PHLEBOTOMINE SANDFLY (DIPTERA: PSYCHODIDAE) BEHAVIOUR AND UTILIZATION OF TERMITE HILLS AND ANIMAL BURROWS IN BARINGO DISTRICT, KENYA.

by

PHILIP M. NGUMBI

A Thesis submitted in partial fulfillment for the Degree of Master of Science in Zoology.

(Entomology)

University of Nairobi.

1995

Declaration

This Thesis is my original work and has not been presented for a degree in any other University.

Philip M. Ngumbi

This Thesis has been submitted for examination with our approval as Supervisors

Dr. L.W.Irungu — Date 29/3/96

Dr. L. L. Robert — Date 20/2/96

Dedication

Dedicated to

My wife Scholastica Nzisa, our children and my brother, Dr. Paul Muli Ngumbi, for all the support you gave me during my study.

Acknowledgements.

I would like to express my sincere thanks and gratitude to all those who, in one way or the other, made this Thesis a success. This expression of gratitude goes particularly to my Supervisors, Dr. L. W. Irungu and Dr. L. L. Robert for their assistance and guidance during the period of the study. I wish to thank the Directors of Walter Reed Project (WRP), Drs. C. R. Roberts, A. J. Johnson and D. M. Gordon for having made funds available for my study at the University of Nairobi. I am specially grateful to the Director of Kenya Medical Research Institute (KEMRI) Dr. Davy K. Koech for his encouragement to pursue the study. I would unreservedly like to thank Dr. R. Copeland for his assistance with data manipulation in the computer. I would also like to thank Mr. C. O. Anjili for his good advice. Thanks also goes to the two technicians from Medical Research Centre (MRC) Messrs., Alex Muema and Noah Mutiso and also our Walter Reed Project driver, Mr. Reuben Rugwe. Thanks also goes to the staff of Kenya Agricultural Research Institute (KARI) at Marigat for providing me with meteorological data for the period of the study. I wish to give special thanks to Ms. Agnes Ng'ang'a for assisting me with the typing and printing of my Thesis.

Lastly, I would like to thank my family, relatives and friends without whose support and encouragement this study would never have been a success.

Table of contents

List of Figures.

Figure

List of Tables.

Table

List of Plates.

Plate

Abstract.

A field based project to study the behaviour and utilization of termite hills and animal burrows by phlebotomine sandflies in Baringo District, Kenya was undertaken. The trapping of sandflies was done in two areas; around Perkerra and Rabai Primary Schools. Sandflies were trapped with sticky papers inserted into entry-exit traps (Yuval *et al.*,1986). The traps were set from 1800h to 2400h and again from 2400h to 0600h. For every six hours,12 traps were set out at each of the two trapping sites. In a full night's collection a total of 24 traps were set.

In both areas sandfly abundance and activities were studied in termite hills and animal burrows. Perkerra produced more sandflies than Rabai, 7,132 (68.6%) and 3,166 (31.4%), respectively. Perkerra area had more *Phlebotomus duboscqi* than Rabai, 57 and 22, respectively. Rabai seemed more suited for the breeding of *P. martini* than Perkerra, 376 and 66, respectively. More sandflies were caught in termite hills (59.1%) than in animal burrows (41.0%). *P. martini* was found to be more abundant in termite hills than animal burrows, (3.6%) and (0.6%), respectively. *P. duboscqi* were more abundant in animal burrows than in termite hills, 0.7% and 0.02%, respectively. Among the *Sergentomyia* species; *S. schwetzi; S. antennata* and *S. bedfordi* were more abundant in termite hills. *S. clydei, S. adleri* and *S. squamipleuris* were caught in greater numbers in animal burrows. *P. rodhaini* a species which is closely related to *P. duboscqi*, was more abundant in animal burrows. Sugar feeding by phlebotomine sandflies occured in the two trapping periods (i.e 1800h-2400h and 2400h-0600h).

Most sugar-fed sandflies were collected after midnight in both animal burrows and termite hills. Bloodfeeding patterns of female sandflies in both Perkerra and Rabai showed that more bloodfed sandflies were caught in the first half of the night. At Perkerra, where a total of 74 bloodfed sandflies were caught, 65 (87.8%) were caught in the first half of the night compared to 9 (12.2%) caught in the second half. With a total

of 73 bloodfed female sandflies collected at Rabai, 53 (72.6%) were captured in the first half of the night and 20 (27.6%) in the second half. Out of a total of 1,661 female sandflies captured in animal burrows, 164 (9.9%) were parous and 1091 (65.6%) were nulliparous. In termite hills, where a total of 2,593 female sandflies were collected, 255 (9.8%) were parous and 2,132 (82.2%) nulliparous. Also in animal burrows, where a total of 2,640 male sandflies were collected, 2,443 (92.5%) had rotated genitalia and 197 (7.5%) unrotated. For those males trapped in termite hills, where a total of 3,499 males were trapped, 3,241 (92.6%) had rotated genitalia and 258 (7.4%) unrotated.

By the end of the study period, it was observed that resting sites of the vectors of leishmaniasis could be identified. Their feeding behaviours were also established with the information on sugar and bloodfeeding

patterns. This may lead to control measures being aimed at these resting sites.

CHAPTER I

INTRODUCTION AND LITERATURE REVIEW

1.1 Introduction

Leishmaniasis is a disease of both humans and animals. The causative agent being a protozoan parasite belonging to the class Zoomastigophora; order Kinetoplastida, suborder Trypanosomatina, family Trypanosomatidae and genus *Leishmania*. The genus *Leishmania*, is closely related to the Trypanosomes and together are loosely grouped as "haemoflagellates" (Laison,1982). It is generally felt that the leishmanias form an ancient group of parasites, originating from flagellates which were at one time peculiar to insects. However, with the development of a haematophagous habit by the primitive insect host, and then the migration of the flagellates to the biting mouthparts, the leishmanias gained entrance into certain vertebrates (Minter, 1976; Markell and Voge, 1976). Parasites located in the cells of the skin or blood of these animals now act as a reservoir of infection for certain sandflies (Diptera: Psychodidae: Phlebotominae). Like other members of the Trypanosomatidae, *Leishmania* do not undergo a sexual cycle and multiplication in both the sandfly and the vertebrate is limited to binary fission. The genus *Leishmania* has several sub-genera and species, all of which are vector-transmitted either cyclically or mechanically (Laison1982; Marinkelle 1980).

