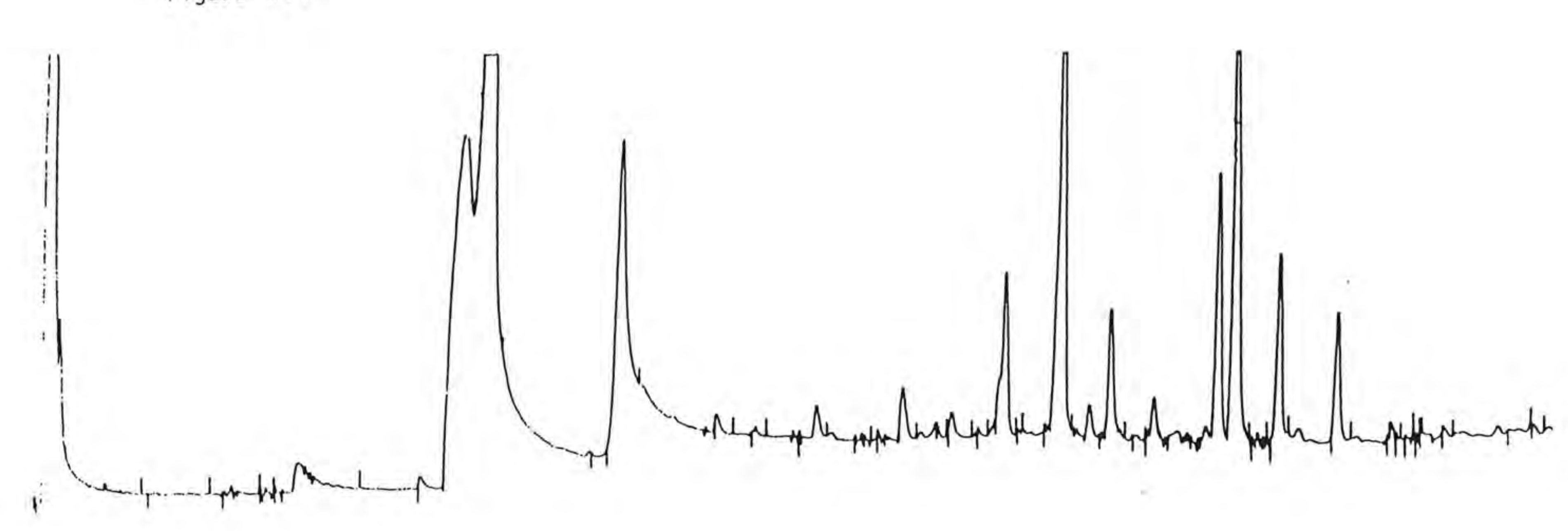


Figure 4.I Chromatogram from the IO-min hexane extract of a whole fly, female *G.f.fuscipes*. Extract not concentrated.

Figure 4.2

Figure 4.2 Chromatogram from the 10-min hexane extract of one wing, female *G.f.fuscipes*. Extract concentrated.

Figure 4.3

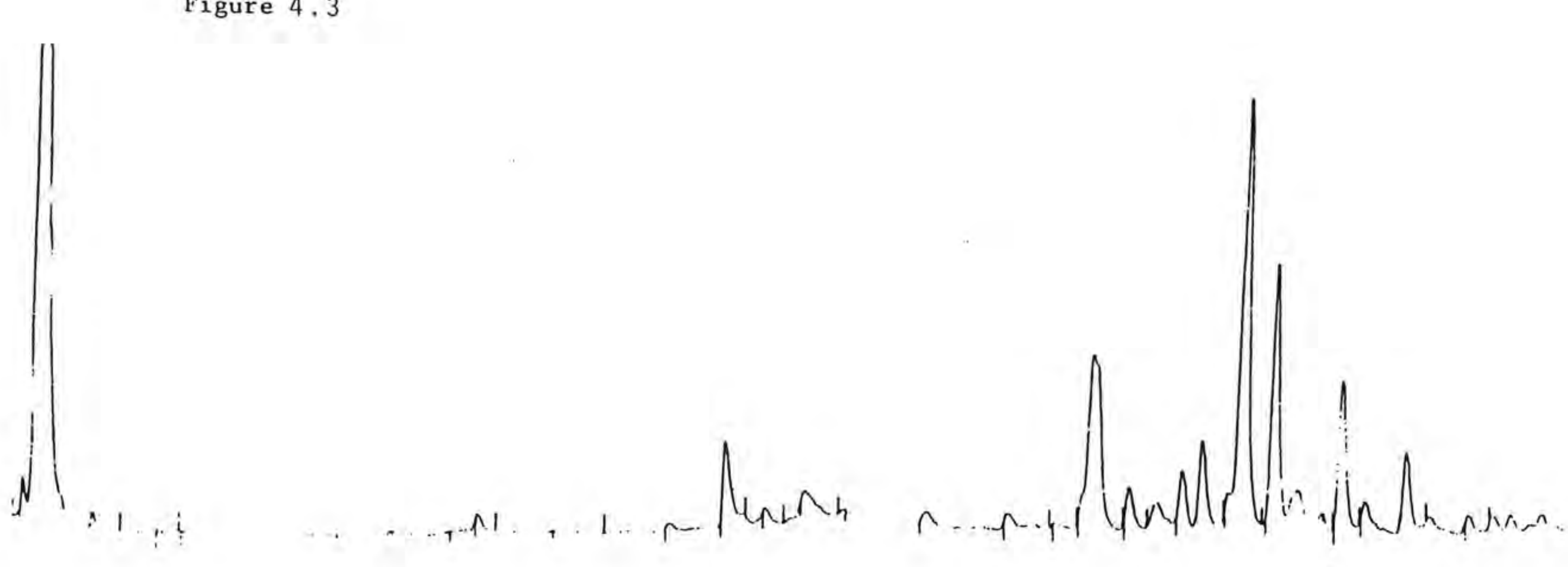


Figure 4.3 Chromatogram from the 10-min hexane extract of a puparial shell, male G.f.fuscipes. Extract concentrated.

Figure 4.4

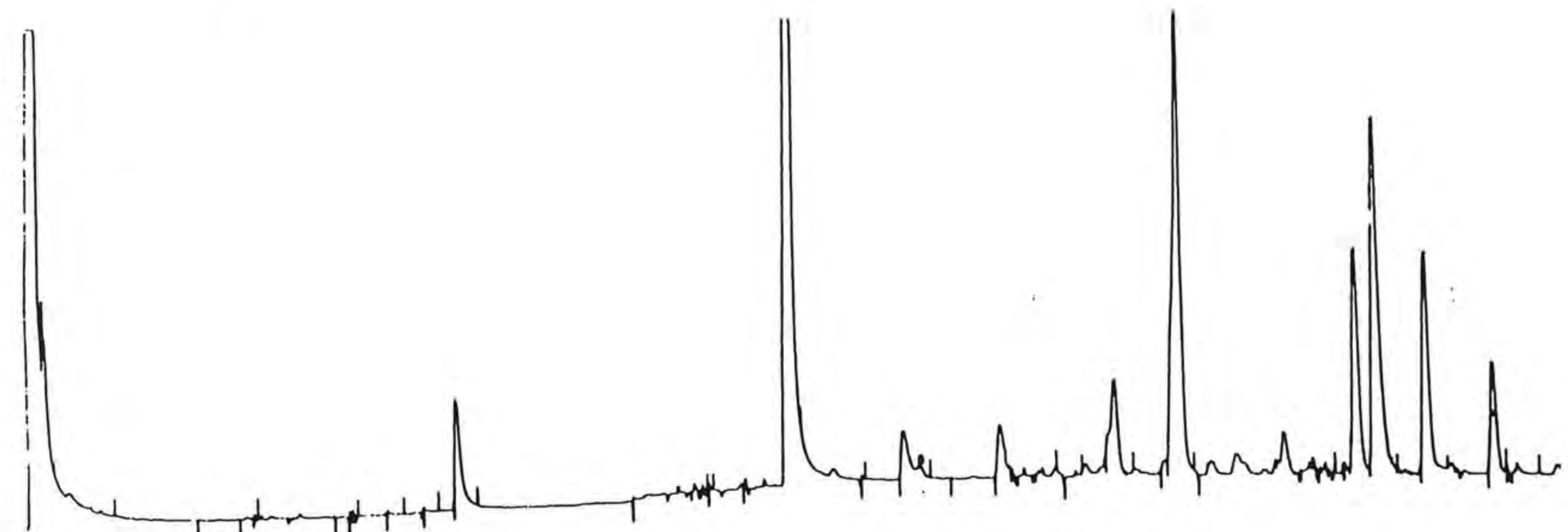


Figure 4.4 Chromatogram from the 6-hour hexane extract of one leg, female
G.f.fuscipes. Extract concentrated.

Figure 4.5 Retention Time versus 5 chromatograms of known Carbon numbers.