Visceral leishmaniasis (VL) is, also known as Kala-azar, caused by *Leishmania donovani donovani* in India and Bangladesh, by *L. donovani infantum* in the Mediterranean area and probably other parts of the Old World, by *L. donovani chagasi* in the New World and by *L. donovani* s.l. in other areas such as the Sudan and eastern Africa. This disease has a similar clinical outlook in all countries. The disease can attack individuals of all ages but it has been observed that *L. donovani infantum* and *L. donovani chagasi* are most

commonly seen in infants and young children,whereas *L. donovani donovani* is equally common in older children and young adults (Marinkelle 1980; Shaefer *et al.*1994). The disease is manifested by gradual onset of irregular fevers, sometimes a fever that appears twice daily, progressive spleno-hepatomegally, increasing anaemia often accompanied by an excellent appetite, general body weakness, and enlarged abdomen due to the sizes of the spleen and liver (Marinkelle 1980). In the absence of treatment, death ensues within a few months after the onset of symptoms (Kager *et al.*1983). Post-kala-azar dermal leishmaniasis (PKDL) is rather common in India and eastern Africa. The dermal lesions appear on some kala-azar patients one to several years after treatment and respond very poorly to specific treatment (Kager *et al.*1983). In the Sudan, *L. donovani* s.l. is probably responsible for the mucosal lesion sometimes seen in adult human males and the dermal lesions seen on rare occasions (Marinkelle 1980).

With the exception of India, Bangladesh, Iraq and probably eastern Africa, canines are the main reservoir of the subspecies of *L. donovani* (Killick-Kendrick 1990). Transmission occurs usually under rural conditions but sylvatic and occasionally urban epidemics sometimes occur.

Anthroponotic or urban cutaneous leishmaniasis (ACL) which is caused by *L. tropica*, is found in southern Europe, northern and western Africa and Asia from Turkey to western India, but has almost been eradicated in the former Soviet Union (Marinkelle 1980; WHO 1988). *L. tropica* produces painless ulceration of the skin often leading to disfiguring scars. Ulcers normally heal spontaneously after a year or more. Lesions of the face and fingers are often painful and disfiguring. Recently, epidemiological work on *L. tropica* foci in Kenya has shown that the disease is more widely distributed in the central Rift Valley and the highlands to the east, which include the Aberdare ranges than earlier expected (Lawyer *et al.* 1991; Johnson *et al.* 1993; Sang *et al.* 1993).

Zoonotic or rural cutaneous leishmaniasis (ZCL) is caused by *L. major*. The disease is common in parts of southern Russia, Iran, Pakistan, and most countries of middle Asia, Saudi Arabia and Yemen (Marinkelle 1980; Killick-Kendrick 1990). It also occurs in north and west Africa and other parts of Africa. In Kenya, it has been observed to occur in

Baringo District (Heisch 1957; Wijers & Minter 1962; Mckinnon & Fendall 1955; Leeuwenburg 1981; Beach *et al.* 1984). The skin lesions are painless and self healing in less than six months. The lesions are also numerous and often leave disfiguring scars. Rodents are the suspected reservoirs of this disease (Githure *et al.* 1984). Transmission occurs under rural conditions in dry areas.

Diffuse cutaneous leishmaniasis (DCL) is caused by *L. aethiopica* and is present in the highlands of Ethiopia and Kenya (Mutinga 1971 & 1975). Usually DCL does not respond well to treatment but some cases are responsive. Hyraxes (*Procavia* spp. and *Heterohyrax brucei*) are the suspected reservoir hosts (WHO 1980). Transmission occurs in defined foci when people enter the territory of the reservoir host, usually near cliffs or large fig trees.

Vectors of the leishmaniases fall under the class Insecta, order Diptera, family Psychodidae, subfamily Phlebotominae. Genera *Phlebotomus* and *Sergentomyia* occur in the Old World, and *Lutzomyia* and *Brumptomyia* in the New World. Whereas the genus *Phlebotomus* transmits *Leishmania* parasites in the Old World, it is those sandflies of the genus *Lutzomyia* in the New World that are of medical importance. A number of *Sergentomyia* spp in Kenya had been suggested to transmit *L. donovani*. *S. clydei* Sinton and *S. garnhami* (Heisch, Guggisbergi, and Teesdale 1956) were first considered as possible vectors of *L. donovani* by Heisch (1956). Further investigations on these two species disqualified them as vectors of kala-azar (Heisch *et al.*1956; Wijers and Minter 1962; Wijers 1963 and Minter

1964). Mutinga *et al.* (1984) demonstrated the existence of *S. garnhami* in Baringo district which had previously been thought not to occur there. They trapped a number of *S. garnhami* at Loboi, which is a kala-azar focus, and found two of these sandflies infected with leptomonads in the anterior mid-gut.

Phlebotomine sandflies, comprised of about 600 species, are small (1.5-2.5 mm), hairy flies with long slender legs. Their wings are also hairy and usually held in a vertical 'V'-shaped position over their backs when resting. They have filamentous antennae which are similar in both sexes. Sandflies feed on juices or secretions of plants. However, the female sandfly needs one or more blood meals to complete the maturation of each batch of eggs

(Ashford 1974; Kaddu *et al.* 1992; Schlein *et al.* 1986). The mouth parts are similar in both sexes and consist of a pair of toothed mandibles and maxillae, with a median labium and hypopharynx. The labrum is short and forms part of the head. The mouth parts are flanked on each side by hairy maxillary palps.

The distribution of sandflies is mainly tropical and subtropical but extends into north temperate latitudes as far as southern Canada. Southern limits are less well defined but sandflies occur in southern parts of Australia and in South America to about 40^{o}S. The vertical distribution of sandflies extends to 2,800m, or more (Peru and Ethiopia) in warm parts of the World (WHO1980).

Sandflies are active in the twilight hours and most species seek shelter in dark, moist places in daylight hours. The most common daytime resting places include dark corners and crevices of houses, in pit latrines, crevices in stone walls, soil and rock crevices, animal burrows and in ventilation shafts of termite hills (Minter 1964; Bray 1983; Basimike *et al.* 1992; Johnson *et al.* 1993 and Mutinga *et al.* 1989).