Figure 4.5

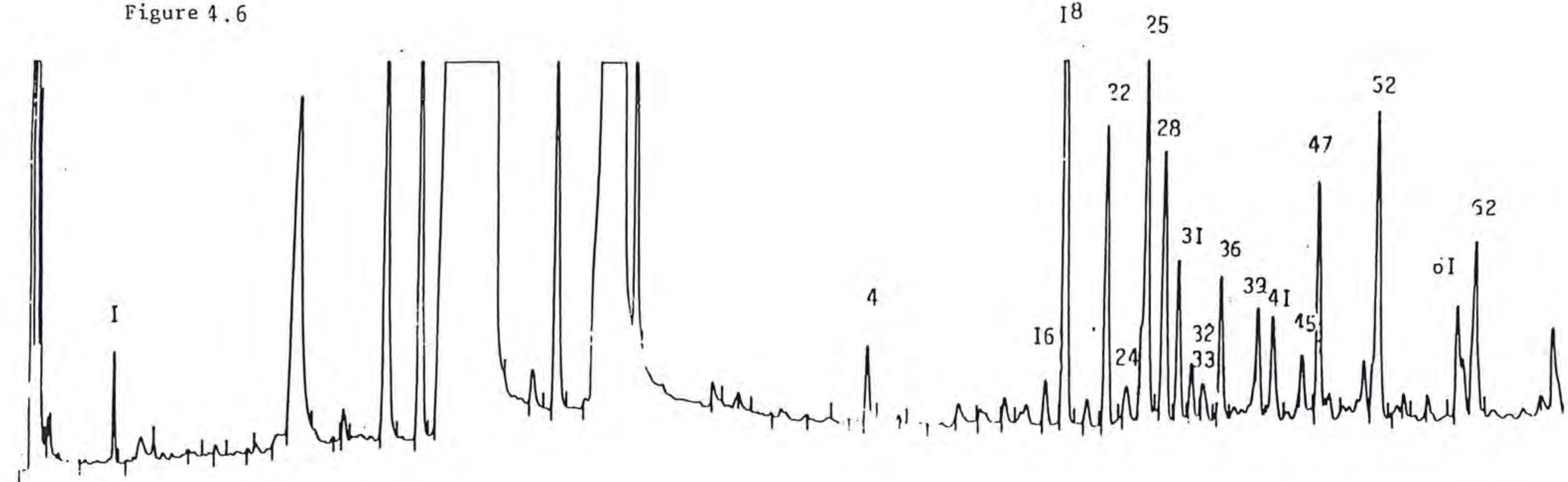


Figure 4.6 Chromatogram of female <u>G.fuscipleuris</u>

Figure 4.7

Figure 4.7 Chromatogram of male *G.fuscipleuris*

Figure 4.8

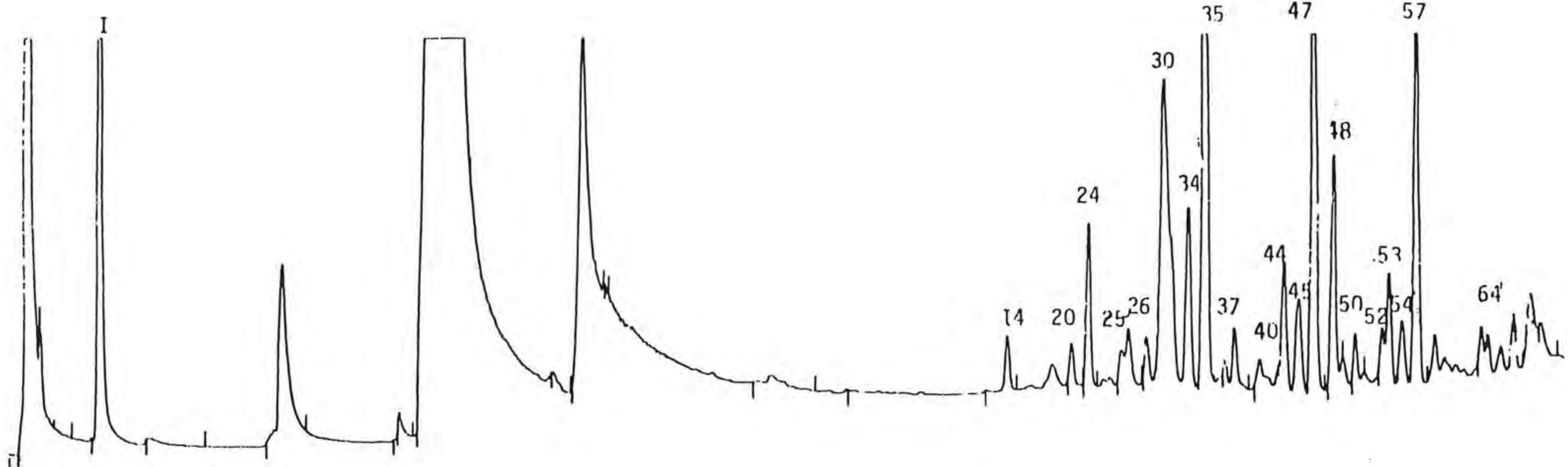


Figure 4.8 Chromatogram of female <u>C.medicorum</u>

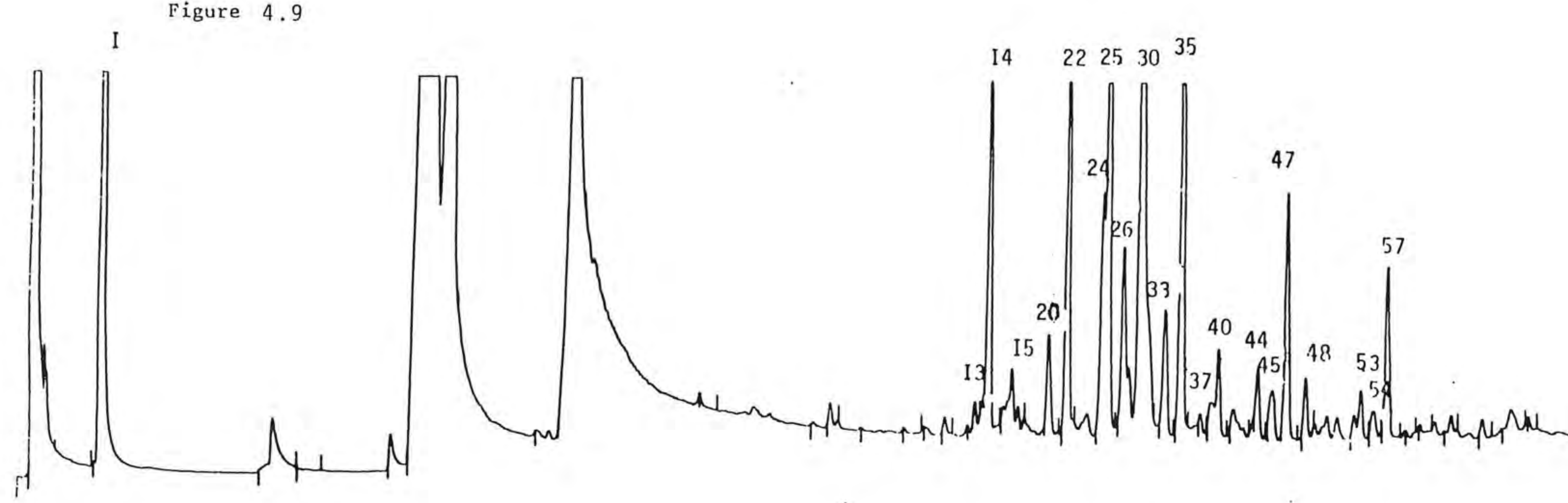


Figure 4.9 Chromatogram of male G.medicorum

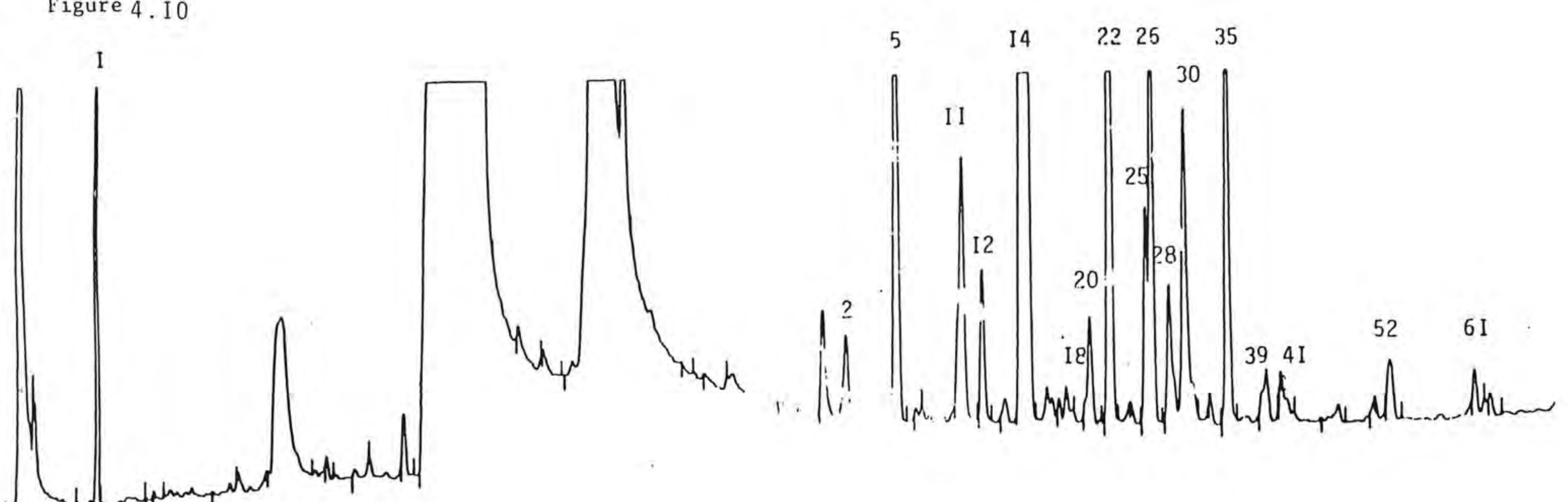


Figure 4.I0 Chromatogram of female <u>G.f.congolensis</u>

Figure 4.II

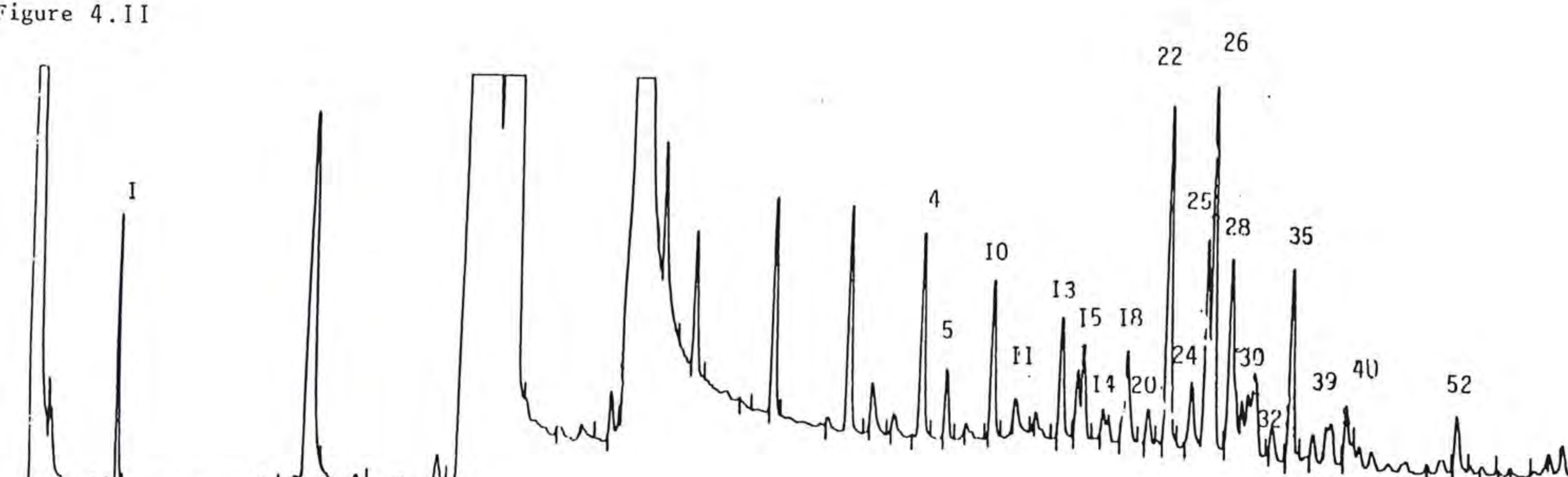


Figure 4.II Chromatogram of male <u>G.f.congolensis</u>

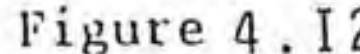


Figure 4.I2

Figure 4.I2 Chromatogram of female G.f.fusca

Figure 4.I3

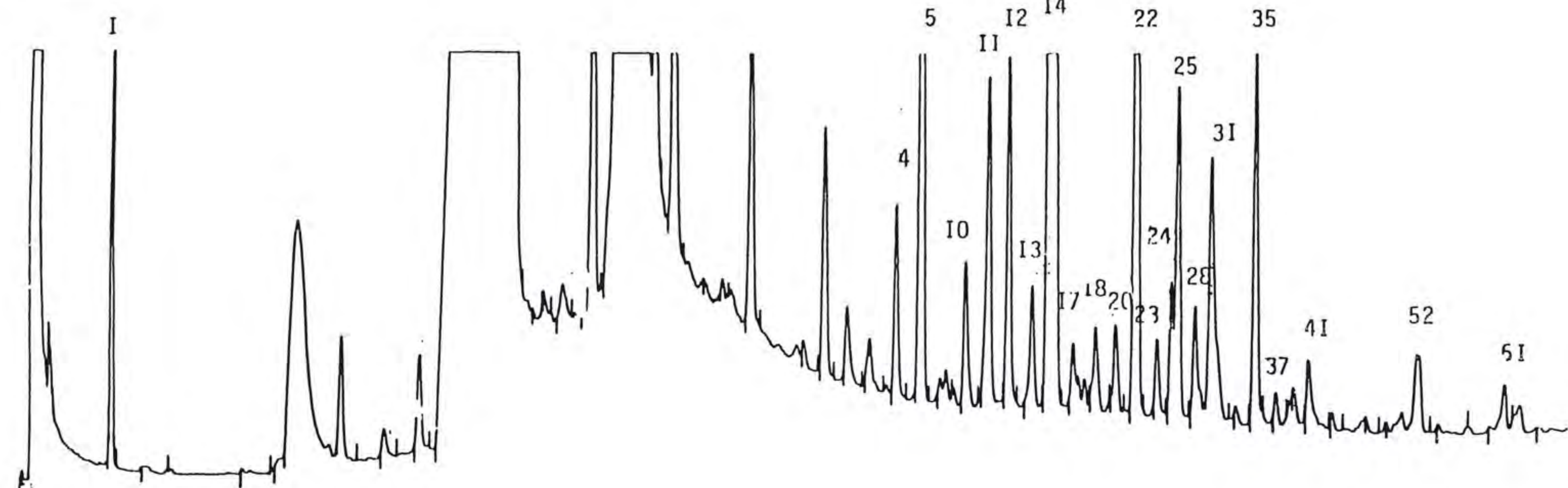


Figure 4.I3 Chromatogram of male _C.f.fusca_

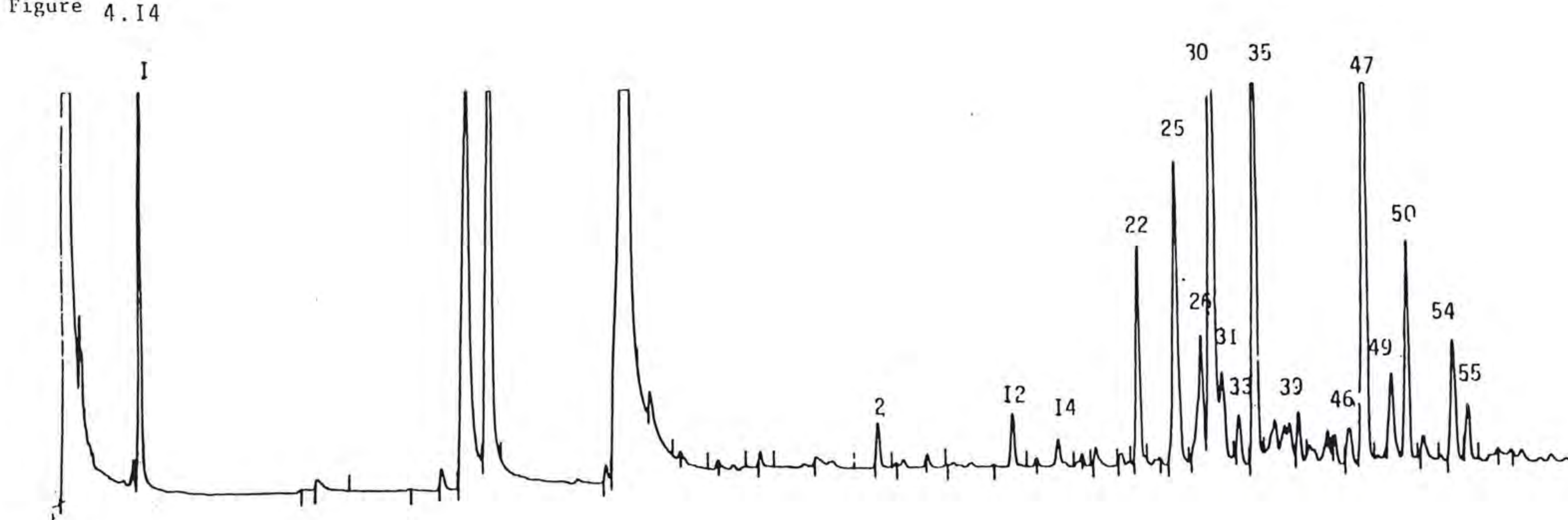


Figure 4.I4 Chromatogram of female <u>G.brevipalpis</u>

Figure 4.I5

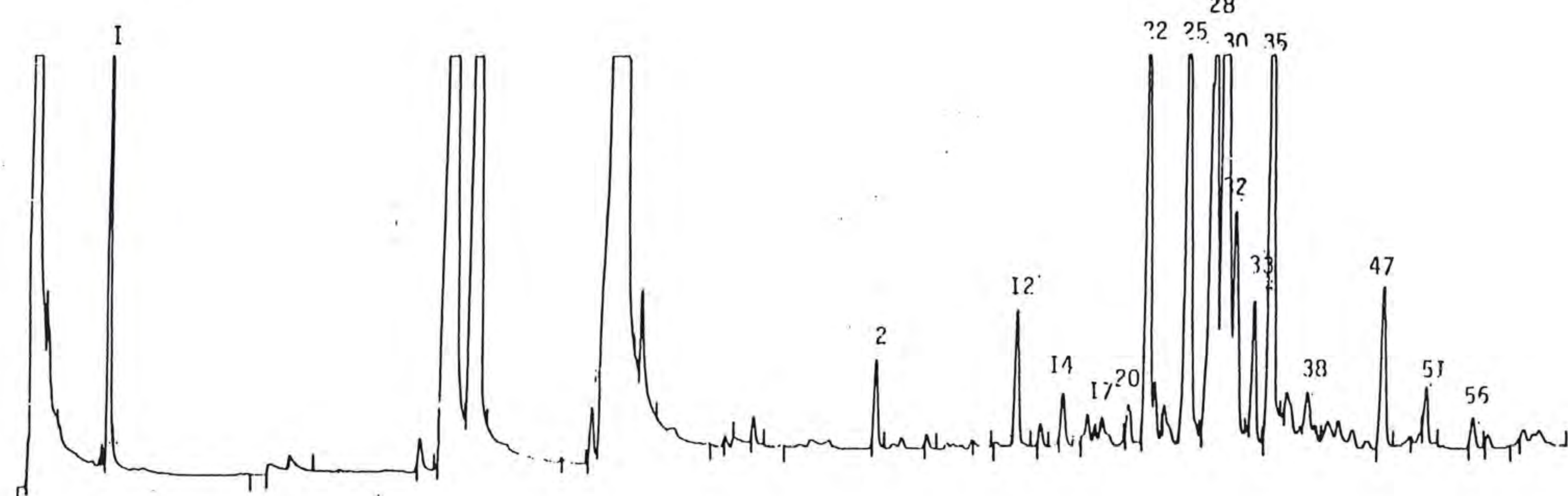


Figure 4.I5 Chromatogram of male G.brevipalpis

Figure 4.I6

Key:

× G.fuscipleuris

◇ G.medicorum

▽ G.f.congolensis

\+ G.f.fusca

□ G.brevipalpis

Figure 4.16

Distribution of individuals of <u>fusco</u> group species in the space of discriminant functions 1 and 2

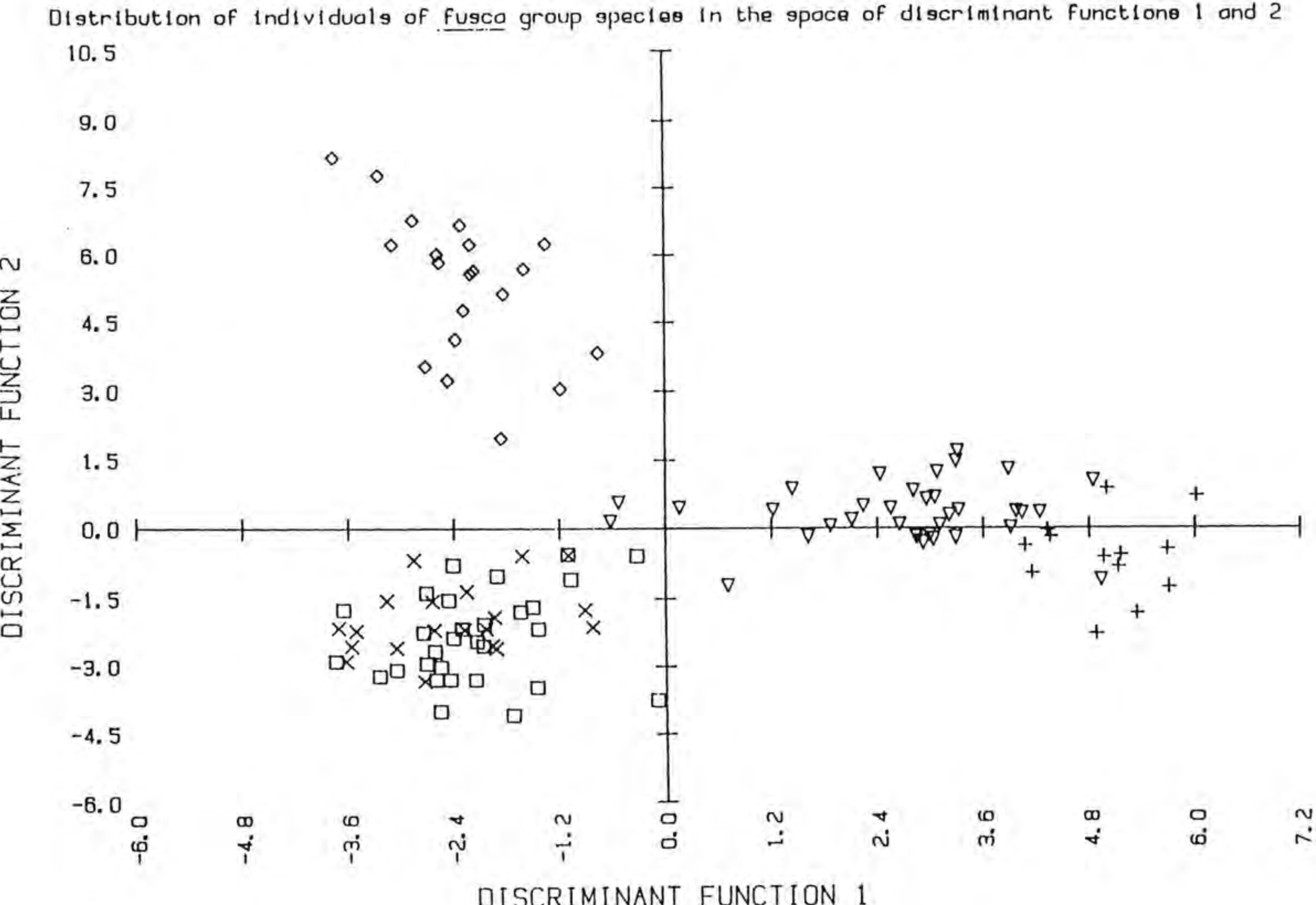


Figure 4.I7

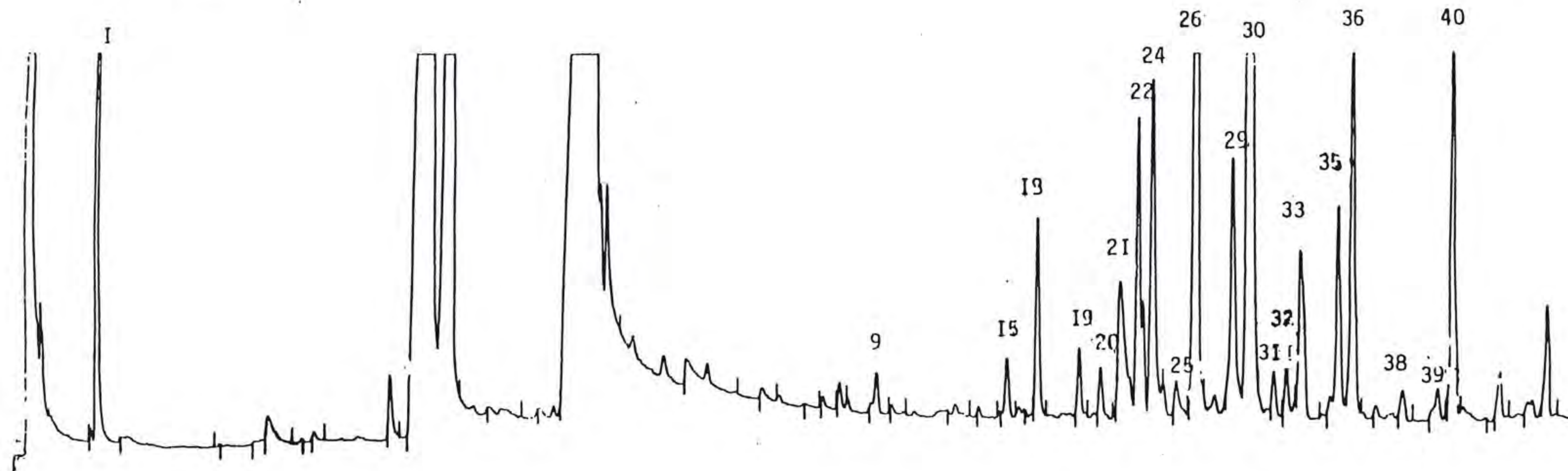


Figure 4.I7 Chromatogram of female G.pallidipes from Apach, Lango, Uganda

Figure 4.I8

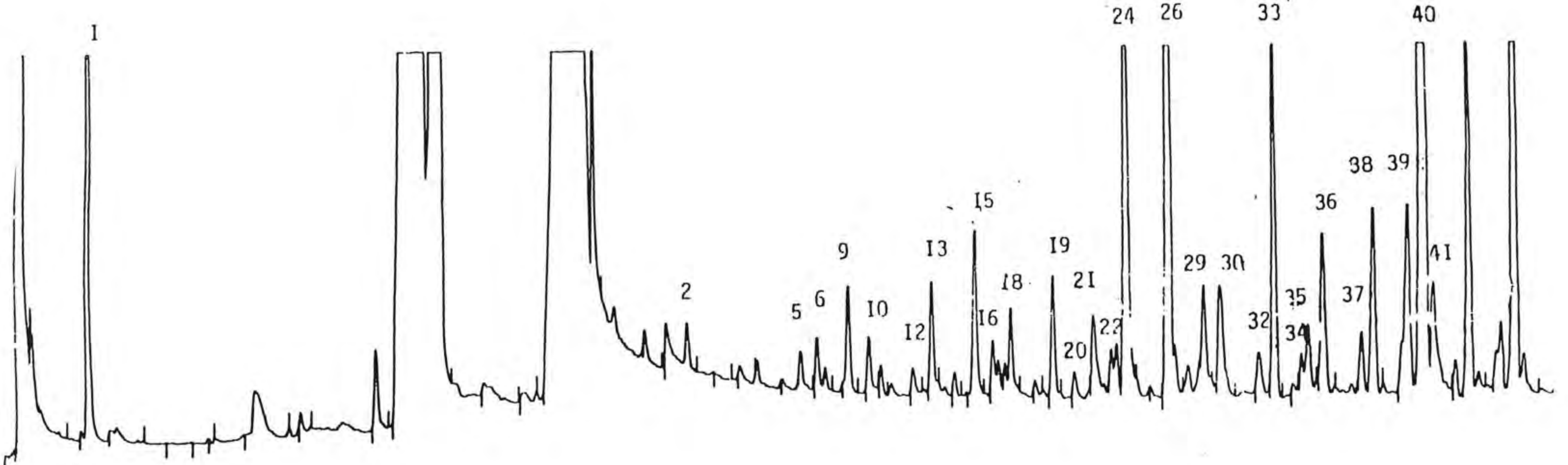


Figure 4.I8 Chromatogram of male _G.pallidipes_ from Apach, Lango, Uganda

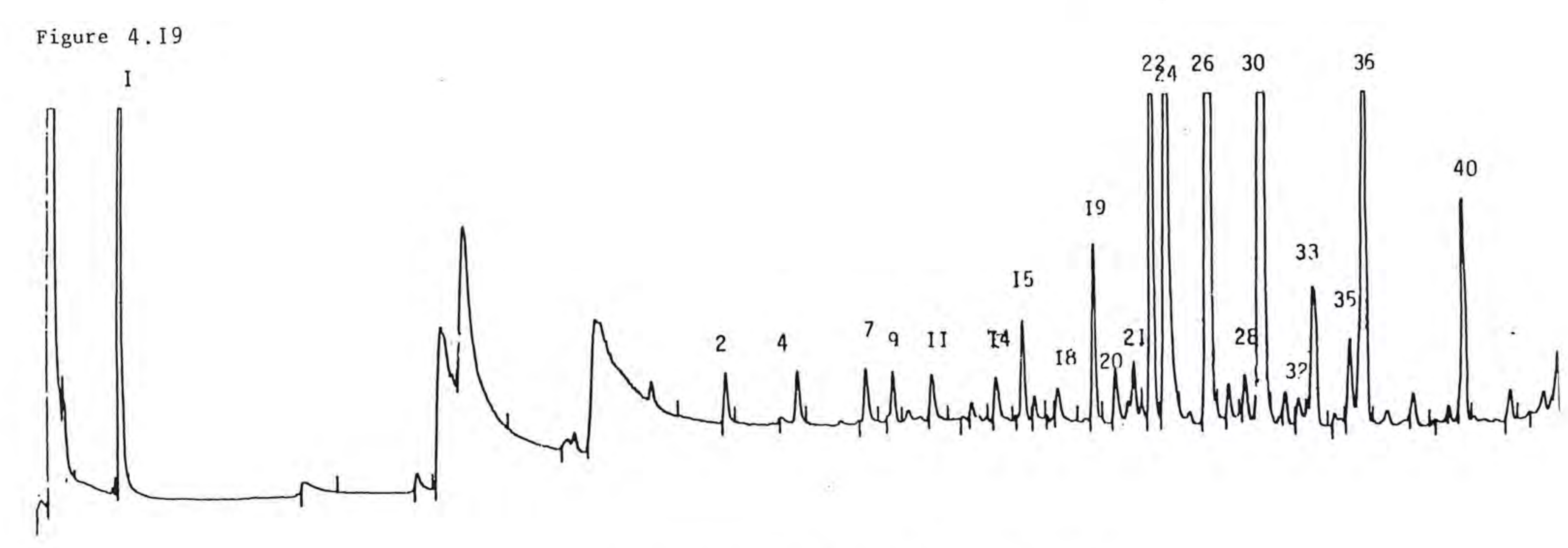


Figure 4.I9 Chromatogram of female G.pallidipes from Lugala, Busoga, Uganda

Figure 4.20

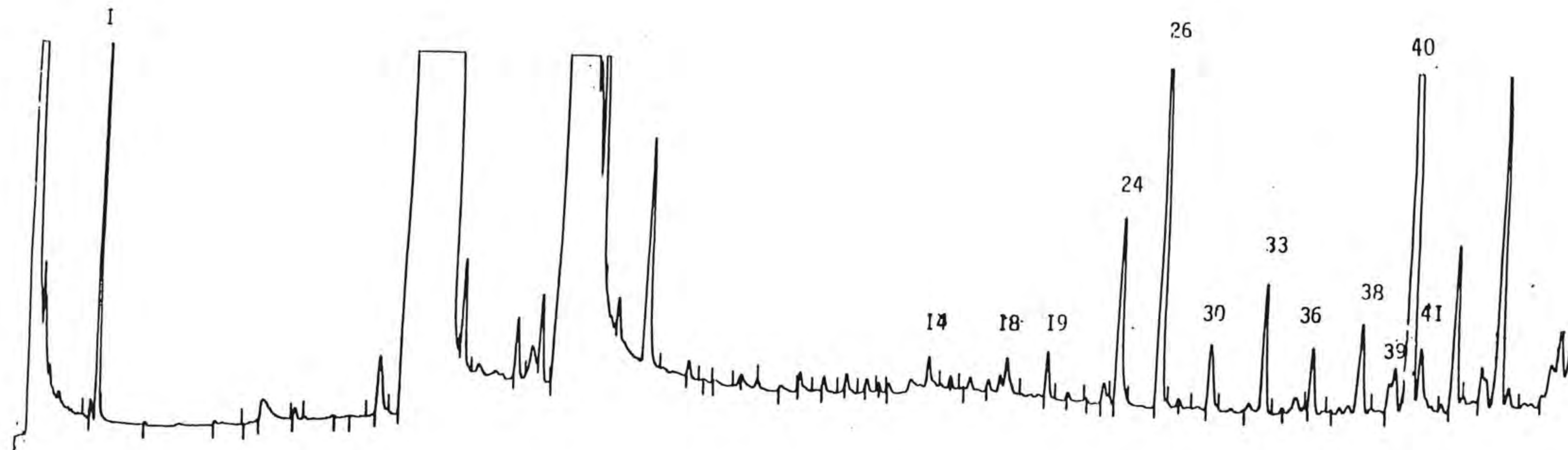


Figure 4.20 Chromatogram of male G.pallidipes from Lugala, Busoga, Uganda

Figure 4.2I

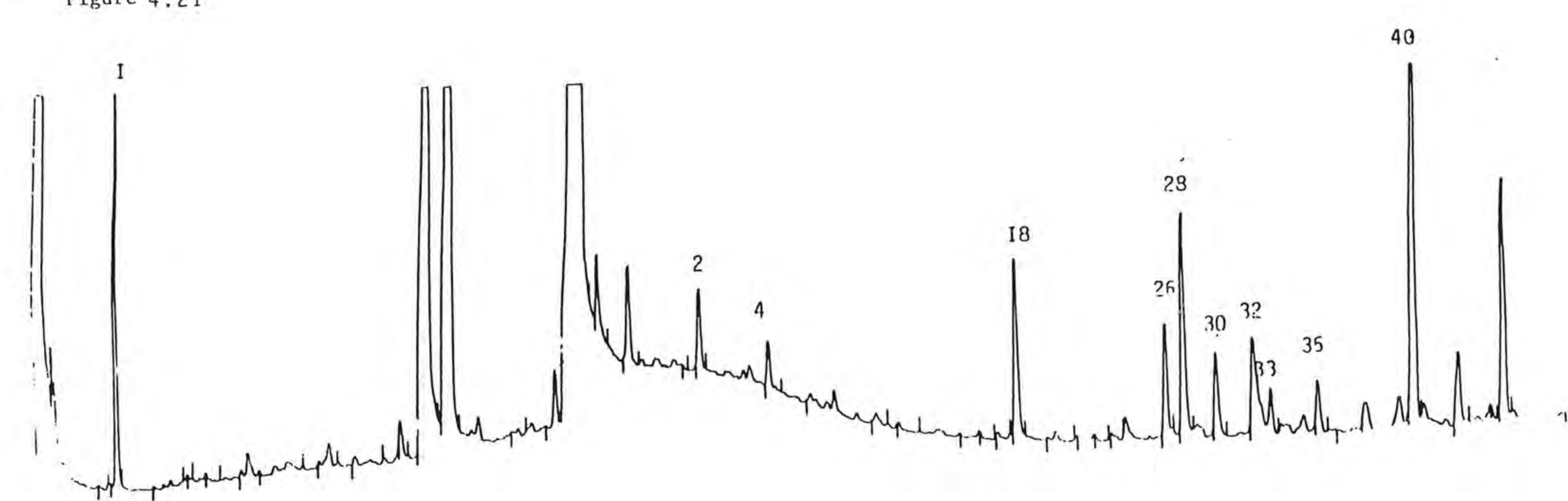
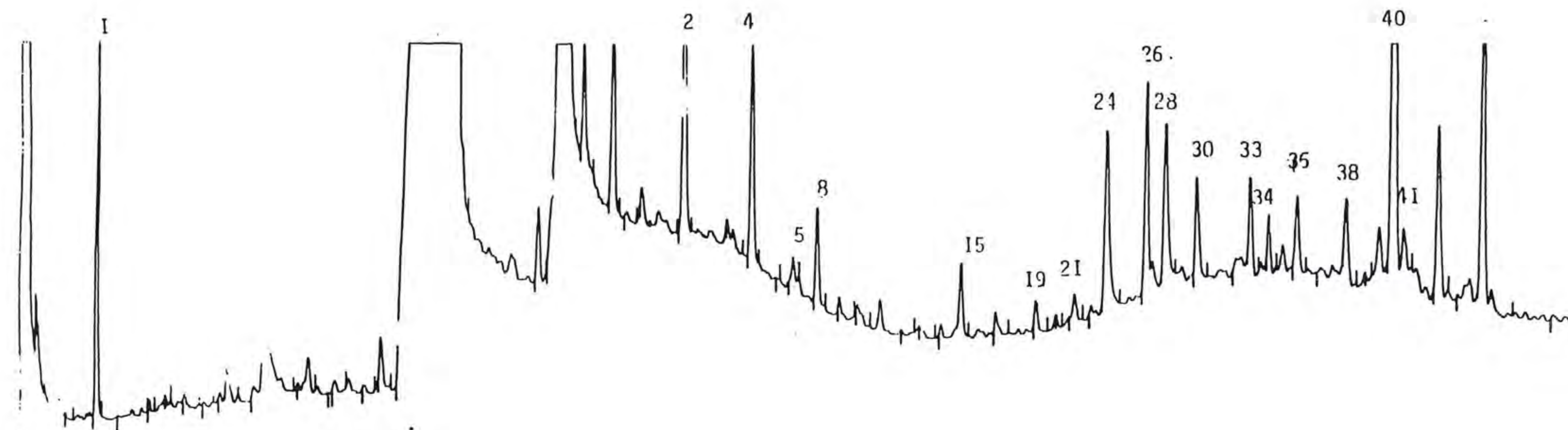


Figure 4.2I Chromatogram of female G.pallidipes from Budongo, Bunyoro, Uganda

Figure 4.22



Fiqure 4.22 Chromatogram of male *G.pallidipes* from Budongo, Bunyoro, Uganda

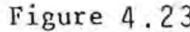


Figure 4.23 Chromatogram of female G.pallidipes from Zimbabwe

Figure 4.24

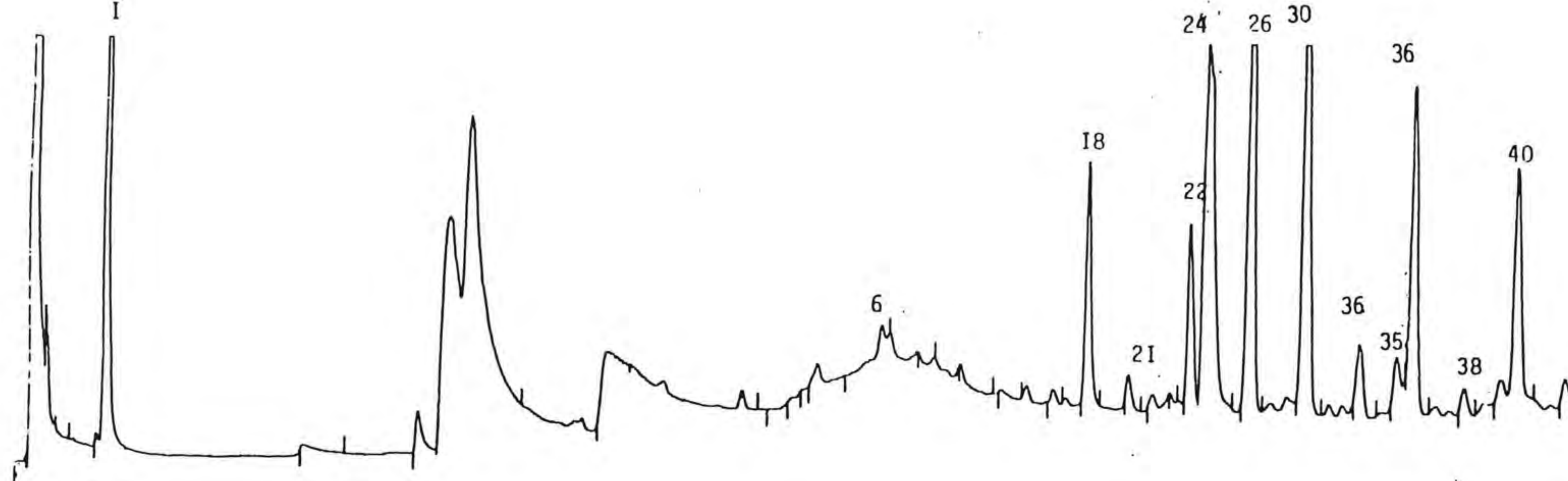


Figure 4.24 Chromatogram of male <u>G.pallidipes</u> from Zimbabwe

Figure 4.25

Key:

□ Apach

◇ Lugala

△ Budongo

▽ Zimbabwe

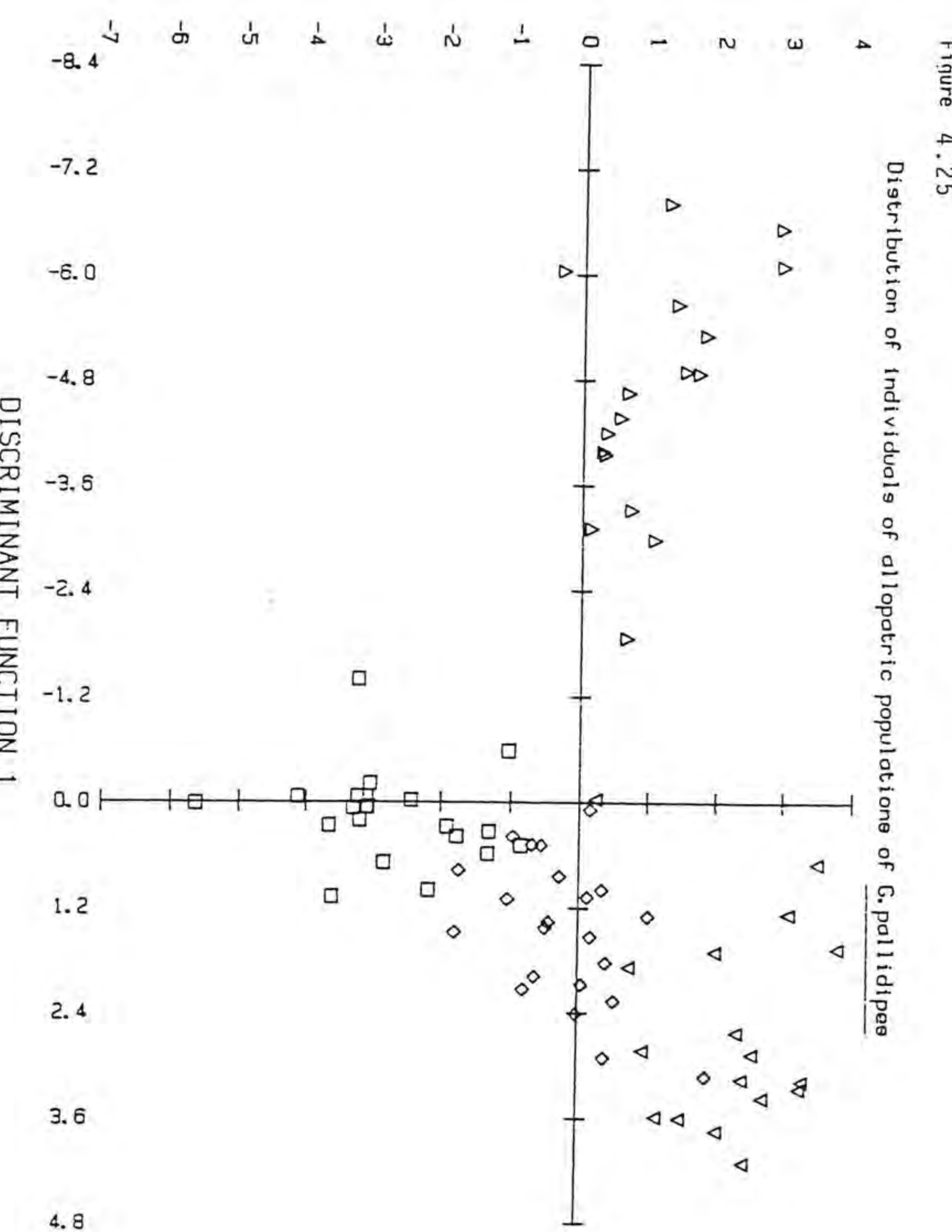


Figure 4.25 Distribution of individuals of allopatric populations of G.pallidipes