Sandflies are weak fliers and their normal flight is more like a slow and leisurely hop usually of less than a metre. Flight range of sandflies was studied by Killick-Kendrick *et al.* (1984) in a "mark-release-recapture" experiment that reported distances which sandflies can travel in a single night as a few metres, or less than a kilometre in general. Yuval *et al.* (1988) found that *P. papatasi* could travel from 200m-2000m in a single night. Nonetheless, certain species can cover long distances, sometimes more than a kilometre overnight (Mutinga *et al.* 1990). Movements of upto 100m during the night are not uncommon. Perhaps such long range travel is mainly the result of passive carriage by air movements rather than of sustained flight, although most species cease activity in the lightest perception of a breeze. It is thought that most sandflies live and die within a few tens of metres from their place of birth (Killick-Kendrick *et al.* 1984).

Female sandflies of different species feed on the blood of a wide range of warm- and cold-blooded hosts which include : man, cats, dogs, rodents and other small mammals, cattle, wild carnivores such as jackals and foxes bats, birds, lizards, tortoises, snakes, frogs and toads (Mutinga *et al.* 1990; Ngumbi *et al.* 1992). Each species probably has a limited

range of preferred hosts: members of the genus *Sergentomyia* often favour birds and reptiles; while those of the genus *Phlebotomus* frequently favour mammals and sometimes, man. Sometimes, some species such as *P. papatasi* in the Near and Middle East, readily enter houses at night and man becomes a preferred host (WHO 1980).

Kala-azar was unknown in Kenya before the end of the second World war. Epidemics of visceral leishmaniasis resulted in some parts of Kenya when the soldiers of the King's African Rifles returned from war zones in the Sudan and Ethiopia (Cole *et al.* 1942; Anderson, 1943). Studies on the epidemiology of kala-azar in East Africa by Kirk (1956), Kirk and Lewis, (1947) and Heisch (1954) indicated that the disease is a zoonosis and that the causative agent is propagated by sandflies living in close association with an animal reservoir. Adler (1963) and Perkins *et al.*(1988) confirmed these findings. The Synphlebotomus group, *Phlebotomus martini* Parrot,1936 *P. vansomerenae* Heisch, Guggisbergi and Teesdale 1956 and *P. celiae* Minter 1962 are important in the transmission of visceral leishmaniasis in Kenya (Heisch *et al.* 1956,1962; Mutinga & Ngoka 1978; Perkins *et al.* 1988). The vector of cutaneous leishmaniasis which is caused by *L.major* is *P. duboscqi* (Beach *et al.*,1984; Chance *et al.* 1978). Vectors of *L. aethiopica* are; *P. pedifer, P. longipes* and *P. aculeatus* (Mutinga 1971, 1975; Kaddu & Mutinga 1981; Sang *et al.* 1983; Ngoka *et al.* 1975). *Leishmania tropica* is transmitted by *P. guggisbergi* (Lawyer *et al* ,1991).

Different sandfly species live in various habitats (Basimike *et al.* 1992; Mutinga *et al.* 1984,1990; WHO,1990). Certain species like *P. martini* Parrot has been reported to live in termite hills and *P. duboscqi* Neveu-Lemaire, in animal burrows (Robert *et al.* 1994). Other *Phlebotomus* species live in caves and dense forests on the highlands. These include *P. guggisbergi* Kirk & Lewis, *P. elgonensis* Ngoka, Madel & Mutinga, *P. pedifer* Lewis, Mutinga & Ashford, *P. aculeatus* Lewis (Bray 1982; Mutinga *et al.* 1986, 1989). Breeding sites of sandfly species in Kenya include termite hills, animal burrows, human dwellings and animal enclosures Mutinga *et al.*(1986). Adult sandflies live in varying and obscure habitats. The difficulties encountered in rearing sandflies for research purposes has led to very little knowledge being known about them (Lewis,1974; Killick-Kendrick 1984,1990; Mutinga *et al.* 1984, 1990; WHO 1990). Rodents are considered as reservoirs of certain *Leishmania* spp. in

Kenya and the most likely place of their contact with sandflies is in their burrows during resting times (Heisch, 1961; Githure *et al.* 1986). Rodents provide blood meals for sandflies which live in the burrows. The burrows also shelter sandflies from dryness and extreme temperatures that prevail above the ground during the day (Naggan *et al.* 1970; Gunders 1974).

To achieve a meaningful level of control of leishmaniasis, research must be conducted taking into consideration the ecological behaviour of the vectors, the interaction of the vector and the reservoir hosts, the vector-reservoir-host relationships and the development of the parasite in the vectors, reservoirs and hosts. This view was expressed by Lane (1991) who pointed out that the single most important constraint in assessing the value of vector control in leishmaniasis control is the lack of well documented examples of intervention. Information is usually in short ineresting pieces and therefore it is not possible to precisely evaluate the significance of sandfly control in disease reduction. The control of arthropod-borne diseases may be achieved under ideal conditions, perhaps by elimination of the principal sources of food of the vector for example rodents, or by removing the source of infection like, infected mammals and may be at the same time, knowing other sandfly behaviours (Weitz 1960). Possible methods of the control of arthropod-borne diseases include: a) Detection of cases and rapid treatment of patients. b) Control of the specific vector in and near human dwellings by periodic spraying of insecticides. c) Repair of cracks in mudwalls and removal of rubbish around houses. d) Organization of good reporting and recording systems for VL. Public health education by press, posters,radio, and television is valuable here. e) Elimination of infected dogs. f) Eradication of desert rodents over a radius of 2-3km around the focus by use of poisoned bait or deep ploughing.

Marigat Location in Baringo District where the study was conducted, has two sandfly genera; *Phlebotomus* and *Sergentomyia*. The former genus contains two important vector species: *P. martini* which transmits visceral leishmaniasis caused by *Leishmania donovani* and *P. duboscqi* which transmits cutaneous leishmanisis caused by *L. major* (Githure *et.al.* 1984,1986; Muigai *et al.* 1987; Mutinga *et al.* 1981,1984; Perkins *et al.* 1988). Sandflies of the genus *Sergentomyia* do not transmit diseases of medical importance to

humans. These include the following species *S. schwetzi* (Adler, Theodor & Parrot), *S. antennata* Newstead, *S. clydei* Sinton, *S. bedfordi* Newstead, *S. africana* Newstead, *S. squamipleuris* Newstead, *S. adleri* Theodor and *S. inermis* Theodor.

As a method of control, spraying of tertmite hills has been tried in an attempt to control *P. martini* (Mutinga *et al.* 1980, 1986). This has not been evaluated adequately and therefore no effective vector control measures can be recommended for *P. martini* or *P. orientalis* Parrot (WHO 1988). For the control of sandflies, identification of sandfly breeding sites as well as the importance of termite hills as breeding sites are important parameters to consider (WHO 1988). Schlein *et al.*(1989) showed how differential, attraction of dispersing and breeding sites of populations of *P. papatasi* Scopoli respond to manure and water baits.