Figure 4.26

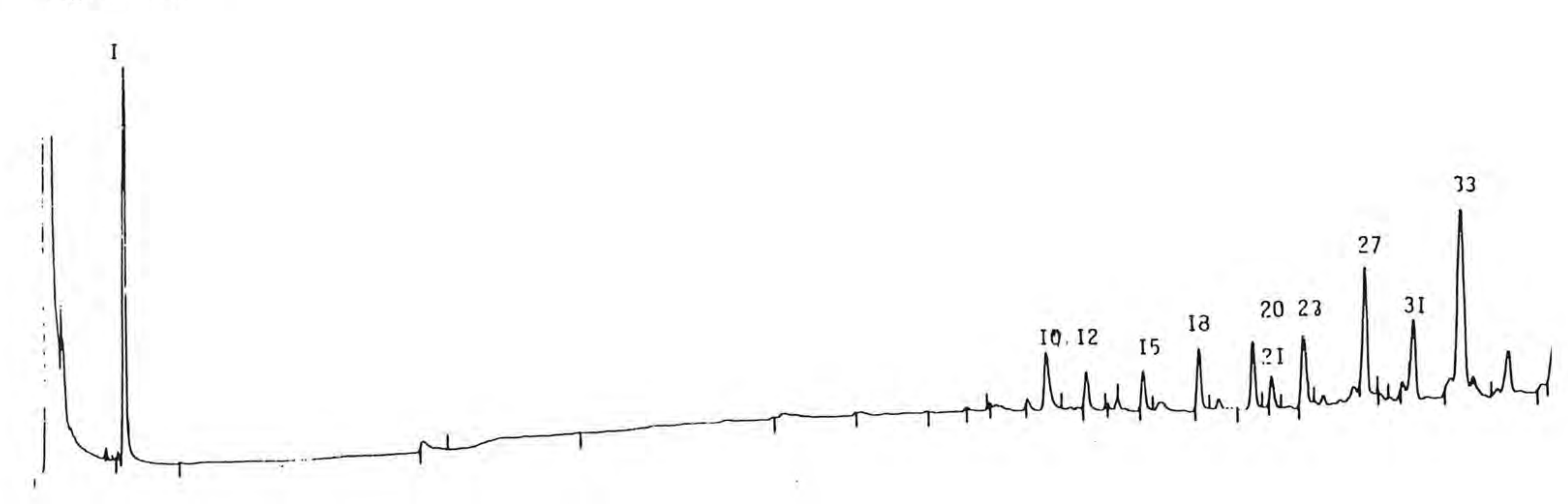


Figure 4.26 Chromatogram of female *G.f.fuscipes* puparium

Figure 4.27

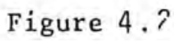


Figure 4.27 Chromatogram of male *G.f.fuscipes* puparium

Figure 4.28

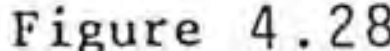


Figure 4.28 Chromatogram of female I-day old *G.f.fuscipes*

Figure 4.29

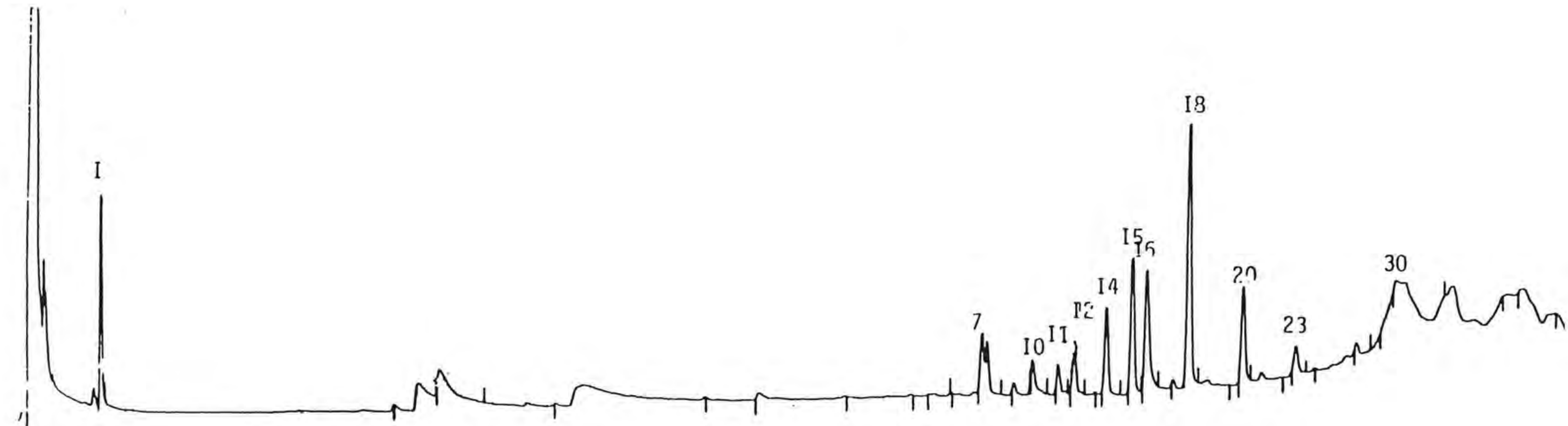


Figure 4.29 Chromatogram of male I-day old <u>G.f.fuscipes</u>

Figure 4.30

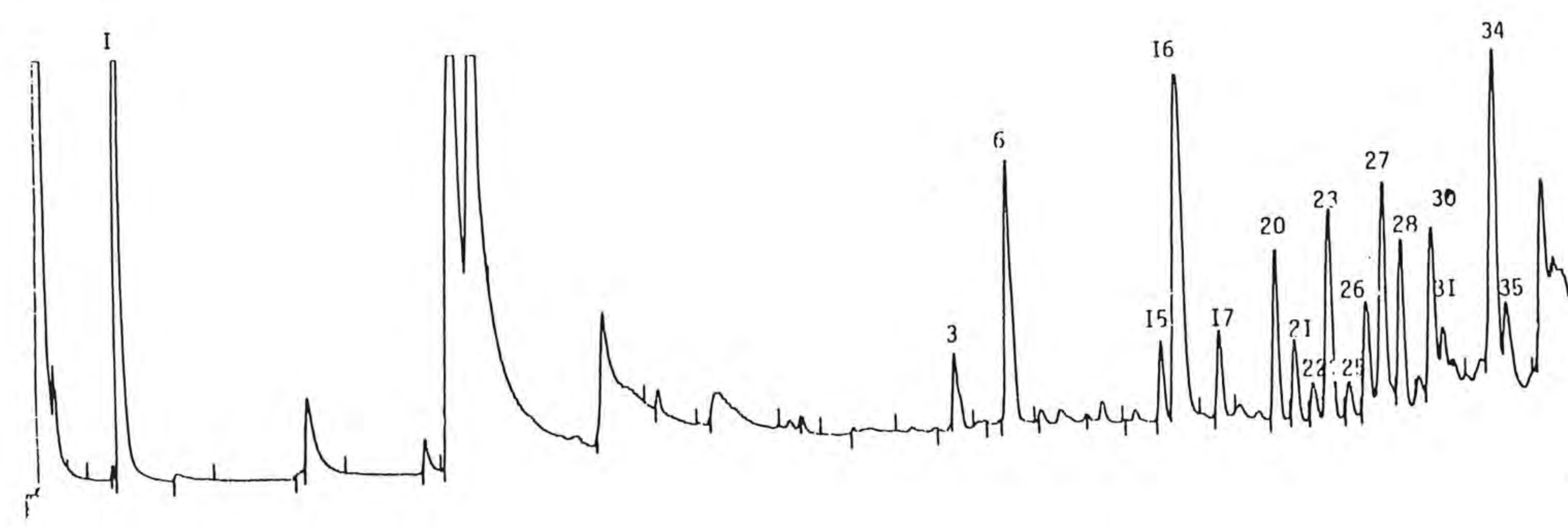


Figure 4.30 Chromatogram of female 30-day old <u>C.f.fuscipes</u>

Figure 4.3I

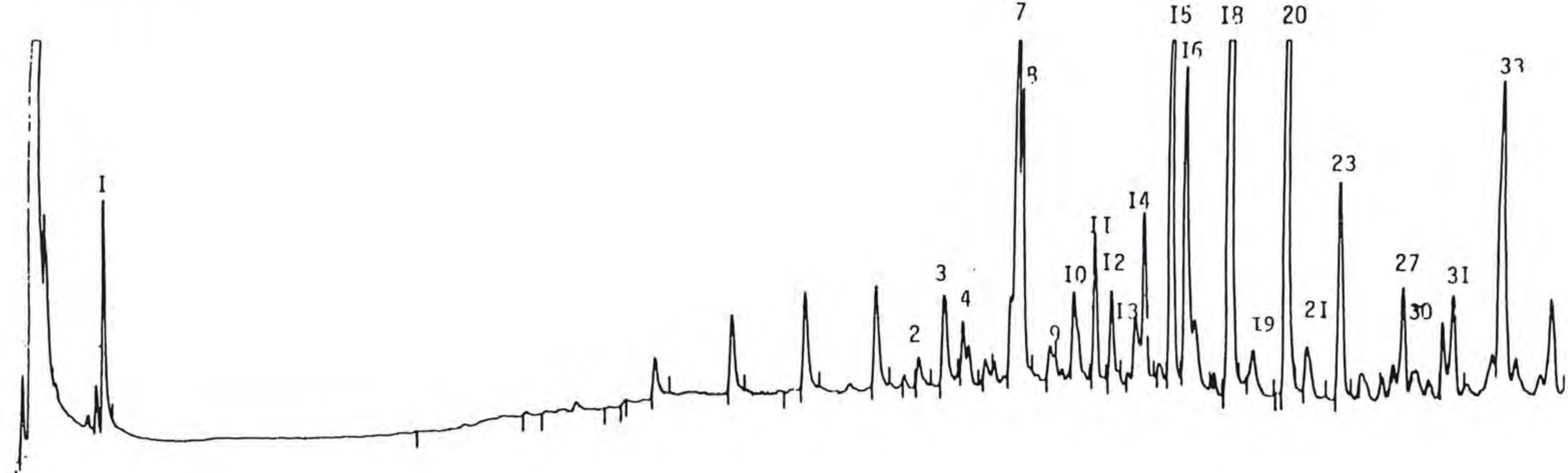


Figure 4.3I Chromatogram of male 30-day old G.f.fuscipes

Figure 4.32

Key:

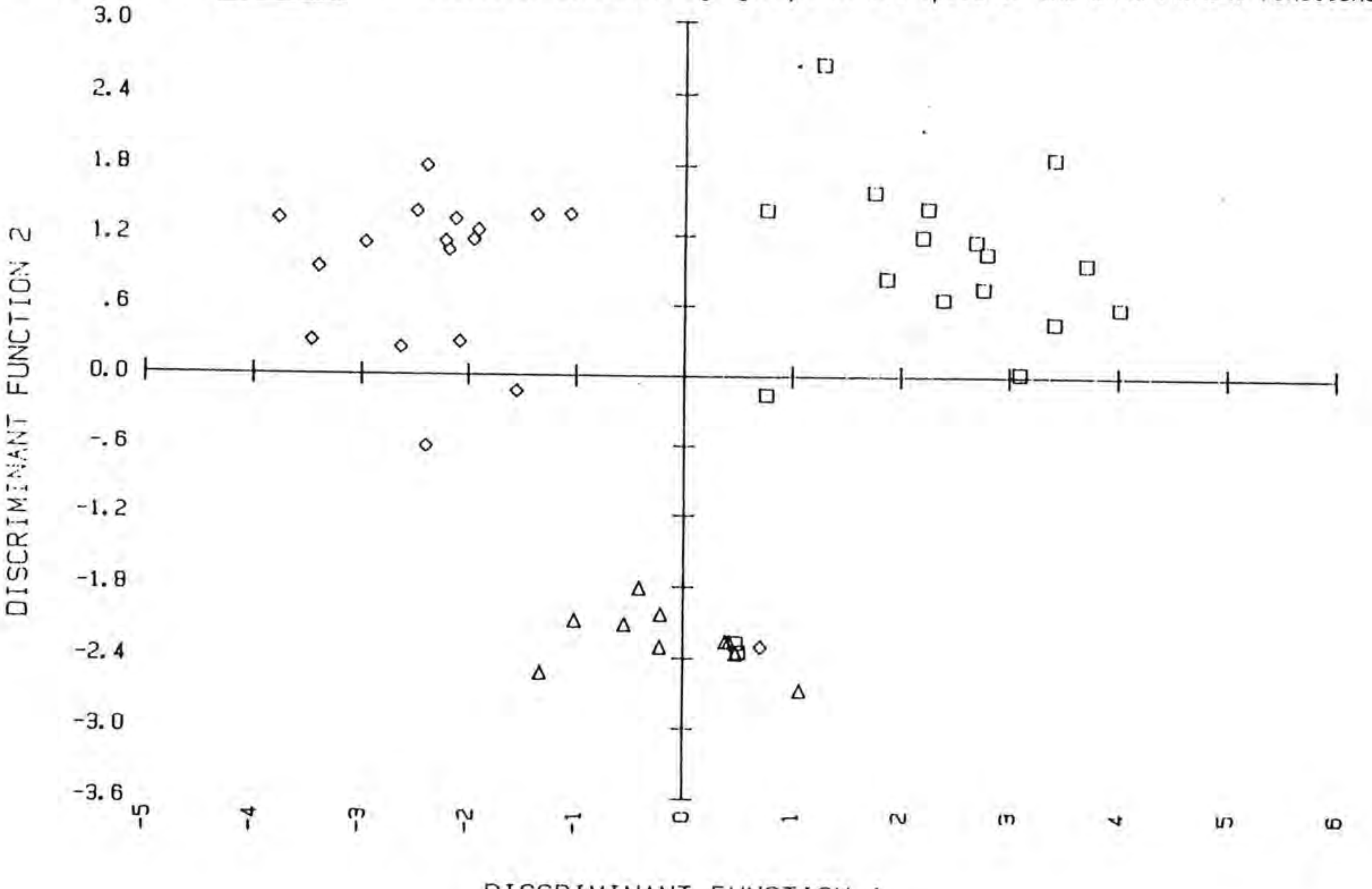
△ Puparia

◇ I day old

□ 30 day old

Figure 4.32

Distribution of G. fuscipes individuals of different age groups in the space of the discriminant functions



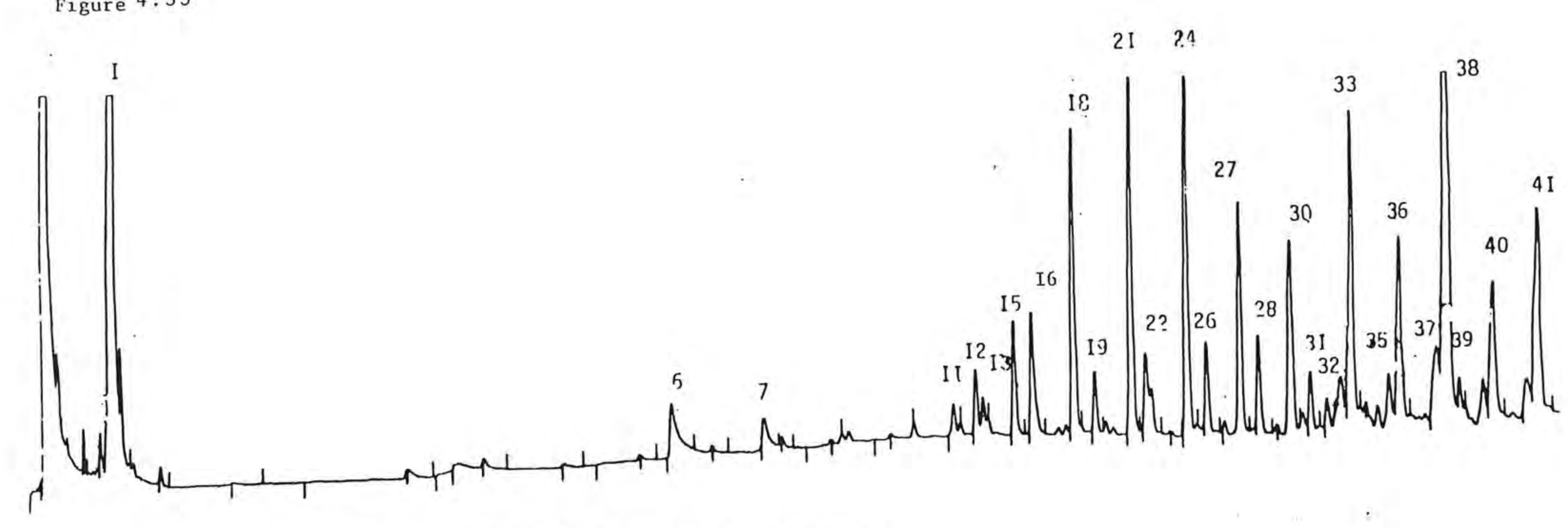


Figure 4.33 Chromatogram of female <u>G.f.fuscipes</u> puparium

Figure 4.34

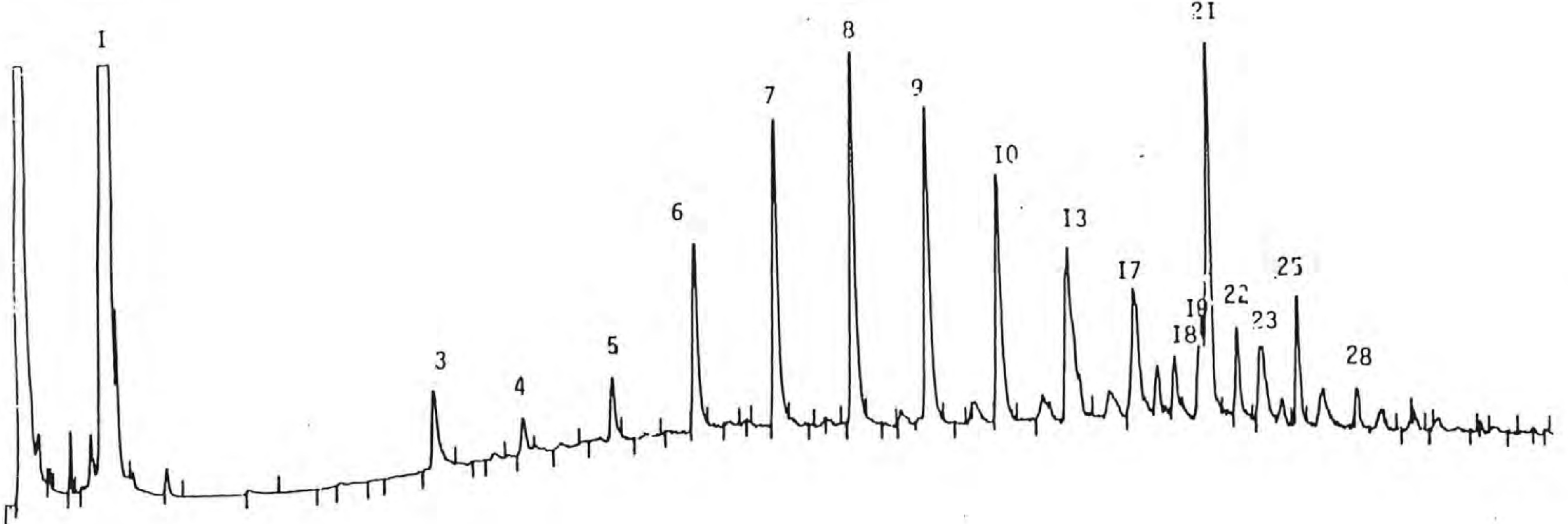


Figure 4.34 Chromatogram of male C.f.fuscipes puparium

Figure 4.35

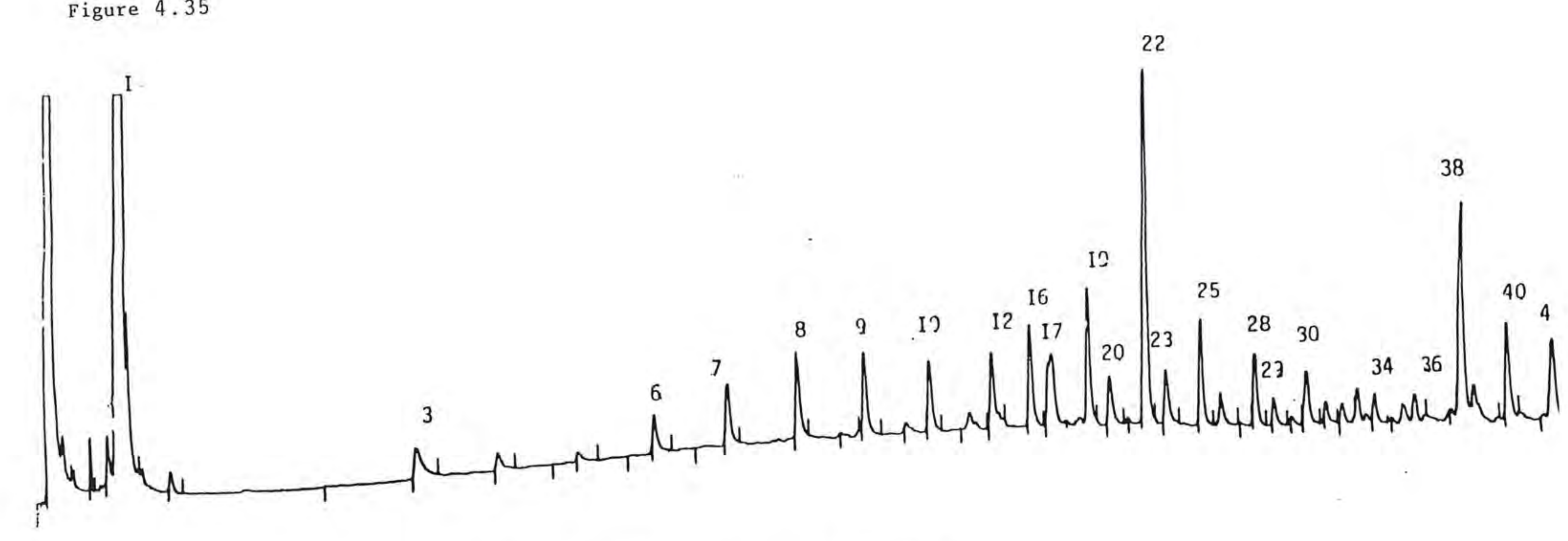


Figure 4.35 Chromatogram of female _G.p.gambiensis_ puparium

Figure 4.36

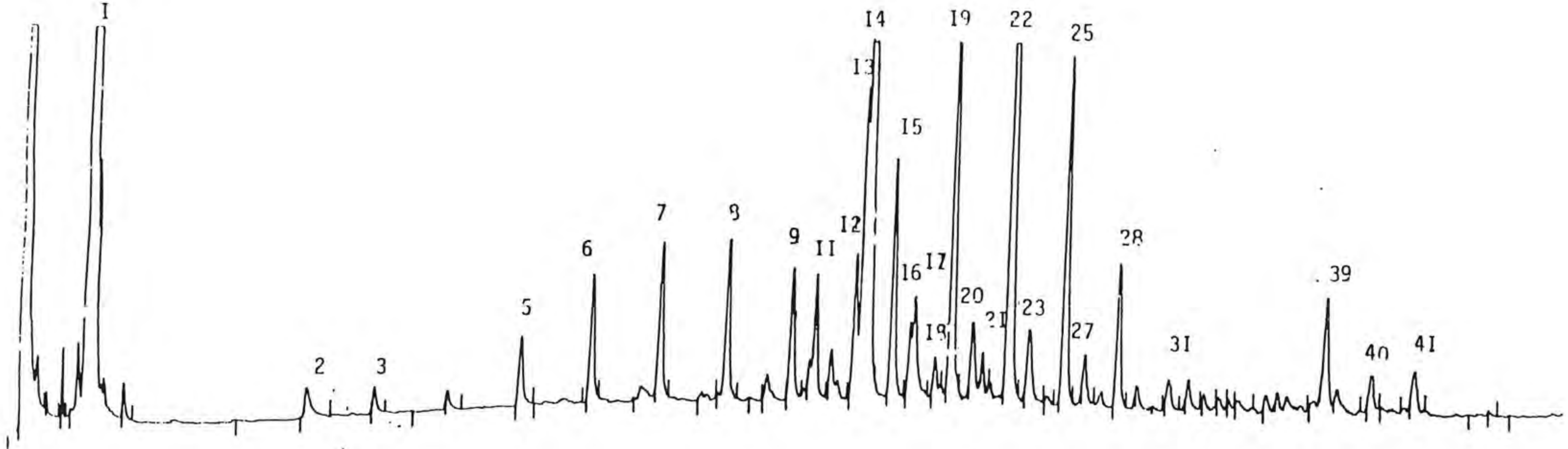


Figure 4.36 Chromatogram of male _G.p.gambiensis_ puparium

Figure 4.37 Histogram showing the frequency distribution of discriminant scores for the two species.

Key:

G.f.fuscipes puparia

G.p.gambiensis puparia

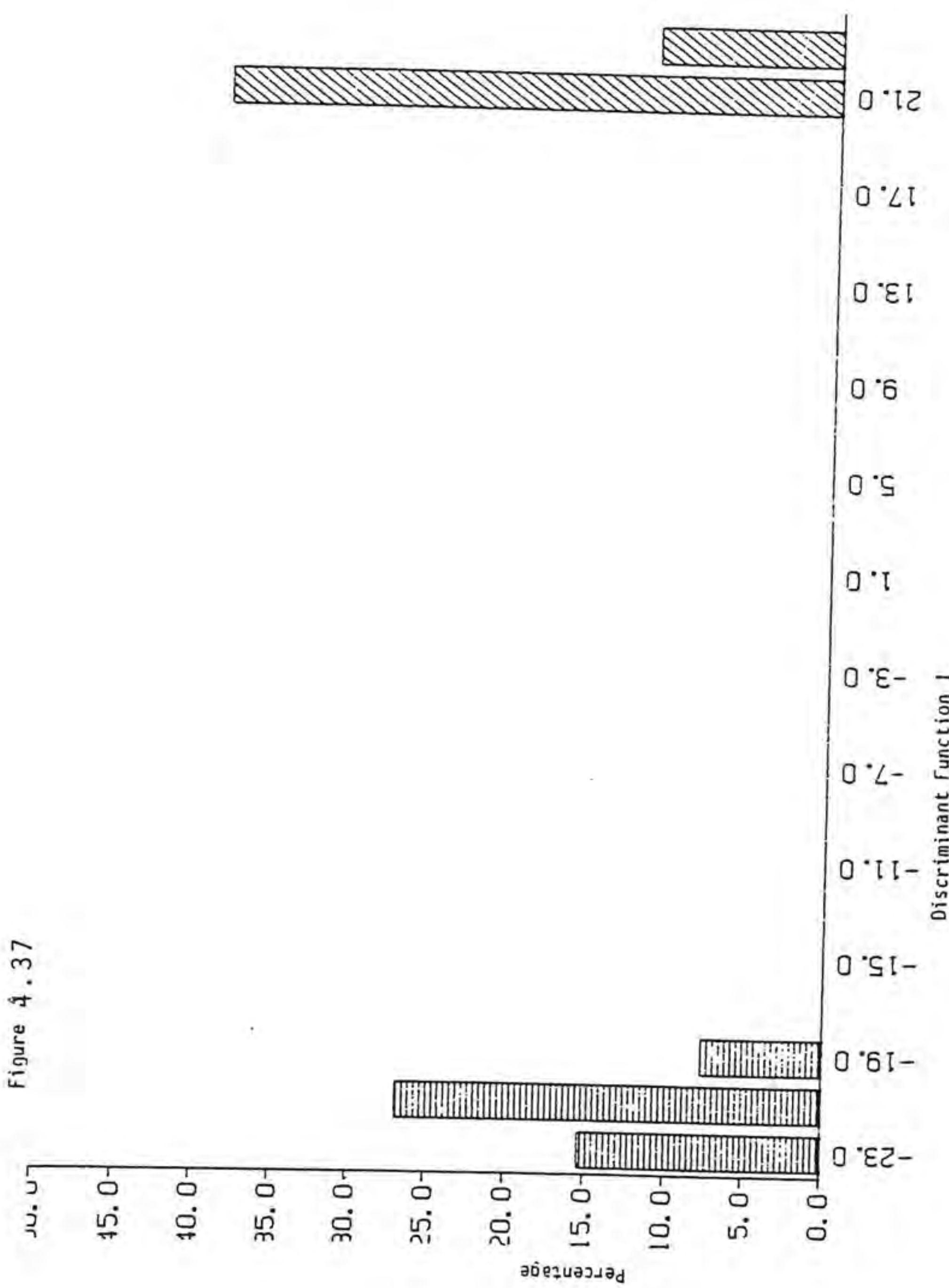


Figure 4.37

CHAPTER 5

LABORATORY STUDIES ON VIRGIN GLOSSINA

5.1 Introduction

Members of the genus Glossina reproduce by adenotrophic viviparity. Each fly has a pair of ovaries, each with two polytrophic ovarioles. The follicles of the right ovary are known as A and B and those of the left, C and D. Follicle A develops first and is mature by about the 10th day. The mature egg is ovulated into a common oviduct which enlarges to form a uterus. The first larva is produced by about the 20th day. While this larva is developing, follicle C is maturing and ovulation occurs soon after the first larva is extruded. Follicle B then begins to develop followed by follicle D so that in the ovarian cycle eggs, and eventually larvae, are produced at about 10-day intervals. These cycles of ovarian development are widely used as a means of ageing female flies up to about the age of 80 days (Saunders 1962; Challier 1965).

Insemination and fertilisation have already been briefly discussed (Chapter 1). A full account of tsetse reproductive physiology has been given by Saunders (1970), Langley (1977) and Tobe and Langley (1978).