Sandflies have been noticed to oviposit in areas that contain earlier colony remains and larval rearing medium. This acts as an attractant for sandflies to select certain areas as breeding sites as pointed out by Dia-Eldin *et al.* (1992). It could be possible to find a way of intervention in the life cycle of a sandfly if one can get the basic knowledge of how they behave within their ecosystem. This basic information can be enhanced by studying the following parameters in the population: species, sex, ovarian development, sugar feeding patterns, rotation of male genitalia, bloodfed and where and when caught. It is assumed that it will be possible to draw useful conclusions as to why sandflies will be found at particular areas in their ecosystems.

1.1.1 Objectives

The main objectives were to study the behaviour and utilization of termite hills and animal burrows by phlebotomine sandflies. The specific objectives were to:

1. Determine which of the two sandfly resting sites (animal burrows and termite hills) is more popular than the other and to which sandfly species.
2. Determine time of day, period during autogeny sandflies feed on sugar.
3. Determine whether termite hills and animal burrows act as breeding sites by observing the following parameters in sandflies: species, sex,

ovarian development, presence of bloodmeal and genitalia rotation.

1.1.2 Justification

Leishmaniasis is a global health problem as shown by the list of major diseases of the World (WHO/TDR, 1993). This disease affects people inhabiting Old and New World countries. In fact, the leishmaniases have been reported from 80 countries and probably some 400,000 new cases occur each year (Marinkelle,1980). The vectors of the disease have to be extensively studied and some control methods developed from these studies (Lane, 1992). The vectors of leishmaniasis have shown no signs of developing resistance to insecticides (WHO, 1988), which facilitates control measures when sandfly developmental stages are properly studied to show the most vulnerable stage to be attacked in a control programme.

Knowledge of sandfly behaviour in termite hills and animal burrows can be of great advantage in dealing with sandfly species that are suspected to live in these areas. The experimental area was chosen because of its abundant numbers of sandflies and the endemicity of leishmaniasis throughout the year (Heisch 1957; Heisch *et al.* 1959; Mutinga & Ngoka 1978; Perkins *et al.* 1988; Robert *et al.* 1994) . It is hoped that information obtained from this study will be of help in controlling sandfly populations.

1.2 Literature Review

1.2.1 Sandfly population structure

When sandfly populations are subjected to stressful conditions, their structure is changed. These changes depend mainly on the source and magnitude of the stress. For instance, insecticidal sprays cause death to most or all of sandflies in an area. In many countries, malaria control campaigns led to reduction in sandfly densities and in leishmaniasis morbidity, while resurgence of leishmaniasis morbidity was observed in numerous areas after cessation of these campaigns (WHO, 1980; Marinkelle, 1980)

Other factors that change the normal population structure are climatic conditions, seasonal abundance, the longevity of the vector, the duration of life-cycle and diapause period, the number of blood meals per gonotrophic cycle and autogenous behaviour of the fly (Marinkelle 1980).

During most control programmes, information collected is not adequate for the evaluation of the success of the control of leishmaniasis (Lane, 1991; Weitz, 1960). For successful control of sandflies, identification of sandfly breeding sites as well as the importance of termite hills as breeding sites are important parameters to consider (WHO,1980).

Minter (1964) noted that *P. martini*, which transmits pathogens responsible for kala-azar, are abundant in termite hills between December and April. Basimike (1992) reported that *P. martini* were more abundant in animal burrows than in termite hills in Baringo District. This information calls for control measures to be targeted at the termite hills and animal burrows.

Since successful control programmes of leishmaniasis depend on knowledge of sandfly behaviour, it is important to acquire as much knowledge as possible about them. Some of this knowledge has to come from studies done on sandflies in their natural habitats. This study on abundance of sandflies in termite hills and animal burrows is aimed at providing useful information that can be used in controlling sandfly transmitted diseases.

The ecological conditions of areas around Perkerra and Rabai Primary Schools give ample opportunities for the study of the behaviour of the phlebotomine sandflies in an area that is active with leishmaniasis. For this study, an entry-exit trap, was made to capture sandflies when entering or leaving the termite hills and animal burrows (Yuval *et al.*1986). By trapping sandflies in the termite hills and animal burrows it was possible to study the sandfly activities at different times or periods of the night. The objectives of this study are centred around the determination of the abundances of sandflies in termite hills and animal burrows, periods when sandflies feed on sugar, whether termite hills and animal burrows act as breeding sites by observing parameters like: species, sex, ovarian development, presence of a blood meal and male genitalia rotation.

1.2.2. Development of *Leishmania* in a sandfly.

Sandflies are 'pool-feeders', which means that they pierce the skin of the vertebrate host with their cutting mouth parts and create a tiny pool of blood which they suck into their guts. Should the host be infected with *Leishmania*, the free or intra-cellular amastigotes may be taken up with the blood meal. During the first 72 hours in the insect gut (Bates 1994; Lawyer *et al.* 1990), the parasite elongates and the rudimentary flagellum grows out into a long whip-like structure that extends from the flagellar pocket. The resulting parasite, called a promastigote, is very motile and undergoes many multiplications by longitudinal binary fission.

The size of the flagellate is dependent upon the species or subspecies of *Leishmania*. Laison (1982) reported the size of *Leishmania* promastigote to vary from 16.0-40 μm long (including the flagellum) by about 1.5-3.0 μm wide. The flagellum and kinetoplast are situated at the anterior end of the organism and the nucleus is more or less centrally placed. The parasites by the third day become elongated and active in the midgut while the short forms remain attached to the gut wall occasionally forming epimastigotes. On the fourth day they occur in masses at the proventriculus and the fly is ready for a second blood meal. This method of development is termed 'foregut' development and is the method for all mammalian species of *Leishmania* except *L. braziliensis* sub-species and *L. peruviana* in which development takes place in the "hindgut" and is followed by a foreward migration. Transmission takes place when the infected sandfly attempts to feed and the promastigotes are injected via the proboscis into the skin of the host. In the vertebrate's body the promastigotes change into amastigotes.