There are conflicting reports regarding ovulation in virgin Glossina. The controversy centres on whether mating is a necessary pre-requisite for ovulation to take place in Glossina. Odhiambo (1971), working with G. pallidipes,

and Dodd (1971), with G. morsitans, reported that the act of mating was a prerequisite for the first ovulation and the initiation of the reproductive cycle. Foster (1974) found that ovulation in G. austeni was associated with the median neurosecretory cells and nervous system. Saunders and Dodd (1972) and Chaudhury and Dhadialla (1976) described the mechanisms of nervous and hormonal control of ovulation which they thought was triggered by the act of mating. These observations were not in agreement with those of Mellanby (1937) who found ovulated eggs in virgin G. palpalis. Vanderplank (1947) found that ovulation took place normally in most virgin G. morsitans, G. swynnertoni, G. pallidipes and G. palpalis but he found some flies with eggs accumulated in the ovaries. Leegwater-van der Linden (1981,1982) found normal sequential ovulation in about 90% of virgin G. pallidipes and only 1% of the flies retained all their eggs in the ovaries. In most flies eggs were produced every 8 days. The eggs were extruded from the uterus of flies of at least 15 days of age. Harley (1966) found normal ovulatio and abortion of infertile eggs in two species of G. f. fuscipes in the field. Okoth (unpublished) found about 0.4% of wild-caught G. f. fuscipes showing signs of abnormal ovarian development; follicles A and C were full developed, there were traces of spermatozoa in the spermathecae but there were no signs of any previous ovulation. One explanation for this abnormality is late mating.

The present study was designed to provide further

information on ovulation in virgin G. f. fuscipes, G. palpalis gambiensis, G. tachinoides and G. m. morsitans and on abnormalities in reproduction due to late mating.

5.2 Materials and Methods

Puparia of G. f. fuscipes, G. p. gambiensis and G. tachinoides were obtained from the Institute d'Élevage et Médecine Vétérinaire des Pays Tropicaux (IEMVT) and of G. m. morsitans from Tsetse Research Laboratory, Langford, Bristol.

The puparia were placed individually in tubes of 2.5 x 7.5 cm covered with netting and kept in an incubator at 24°C and 80% R.H. Hatched females were reared in individual tubes which were changed at least twice a week. The flies were offered blood meals from the ears of rabbits every other day. Samples of virgin flies of different age groups beginning at 20 days-old were dissected and each fly's reproductive condition was recorded. Other samples of virgin females aged 10-60 days were paired with 5-10 day old males overnight then separated and dissected after 20 days or when they larviposited, whichever came first.

Uterine and follicular length were measured using a calibrated micrometer eye-piece to determine growth rate. The most developed follicle, and the next most developed were called follicles 1 and 2 respectively. Follicular relics were examined in order to determine whether or not ovulation had taken place. These were mounted in polyvinyl lacto-phenol (Downs 1944).

5.3 Results

5.3.1 Ovulation in virgins

Tables 5.1 and 5.2 show the details of virgin *Glossina* dissected at different ages.

5.3.1.1 Egg retention

A fly was regarded as having retained all its eggs in the ovary if it had no follicular relics. In such flies either:

(i) a mature egg was found in each ovary

or (ii) two mature eggs were found in one ovary (Plates 5.1 and 5.2).

In older flies degenerating yolk was found in eggs held within the ovaries (Plate 5.3)

5.3.1.2 Ovulation

The presence of follicular relics or eggs in the uterus indicated that ovulation had taken place. Virgin flies that ovulated showed one or more of the following conditions:-

(i) up to 5 eggs accumulated in the uterus (Plate 5.4).

(ii) partial (Plates 5.3 and 5.5) or complete (Plate 5.6) degeneration of egg yolk resulting in abnormal eggs or empty chorions. These were found in the uterus and also extruded into the rearing tube (Plate 5.7).

(iii) normal eggs were extruded and these were recovered from the rearing tubes (Plate 5.8, Table 5.1).

(iv) the extrusion of premature larvae; two virgin *G. p. gambiensis* of 60 and 80 days old extruded small premature larvae of the second and first

instars respectively. The second instar larva (Plate 5.9) which measured 2.1 mm, had a developed tracheal system, polypneustic lobes and segments (Saunders, 1970).

5.3.2 Late mating and larviposition

Table 5.3 shows the results of dissecting old virgin *Glossina* 20 days after they were mated. The following observations were made:-

(i) some females showed lack of receptivity, some were forced into copulation by the males while others readily paired.

(ii) Among the flies that managed to pair, only two had larvae in their uteri when dissected after 20 days; both flies had traces of spermatozoa in their spermathecae.

(iii) The remainder of the females had empty uteri in spite of slight insemination.

(iv) No larviposition took place but there was evidence of ovulation in the presence of follicular relics.

As a result of these observations on ovulation, a further study was undertaken to see if the transport of puparia from IEMVT in Paris or Bristol had affected the reproductive physiology of the flies. Pupae were raised from flies hatched at Salford and treated as in the previous study. The results are indicated in Table 5.1

5.4 Discussion and Conclusion

The observation of ovulation in virgin tsetse is in agreement with those of Vanderplank (1947) and

Leegwater-van der Linden (1981, 1982). The tendency of G. f fuscipes to retain its first egg for 20 days (Table 5.2) was also observed in G. pallidipes by Leegwater-van der Linden (1982) and in G. m. morsitans by Gillott and Langley (1981). However, these authors suggested that once this first egg was ovulated, regular ovulation continued at about 8 day intervals. The present study has shown that egg retention can occur after the first ovulation (Plates 5.3, 5.6 and 5.10).

Parthenogenesis, a phenomenon in which eggs and embryos develop without having been fertilised, is known to occur in various insect orders, many examples occurring in Lepidoptera (Cockayne 1938). This phenomenon in Glossina is rare and little studied and requires further investigation. Buxton (1955) quoting Stuhlmann (1907) stated that there is "an early but definite record that two virgin female G. brevipalpis produced normal full-size larvae." The ploidy of the larvae would be of considerable interest.

Another striking phenomenon, hitherto not recognised in Glossina, is the degeneration of egg yolk either in the ovary or in the uterus and the eventual extrusion of empty chorions. It is suggested that this development could have led to the current conflict on ovulation in virgin tsetse. For example, Table 5.1 lists several flies which had empty uteri and follicular relics, an apparent contradiction. It would be difficult to decide whether a virgin fly (e.g. fly 27, Table 5.1) which had a developed follicle A was in its first or second ovarian cycle, without examining the

follicular relics. Challier (1973) reported absorption of eggs in old virgin G. p. gambiensis. The present study illustrates that empty chorions are extruded.

It is also suggested that extruded eggs, chorions and larvae may have been missed as a result of the various methods used in previous studies of this type. They cannot be distinguished by eye and may easily go unnoticed, mixed with faeces or stuck on the side of the rearing tube. Individual rearing of flies in tubes, not cages, and regular examination and changing of tubes, preferably daily, increases the chances of finding such products of ovulation.

These results show no environmental effect on pupal life during the transportation and it was therefore concluded that the observations made were inherent.

None of the flies of advanced age which mated larviposited. Leegwater-van der Linden (1982) recorded a prolonged interval between mating and larviposition in G. pallidipes which were mated at ages greater than 13 days. She attributed this to a missed first ovarian cycle. This could partly explain the observations made in this study but the delay of 20 days greatly exceeds the length of a normal cycle. One reason could have been that degenerating egg yolk from the first cycle in the virgin flies could have prevented fertilization of eggs from the next cycle. Two flies had larvae and one an egg in the uterus, these also showed traces of spermatozoa in the spermathecae. The same flies showed follicular relics when dissected. It is difficult to tell whether ovulation in

the flies took place before or after mating and whether the larvae were fertilized or were the result of some parthenogenetic process. Other flies with traces of spermatozoa in the spermathecae had empty uteri.

The unwillingness of old virgin females of G. palpalis to mate was observed by Jordan (1958). This lack of receptivity may be due to abnormalities in the female reproductive system. These observations cast doubt on the significance of late mating which usually occurs (Nash 1955). The multiple mating which occurs in the early life of the female (Gillott and Langley 1981) is of much greater importance.

It is concluded that in most cases normal ovulation in virgin Glossina takes place. In some cases eggs pile up in the ovaries and uterus and these are either eventually extruded or their egg yolk degenerates and the chorions extruded. Larvae may also develop from the unfertilized eggs. Late mated flies did not larviposit.

These studies provide a basis for further investigation into abnormalities in virgin females, into the effects of late mating on and larval development in relation to the significance of multiple mating in the field.

Table 5.1 Virgin *Glossina* dissected at different ages

Serial No.	Species	Age dissected (days)	Uterine content	Follicle 1 length (mm)	Follicle 2 length (mm)	No. of ovulations/ relics	No. of eggs /larvae observed
1	*G. f. fuscipes*	20	empty	1.61	0.30	0	0
2	"	20	empty	1.40	1.33	0	0
3	"	20	empty	1.33	0.80	0	0
4	"	20	empty	1.49	0.31	0	0
5	"	20	empty	1.10	0.82	0	0
6	"	20	empty	1.38	1.38	0	0
7	"	30	egg	0.46	0.25	1	1
8	"	30	empty	0.59	0.55	2	1
9	"	40	empty	0.12	0.60	2	0
10	"	40	egg	1.49	0.34	3	0
11	"	40	empty	0.69	0.00	2	0
12	"	40	egg	1.26	0.32	2	0
13	"	40	egg	1.38	1.26	1	1

Table 5.1 (Contd)

Serial No.	Species	Age dissected (days)	Uterine content	Follicle 1 length (mm)	Follicle 2 length (mm)	No. of ovulations/ relics	No. of eggs /larvae observed
14	<u>G. f. fuscipes</u>	40	2 eggs	0.34	0.00	2	0
15	"	40	empty	1.42	0.69	3	0
16	"	40	egg	1.42	0.00	1	1
17	"	40	egg	0.46	0.00	1	1
18 xx	"	40	2 eggs +	1.54 +	1.38	2	2
19 xx	"	40	egg +	1.26	1.26	1	1
20	"	50	empty	1.49	1.46	1	0
21	"	50	empty	1.38	0.00	3	0
22	"	50	2 eggs	1.38	1.64	2	2
23	"	50	empty	0.52	0.23	2	0
24 xx	"	50	empty	0.23	0.00	1	1
25 xx	"	50	empty 2 eggs	1.58 1.38	1.03	1	0
26	"	60	egg	0.46	0.00	1	1

Table 5.1 (Contd)

Serial No.	Species	Age dissected (days)	Uterine content	Follicle 1 length (mm)	Follicle 2 length (mm)	No. of ovulations/ relics	No. of eggs /larvae observed
27	G. f. fuscipes	60	empty	1.38	0.46	4	0
28	"	80	empty	1.38	0.03	4	0
29	G. p. gambiensis	20	empty	1.15	1.05	1	1
30	"	20	empty	1.49	1.38	0	0
31	"	20	egg	1.54	0.80	1	1
32	"	20	empty	1.72	1.72	1	0
33	"	20	2 eggs	0.23	0.29	2	2
34	"	40	egg	1.15	0.34	2	1
35	"	40	egg	0.50	0.00	1	1
36	"	40	empty	1.03	0.34	2	1
37 xx	"	40	empty	1.15	0.00	2	2

Table 5.1 (Contd)

Serial No.	Species	Age dissected (days)	Uterine content	Follicle 1 length (mm)	Follicle 2 length (mm)	No. of ovulations/ relics	No. of eggs /larvae observed
38 xx	<u>G. p .gambiensis</u>	40	egg	2 eggs 1.38 1.26	1.36	1	1
39 xx	"	50	2 eggs +	1.49 +	1.38	1	1
40 xx	"	50	empty	2 eggs 1.38 1.38	2 eggs 1.38 1.49	0	0
41 xx	"	50	egg	1.49	0.80	1	1
42 xx	"	50	empty	2 eggs 1.49 1.54	0.00	0	0
43 xx	"	50	empty	1.26	1.46	1	1
44 xx	"	50	egg	1.26	0.46	1	1
45 xx	"	50	empty	2 eggs 1.15 1.10	1.58 +	0	0
46 xx	"	50	empty	0.08	2 eggs 1.49 1.58	1	0
47	"	60	2 eggs	0.80	0.00	2	2
48 **	"	60	empty	1.38	0.57	1	1

Table 5.1 (Contd)

Serial No.	Species	Age dissected (days)	Uterine content	Follicle 1 length (mm)	Follicle 2 length (mm)	No. of ovulations/ relics	No. of eggs /larvae observed
50	G. p. gambiensis	70	5 eggs	1.26	1.127	5	5
51 **	"	80	empty	0.23	0.000	1	1
52	G. tachinoides	40	egg	1.16	0.575	1	1
53	"	40	empty	1.15	0.000	1	0
54	"	60	empty	0.57	0.000	1	0
55	"	60	empty	2 eggs 1.38 1.38	0.230	1	0
56	G. m. morsitans	20	empty	1.49	0.380	0	0
57	"	20	empty	2 eggs 1.38 1.38	1.380	0	0

* includes eggs/larvae seen in the uterus
xx inbred virgin flies
** extruded larvae
\+ degenerating egg yolk

Table 5.2 Summary table for ovulation in virgin Glossina

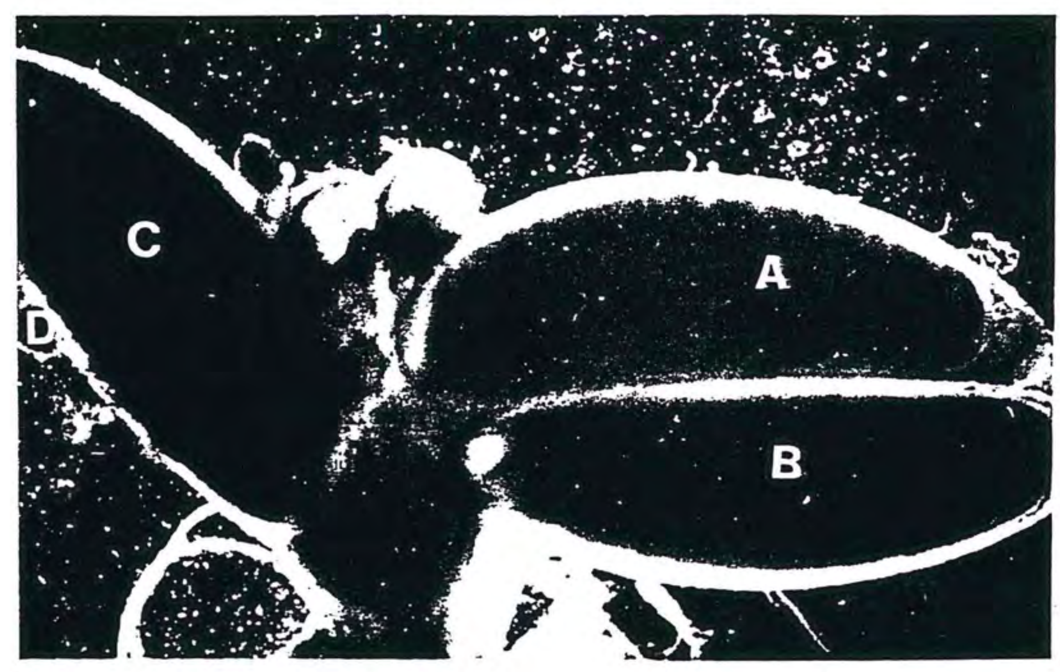
Species	No. of flies dissected	Age (days)	Egg retention %	Ovulation %
G. f. fuscipes	6	20	100	0
	22	30-80	0	100
G. p. gambiensis	5	20	20	80
	18	40-80	22.2	77.8
G. tachinoides	-	20	-	-
	4	40-60	0	100
G. m. morsitans	2	20	100	0
	57		22.8	77.2

Table 5.3 Virgin *Glossina* mated at advanced ages and dissected after 20 days

Serial No.	Species	Age mated (days)	Age dissected (days)	Larvae produced	Uterine content	Follicle 1 length (mm)	Follicle 2 length (mm)	Spermathecae content	No. of relics
1	*G. f. fuscipes*	10	20	Nil	empty	0.98	0.46	0*	1
2	"	20	40	Nil	egg	0.28	0.50	traces	1
3	"	20	40	Nil	2nd larva	1.10	0.34	traces	1
4	"	20	40	Nil	egg	0.39	0.00	0*	1
5	"	20	50	Nil	empty	–	–	–	–
6	"	50	70	Nil	empty	0.34	0.00	0	4
7	"	50	70	Nil	empty	1.65	0.46	0*	4
8	*G. p. gambiensis*	60	80	Nil	1st larva	0.57	0.00	traces	2
9	"	60	80	Nil	empty	1.26	0.00	0	4
10	"	60	80	Nil	empty	0.11	0.00	0	4
11	"	60	80	Nil	empty	1.03	0.23	traces	4
12	"	60	80	Nil	empty	–	–	–	–
13	*G. tachinoides*	60	90	Nil	empty	1.26	0.80	traces	4
14	"	60	90	Nil	empty	1.38	0.46	0	4
15	*G. m. morstans*	20	40	Nil	empty	1.15	0.50	0	0

Plate 5.1 Ovaries of *Glossina* showing egg retention: One mature egg in the left and two in the right ovaries (A, B, C, and D are follicles; S = Spermathecae)

Plate 5.2 Ovaries of *Glossina* showing egg retention: two eggs in each ovary (A, B, C, and D, are follicles; S = Spermathecae)



5.I

5.2

Plate 5.3 Degenerating egg yolk in the ovary and uterus (Three eggs have been ovulated from follicles A_2, B_2 and C_2: Two eggs, A and C are in the uterus and the 3rd one (*) was extruded; cf. Table 5.1, No.39)

Plate 5.4 Two eggs accumulated in the uterus (Follicles A_2 and C_2 ovulated)

5.3

5.4

Plate 5.5 Egg (E) in the uterus containing degenerating egg yolk (about a half yolk mass present)

Plate 5.6 Empty chorion (CH) in the uterus (Follicles A_2 and C_2 have ovulated; S = Spermathecae)

5.5

5.6

Plate 5.7 Extruded empty chorion

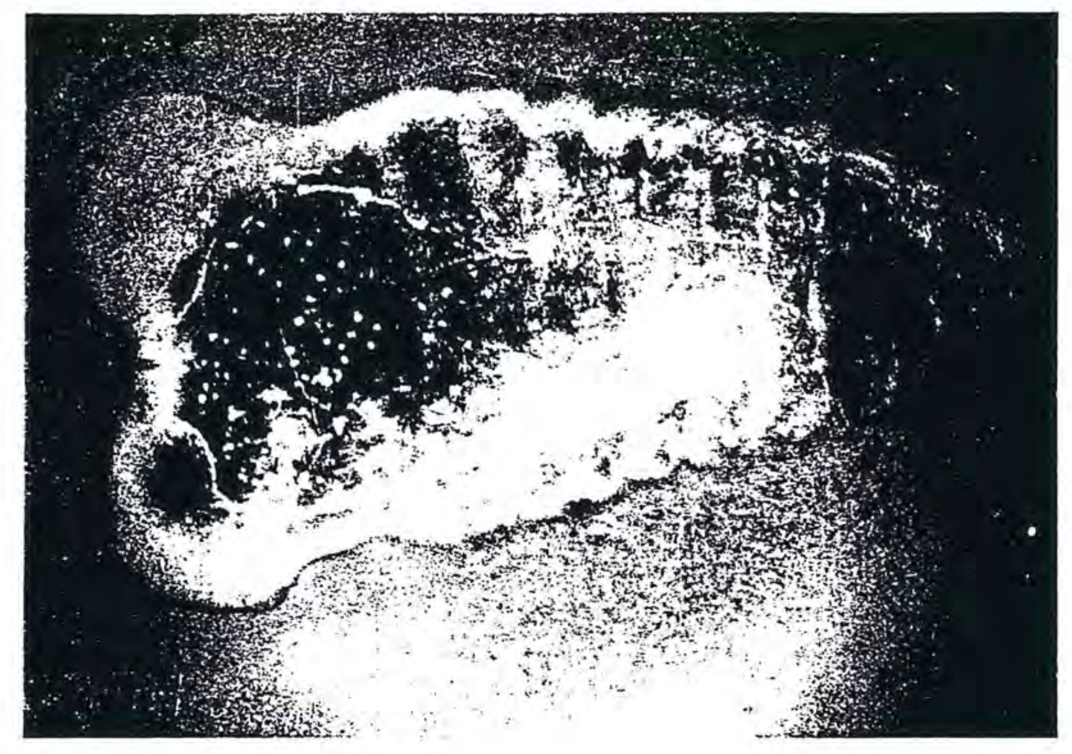
Plate 5.8 Extruded egg found in faeces in the rearing tube

5.7

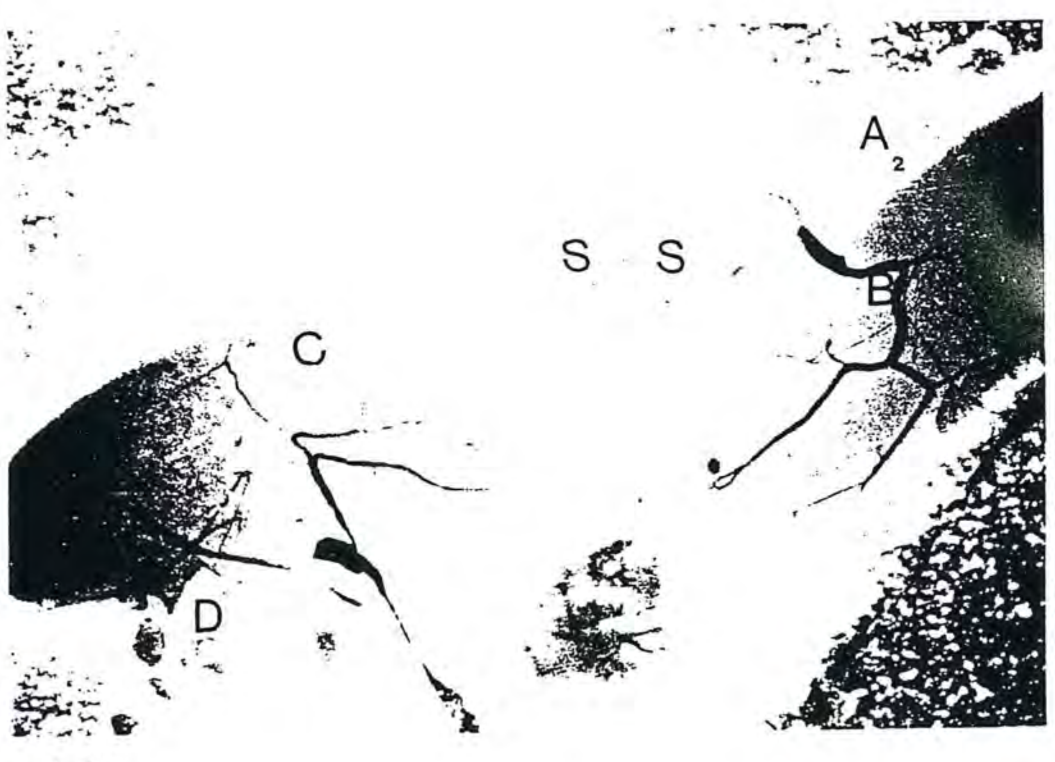
5.8

Plate 5.9 Second instar larva developed from virgin *Glossina*

Plate 5.10 Ovaries showing egg retention after the first ovulation (Follicle A_2 ovulated; B and C developed; S = Spermathecae)



5.9



5.10

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00008 # Peridomestic breeding sites of *Glossina fuscipes fuscipes*
00009 # Newst. in Busoga, Uganda,
00010 # and epidemiological implications for trypanosomiasis
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00015 ## Summary

00016 A search for *Glossina fuscipes fuscipes* puparia near homesteads in the
00017 sleeping sickness focus of Busoga revealed puparia and puparial shells under
00018 *Coffea canephora* (coffee), *Musa* sp. (banana) and *Lantana camara* thickets as
00019 well as under house verandahs and, once, inside a hut. This is the first descrip-
00020 tion of *G. f. fuscipes* breeding sites in a peridomestic habitat. The implications of
00021 these findings in relation to the transmission of the current epidemic of sleeping
00022 sickness in Busoga is discussed.
00023
00024 Key words: *Glossina fuscipes fuscipes;* peridomestic; breeding sites; Uganda.

00025
00026
00027 ## Introduction

00028 It is known that *Glossina fuscipes fuscipes* can extend its distribution from
00029 its normal riverine and lacustrine habitat. For example, Chorley (1944) noted
00030 that, in Uganda, this species could be found near human settlements 12 miles
00031 from the shores of Lake Victoria. Willett (1965) attributed the sleeping sickness
00032 epidemic in Alego, Central Nyanza, Kenya in 1964 to *Trypanosoma rhodesiense*
00033 transmitted by *G. f. fuscipes.* He found that this fly had colonized *Lantana*
00034 thickets and other vegetation surrounding Luo huts. Although he mentioned
00035 that teneral flies and puparia were found under this vegetation he did not
00036 describe any breeding sites or give numbers of teneral flies and puparia found.
00037 (The term "puparium" is used in this paper to include contents of the integu-
00038 ment.) Onyango et al. (1964) working in the same area during the same out-
00039 break, reported that "the significance of *Lantana* in the area was not precisely
00040 determined but it is probable that *Glossina* was breeding in this vegetation type
00041 in some areas".

00042 The present epidemiological situation in Busoga is similar to that in Alego
00043 during the 1960's (Kutuza and Okoth, 1981). The breeding sites of *G. f. fuscipes*
00044 in Busoga have been mentioned in a short communication (Okoth, 1985). This
00045 paper gives details of these breeding sites and discusses them in relation to
00046 epidemiology of sleeping sickness in Busoga.

00047
00048 ## Methods

00049 The area of study was briefly described by Okoth (1985). The general topographic and climatic
00050 conditions of Busoga have been described by Abaru (1985).

00051 The study was carried out for 20 months from March 1983 to January 1985. Sharpened sticks
00052 were used to search for puparia in selected sites. Four searchers were normally assigned to each
00053 sub-area and these groups were rotated weekly. Six artificial breeding shelters were built in selected
00054 sub-areas. These shelters consisted of an open framework of poles with a sloping grass-thatched roof.
00055 They had a floor area of 4×1.5 cm, a back 0.5 m high and a front 1.25 m high.

00056 Initially each group of searchers also carried a biconical trap (Challier and Laveissière, 1973)
00057 which they used to catch teneral flies to indicate the possible proximity of breeding sites. Trapping
00058 was abandoned once teneral flies were caught. Empty puparial shells were examined and any found
00059 with the normal posterior opening or operculum were considered to have hatched a viable fly.
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00061
00062 ## Results

00063 A total of 246 puparia and puparial shells were found in the following
00064 situations: *Lantana* thickets (33), *Coffea canephora* plantation (12), *Musa*
00065 plantation (3), verandahs of human and animal huts (15), inside animal hut (1),
00066 vegetation surrounding homesteads especially *Lantana* hedges (4) and forest
00067 (178). Puparia were found under logs, artificial shelters and individual plants.
00068 Comparing the performance of the artificial shelters with the natural sites,
00069 63.5% of the puparia and puparial shells came from the artificial sites.

00070 Collections tended to be larger during the two dry seasons (January to
00071 March, July to September). All puparial shells were recorded as having hatched
00072 normal flies.

00073
00074 ## Discussion

00075 This study has shown for the first time that *G. f. fuscipes* not only rests in
00076 vegetation surrounding homesteads in Busoga but also that this species breeds
00077 there. Okoth (1980, 1982) studied the composition of the *Glossina* population in
00078 the Busoga fly-belt and found that increases in human activity had reduced the
00079 population of *G. brevipalpis* and *G. pallidipes* to the point where they were rarely
00080 found, whereas the population of *G. f. fuscipes* remained constant. *G. f. fuscipes*
00081 now breeds and rests in peridomestic situations in Busoga. This atypical behav-
00082 iour results in more intense personal man/fly contact even at a very low fly
00083 density and has provided the basis for a man-fly-man cycle of infection which is
00084 the most likely cause of the current Busoga epidemic. Most breeding took place
00085 where coffee, banana, *Lantana* and other vegetation grew under large trees
00086 (*Ficus*, *Mangifera*, *Cassia*, etc.), which provide the appropriate microclimates
00087 required by the fly.

00088 Puparia were found under verandahs of, and within, human habitation as
00089 well as in huts used to shelter animals. The peridomestic behaviour of
00090 *G. f. fuscipes* in Uganda does not appear to be linked to any domestic animal as
00091 occurs in West Africa with *G. tachinoides* and *G. palpalis* with pigs (Baldry,
00092 1964, 1968, 1969; Seketeli and Kuzoe, 1984), so removal of the latter would
00093 probably not reduce the peridomestic population of *G. f. fuscipes*.

00094 When natural habitats and wild hosts are destroyed *G. f. fuscipes* will, in
00095 common with other members of the group, adapt itself to the peridomestic
00096 habitat.

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00098
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