1.2.3 Sandfly population composition and their behavioural patterns

According to life tables, insect populations are usually stable when no added control factors are acting on the population such as density dependent or density independent factors. At this point of stability, the population is said to be at equilibrium and their rate of increasing is usually zero as was reported by Rogers and Randolph (1984). It is therefore obvious that even in the absence of external pressures that limit population growth the population does not increase indefinitely. Some of the factors that limit population growth were discussed by Hargrove (1988). He pointed out such factors as pre-adult and adult survival, interlarval period and pupal duration as density dependent factors regulating population growth. Other regulating factors include those discussed by Rogers and Randolph (1984). Any new births, mortalities of individuals, emigrations and immigrations of individuals, usually change insect population densities.

According to a WHO (1980) report on age composition of sandfly populations, most sandflies show gonotrophic concordance, producing one batch of eggs following each meal. Some species are capable of egg production before the first blood meal, but only one batch will be produced without a blood meal. Occasionally, two partial meals are taken in a single gonotrophic cycle. The normal gonotrophic cycle is estimated to take four to five days.

While there is some controversy over age-determination methods, those used involving dissection of females, are reported to give a reliable indication of whether or not an individual is parous, having survived one or more gonotrophic cycles. (Figure 2. 2). The proportion of parous female sandflies within a sandfly population indicates the epidemiological potential of the population. The highest parous rate occurs in old populations towards the end of a given generation. Therefore maximum parasite transmission can occur when the parous rate is high, provided that the population density is also high (WHO,1980)

In most cases where sandflies have been controlled by insecticides, it was an incidental effect of programmes aimed at other insects. For example, in India and Bangladesh, kala-azar was largely controlled by antimalarial campaigns (WHO 1980). In Israel, control of domestic flies and cockroaches reduced cutaneous leishmaniasis; in Tunisia, the reduction was thought to come from locust control with D D T. In Greece,

antimalarial activities reduced sandfly fever but not visceral leishmaniasis and in Iraq, anthroponotic cutaneous leishmaniasis was controlled, but not visceral leishmaniasis. In other instances there has been no effect on leishmaniasis (WHO,1980). This emphasizes the importance of detailed knowledge of the biology and ecology of sandflies and all other insect pests in the planning of insecticidal operations.

Important vectors of the visceral leishmaniasis in the Old World include *Phlebotomus ariasi* in France, and probably *P. perniciosus*, *P. major* and *P. longicuspis* in the Mediterranean Basin, with *P. smirnovi* and *P. chinensis* in Turkmenia and Kazakhstan. *Lutzomyia longipalpis* is the most probable vector in Latin America (Killick-Kendrick 1978). In Sudan, *P. orientalis* is the vector whereas in Kenya, *P. martini* is the suspected vector. In Bangladesh and India transmission is by *P. argentipes* (WHO 1980; Mutinga *et al.* 1985).

Vectors of cutaneous leishmaniasis in the northern natural foci of Central Asia include *P. mongolensis*. Other sandflies which can take part in the transmission are *P. caucasicus* in areas of fine-grained soils and *P. andrejevi* in the sandier deserts (WHO 1980; Killick-Kendrick 1990). These species are not anthropophilic and risk of human infection is determined by the presence or absence of *P. papatasi* which is confined to the delta-valley landscape with clay soils and a high water table in the southern part of the range of *L. major* (WHO 1980). *P. sergenti* is the vector of *L. tropica* in parts of Mediterranean Basin, North Africa and Iran (Killick-Kendrick 1990). It is abundant in urban areas but uncommon in the rural areas where it is restricted to caves and rocky environments. Buildings in the urban centres provide structures similar to the ones in the natural habitat. *P. longipes* is the vector of *L. aethiopica* in Ethiopia and *P. pedifer* in southern Ethiopia and Kenya (Kirk *et al.* 1947; Killick-Kendrick 1978, 1990; WHO 1980 & Mutinga *et al.* 1985;).

Ngumbi *et al.* (1992) analysed sandfly bloodmeals from Baringo District and found that the majority of bloodfed *P. martini* came from termite hills. Johnson *et.al.* (1993) found that termite hills yielded more sandflies than animal burrows. Bray (1983) working on the reservoirs of cutaneous leishmaniasis in the former Soviet Union discovered that the vast chambered burrows of the great gerbil *Rhombomys opimus* Licht provided conditions for large-scale breeding of sandflies. He also found that the rodent burrows provide the

temperature, humidity and moist breeding ground ideal for the breeding and feeding activities of sandflies

Yuval *et al.*(1986) studied the nocturnal activities of *P. papatasi* and found that the emergence of males with unrotated genitalia were 0-24 hours-old-adults that hatched from burrows between dusk and midnight which pointed to those burrows as *P. papatasi* breeding sites.

It has been suggested by Aussel (1993) that knowledge of the breeding sites of *Leptoconops albiventris* de Meijere (Diptera: Ceratopagonidae) can lead to the control of their populations. In WHO bulletin (1980) it was reported that sandflies usually rest during the day in burrows, tree holes, caves or buildings. They usually leave these shelters at dusk and are active in the open in the evening and at night. Daily temperature fluctuations are minimal in a burrow at a depth of 50 cm, while the surface soil temperature may vary by 40^{o}C (WHO 1980). Sandfly activity commences when the air temperature is equal to the underground temperature usually around sunset. Under suitable environmental and ambient conditions, sandflies remain active throughout the night, but they are sensitive to decreasing temperatures and also air movements (Killick-Kendrick 1978; WHO 1980) .

1.2.4 Distribution of leishmaniases

The genus *Leishmania* has a wide distribution in the tropics and sub tropics, but is apparently absent in South-East Asia and the Pacific. A WHO bulletin (1980) gives the distribution of visceral leishmaniasis caused by *L. donovani* to extend from the Pacific coast of China through Asia and parts of Africa to South and Central America. The distribution of the vectors of visceral leishmaniasis is influenced by factors like: temperature, humidity, altitude, vegetation and fauna. It has also been reported (WHO 1980) that VL is restricted to areas with a dry season of 100-200 days and a significant amount of moisture deficit. The altitude of these areas is generally less than 1,000m above sea level. For at least five months of the year the air temperature is above 15^{o}C and the absolute yearly minimum is not below-16^{o}C.

A large proportion of African biotopes are suitable for VL but the disease is rare or unknown. This is true when one considers that the new disease foci and new sandfly discoveries indicate that there is a lot related to leishmaniasis unknown in the continent. It is believed that VL is more widespread in Africa than is currently known (WHO 1980). The geographical areas that have the highest incidence, sometimes exceeding 120 per 10,000 persons are in Bangladesh, China and India. These areas have more days with temperatures over 20^{o}C and an annual rainfall of 1465 mm (WHO, 1980). Reservoirs are canines, rodents and humans .

Old World cutaneous leishmaniasis can be divided into three separate species:*L. major*, *L. tropica* and *L. aethiopica*. *L. major* occurs in southern Mongolia, and the borders of China through Kazakhstan and northern Afghanistan to the Ural River and Caspian Sea. Other areas include Iran, Saudi Arabia, Israel, and North and West Africa. In Asia, the great gerbil *Rhombomys opimus* is the reservoir whereas a number of rodents are reservoirs in Africa (WHO 1980; Githure *et al.* 1984,1986). *L. tropica* overlaps in some parts with *L. major* in Central Asia, Pakistan, Afghanistan, Iran, Iraq, Syria and the Mediterranean Basin. The reservoir is thought to be man (WHO 1980). *L. aethiopica* occurs almost exclusively in the highlands of Ethiopia and Kenya. Reservoirs are *Procavia habessinica*, *P. johnstoni* and *Heterohyrax brucei* (WHO 1980; Mutinga *et al.* 1970; Kungu *et al.* 1972; Sang *et al.* 1983).

Lawyer *et al.*(1989) reported on the current status of leishmaniasis research in Kenya and pointed out the extent and distribution of visceral leishmaniasis (Kala-azar) as well as the three forms of cutaneous leishmaniasis, *L. major*, *L. tropica* and *L. aethiopica*. In Kenya, kala-azar foci were discovered in Baringo and West Pokot Districts of Rift Valley Province (Mckinnon & Fendall 1955; Wijers & Minter 1962; Leeuwenburg *et al.* 1981). Other foci of visceral leishmaniasis include Meru and Kitui districts (Wijers & Minter 1966), Athi River valley, Machakos District (Wijers & Kiilu 1984), Masinga Location in Machakos district (Mutinga 1985) and Kajiado District (Johnson *et al.* 1993). A recent outbreak of kala-azar occured in southern Turkana District in 1986 in which an estimated 60-100 people died (Lawyer *et al.* 1989). Reservoir hosts of visceral leishmaniasis in Kenya have not been

conclusively identified but dogs are suspect (Mutinga 1980). Humans are also considered possible suspect resevoirs (Minter *et al.* 1962; Chulay *et al.* 1985).

Cutaneous leishmaniasis caused by *L. aethiopica* occurs on the forested slopes of Mt. Elgon in Bungoma District (Mutinga & Ngoka 1970; Kungu *et al.* 1972; Sang *et al.* 1983). Reservoirs of this disease include rock and tree hyraxes (*Dendrohyrax arboreus* and *Procavia johnstonni*, respectively) and the giant rat (*Cricetomys* sp.) (Mutinga 1975). Cutaneous leishmaniasis caused by *L. major* was first reported in Baringo and West Pokot Districts of Kenya by Heisch (1957), Heisch *et al.* (1959) and Beach *et al.* (1982). Reservoir hosts include the ground squirrel (*Xerus rutilus)* and gerbils (*Tatera robusta), Arvicanthis niloticus, Taterillus emini, Mastomys natalensis* and *Aethomys kaiseri* (Heisch1957, Heisch *et al.* 1959; Githure *et al.* 1984,1986). *L. major* was also recently isolated from a naturally infected vervet monkey (*Cercopithecus aethiops*) in Kiambu District by Binhazim *et al.* (1987). *Leishmania tropica* is a recent discovery in Kenya which extends from Muruku sub-location in Laikipia District across to Njoro and Utut areas in the Rift Valley (Mebrahtu *et al.* 1987, Lawyer *et al.* 1991, Sang *et al.*1993). Reservoir hosts have not yet been established but rodents and hyraxes are suspected.

1.2.5 Leishmaniasis control.

The consequences of leishmaniasis in terms of mortality and morbidity and the ever increasing human population makes leishmaniasis control a must in order to open up more land for utilization. In order to do this successfully, the stages of epidemiology have to be broken somewhere along the way. This can be achieved by either elimination of the parasite, vectors, reservoirhosts or even interfering with the human hosts through treatment or vaccination. It is estimated that there are some 400,000 new cases of leishmaniasis of all types every year (Marinkelle 1980; Chance 1981).

1.2.5.1 Chemotherapy.

Chemotherapy against leishmaniasis does not offer protection against reinfection (Manson-Bahr 1959). Kager *et al.* (1983) described some of the curative drugs in use today that can be administered to patients suffering from leishmaniases. These include sodiumstibogluconate (Pentostam) from the Pentavalent antimony compounds and Diamidines such as pentamidines and hydroxystilbamidine. Most of these drugs are toxic when used in high dosages.

Manson-Bahr and Heisch (1956) used combinations of Pentostam and Pentamidine to achieve a superior treatment of kala-azar patients compared to treatment with Pentostam or Pentamidine alone.

Forester (1966) reported an alternative treatment with amphotericin B in a number of kala-azar patients that were resistant to Pentostam. Splenectomy has been performed on patients that were resistant to numerous courses of therapeutic drugs like Pentostam, ureastibamine, hydroxystilbamidine, stilbamidine and pentamidine (Manson-Bahr 1959).

A more recent method of leishmaniasis control still in development is a vaccine that can prevent healthy individuals from contracting the disease (WHO 1980).

1.2.5.2 Vector control

Before the discovery of synthetic insecticides at the beginning of the twentieth century, pest control depended on organic compounds extracted from plant materials. Moriaty (1969) outlines the composition of synthetic insecticides. There are three important groups comprising; 1) organochlorines such as dichloro diphenyl trichloroethane (D.D.T.), dieldrin and endosulfan, 2) organophosphates such as malathion, fenithion and parathion; and 3) carbamates such as carbaryl and propoxur.

In areas where biting sandflies are numerous the use of repellents, such as dimethylphthalate and N,N-diethyl-m-toluamide (Deet) is warranted. Bed nets of mesh size 1.8 cm^2, impregnated with dimethyl phthalate or DEET (20ml/m^2) and used as bed nets, can provide complete protection for 17-20 days provided they are packed in plastic bags every morning to keep their insecticidal qualities from diminishing (WHO 1980).

The use of fine mesh sleeping nets also keeps sandflies and other insects away from people. Sandflies are also very susceptible to modern insecticides of all types (WHO 1980) and where economically feasible they should be widely but selectively applied to known resting or breeding places. The most commonly used insecticides include D.D.T, HCH (Lindane), dieldrin, malathion, acetophos and trichlorfon (WHO,1980). Since breeding places and larval habitats in general are inadequately known or inaccessible to direct insecticide application, only the adults can be attacked and, therefore, the residual effect of the insecticide is very important. Domestic pests or vectors such cockroaches, fleas, mites, mosquitoes, sandflies and houseflies have been controlled through a fortunate by-product of antimalarial spraying programmes of habitations, for example; *P. papatasi* in the Middle East and *P. argentipes* in India, (WHO 1980). In areas where sandfly vectors are exophilic, for instance; *P. orientalis* in the Sudan, *P. martini* and related species in Kenya house-spraying is ineffective (Marinkelle 1980).

In towns and villages, the construction of new houses may provide favourable conditions for sandflies, especially *P. papatasi* and *P. martini* which are endophilic. The newest methods of pest management which include genetic control or biological control (using pathogens or parasitoids and predators), have not been developed for application against sandflies (WHO 1980).

Since sandfly biting takes place in most cases at night, people are required to minimise these bites by constructing dwellings in which the sandflies do not readily reach people while sleeping. People are advised to wear thick clothing, use bed netting and window screening to reduce exposure to sandfly bites (WHO 1980). These methods are generally only effective in places where sandfly bites constitute a nuisance, as the fine mesh netting excessively reduces ventilation in hot climates and people are unwilling to take uncomfortable precautions against an invisible threat. Mutinga *et al.* (1986) suggested that people or children who sit near termite hills in the evenings risk contracting leishmaniasis from sandfly bites. In Kenya, certain *L. tropica* cases came from patients who had visited caves where the sandfly vectors are abundant (Lawyer *et al.* 1991). To reduce the spread of leishmaniasis, areas that offer human-vector contact should be avoided.

1.2.5.3 Environmental modification.

Enviromental methods of sandfly control include the eradication of their breeding and resting sites (WHO 1980). These methods work best in places where breeding is concentrated in clearly defined habitats, such as rodent burrows and termite hills. Construction of houses that are fitted with screens or fine meshes can keep out sandflies at night and reduce the number of bites. Attempts have been made to control rodent populations by ploughing their burrows to a depth of 60 cm in the former Soviet Union (WHO 1980). This method has the advantage of destroying sandflies in addition to rodents. However, surviving rodents rapidly construct new burrows and the process may have to be repeated several times over several years to achieve complete eradication, which makes the process very expensive (WHO 1980). Ploughing is most useful therefore in the immediate neighbourhood of settlements, where the risk of human infection is particularly high.

1.2.5.4 Traps and targets.

A need to conserve the environment and avoid excessive high prices of insecticides has led to cheaper and safer methods being sought. One of these cheap methods is the use of sticky paper traps which has been developed and used with some success in Marigat Location, Baringo District (Mutinga *et al.* 1984, 1986). Long sheets of polythene paper are smeared with castor oil, and then tied round the walls of buildings or living houses. When sandflies attempt to fly into the houses, they hit the sticky sides of the papers and get stuck on them-hence never manage to reach the would-be victims sleeping inside the houses. It has been observed that people living or sleeping with animals around them get few sandfly bites because the animals provide the needed blood meal to the sandflies (Mutinga *et al.* 1990).

CHAPTER II

MATERIALS AND METHODS

2.1 Study area

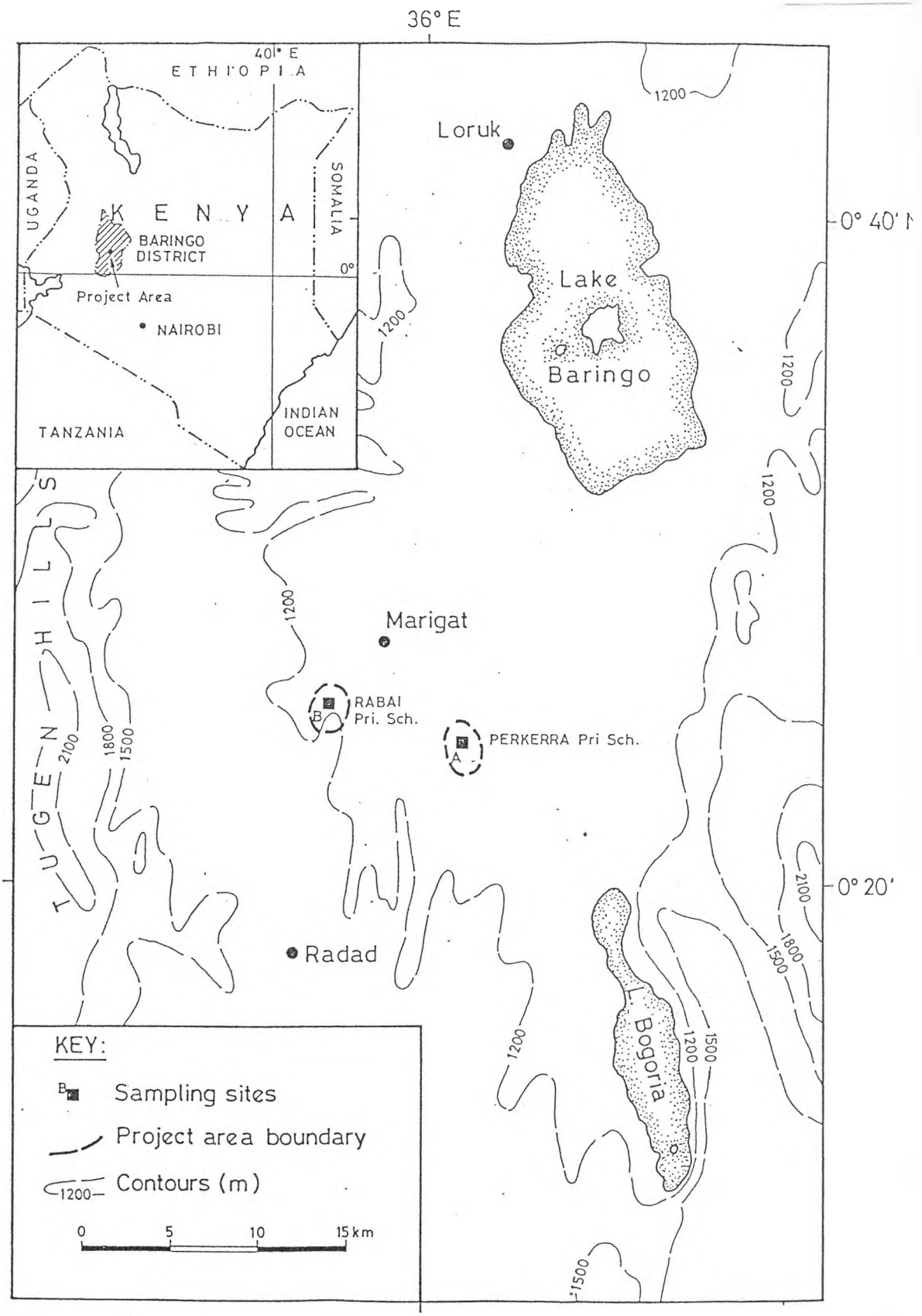
2.1.1 Location

The study was conducted at two sites each covering a radius of about 5km around Rabai and Perkerra Primary Schools. Rabai which lies south west of Marigat town is about 6km away. Perkerra lies to the south east of Marigat town and is approximately 8km from the town. The location of the two sites is approximately (0^{o} 30' N , 36^{o} 0' E). The elevation of the two schools is between 1000 and 1100m above sea level in Baringo District, Rift Valley Province. Marigat town is 250 km northwest of Nairobi, Kenya. The area is bordered by Lake Baringo to the north, Lake Bogoria to the south, the Tugen hills to the west and Laikipia escarpment to the east. This is an area which was subjected to volcanic activities during the formation of the great Rift Valley, which saw the two escarpments to the east and west separating apart and the land in between sinking down (Figure 2. 1).

The Perkerra Primary School trapping site is located next to irrigated farmland, while the Rabai Primary School trapping site, which lies to the north west of Perkerra has no crop growing activities (Plate 2.6). Rabai has scattered homesteads, which have large flocks of sheep and goats (Plate 2.6). The Perkerra site had more small rodents that bred quite well after feeding on the farm produce from the adjacent irrigated plots than did the Rabai Primary School trapping site. Termite hills were readily available at both sites. Animal burrows were more numerous at Perkerra than at Rabai due to the large numbers of rodents at Perkerra.

Fig.2.1

Map of the study area. The location of Perkerra and Rabai Primary Schools.



2.1.2 Geology

The landscape is generally flat and gently slopes into Lake Baringo to the north and Lake Bogoria to the south. The basement rock is hard granite which has undergone extreme heating from deep in the earth's crust. During the formation of the Rift Valley some of these rocks were pushed up. The hills are outcrops of the same granite rocks.

The area is a semi-arid alluvial plain, altitude 1000-1100m, which is extensively overgrazed during the dry season except for occasional weeds, thorn bushes and *Acacia* trees which dot the area (Perkins *et al.* 1988; Basimike *et al.* 1992). Except in the alluvial plains, the rest of the landscape contains granite stones and pebbles that make most areas unsuitable for crop growing. The soils are red and grey with relatively high clay contents. The grey soils are characteristic of low lying areas and shallow depressions. They are made of clay and are high in organic humus where there is good vegetation cover. The area has serious soil erosion caused by wind and heavy torrential afternoon thunderstorms which are frequent during the rainy season.

Tall and weathered termite hills, associated with the transmission of leishmaniasis in Kenya (Southgate & Oriendo 1962; Minter 1963; Wijers & Mwangi 1966) are common throughout the area. The area is endemic for both visceral leishmaniasis due to *Leishmania donovani* (Mckinnon 1962; Schaefer *et al.* 1994) and cutaneous leishmaniasis due to *L. major* (Muigai *et al.* 1987; Heisch 1957).

2.1.3 Climate

Marigat, Fig. 2.3 receives a short rainy season in the months of November and December and a long rainy season from March to August. The long rains are heavier than the short rains. The months of January, February and March are relatively hot and dry. Altitude plays a major role in determining the amount of rainfall received. The hills on the east and west of the study area are wetter than the floor of the Rift Valley. Baringo District is an area where strong winds, having characteristic dust storms, occur in the afternoon and sweep across the semi-arid alluvial plains between lakes Baringo and Bogoria (personal observation).

In the dry season the daily temperatures can rise to as high as 35 ^{o}C. Evaporation on the floor of the Rift Valley is quite high and results in the disappearance of moisture very quickly even during the rainy season. This causes the weeds to wither and die out quickly. The only plants that survive well in this kind of climate are *Acacia* trees which are deep rooted and shrubs that grow along the river banks and in the river beds or under the *Acacia* trees (Perkins *et al.* 1988).

2.1.4 Animals

People living in the study area keep livestock such as cattle, sheep, goats and donkeys as their main source of livelihood. Sheep and goats are the dominant forms of livestock. Wild animals present in the area are ant-eaters, rabbits, porcupines, ground squirrels, hedgehogs, mongooses, bats, vervet monkeys, many small rodents, birds, lizards,tortoises and snakes. Rodents found in this area build many animal burrows (Githure *et al.* 1986).

2.1.5 Sandfly distribution

The study area is infested with important vectors of leishmaniasis such as *P. martini*, *P. duboscqi* and a number of nuisance *Sergentomyia* species. Sandflies occur in Marigat throughout the year with peaks occuring some weeks after the onset of the seasonal rains (Basimike *et al.* 1992; Robert *et al.*1994). In a study done by Killick-Kendrick (1989) on the ecology and entomology of the leishmaniases, he pointed out Pavlosky's concept of landscape epidemiology. He advanced the idea that in foci which are well studied, characteristic features of the landscape are often recognised and can act as markers revealing places where the parasite is circulating. He gave examples as *Acacia* trees in Sudan for VL, termite hills in VL foci of East Africa, oak trees in Southern France and chepond plants in the foci of (ZCL) in Saudi Arabia. These marker features of the landscape are a consequence of, among others, climate and soil, but while recognizable in these and other foci of the leishmaniases in the Old World, they are generally obscured in the complex habitat of the tropical rain forests.

2.2 The trap

The entry-exit trap used in this study was made of a plastic cylinder measuring 42cm long and 8cm in diameter, open at both ends and divided in the middle by a fine mesh partition (20 mesh/cm) (Yuval *et al.* 1986; Perkins *et al.* 1988). Papers coated with castor oil were rolled into cylindrical shapes of diameter 6-7cm. so that they could fit into the traps (Plate 2.1). These cylindrical oiled papers were inserted into each end of the trap. To keep the oiled papers held into position, paper clips were used to hold them firmly on the edges of the trap. The oil also kept the sticky papers stuck in position against, the inner surfaces of the trap. This sticking manner and the holding with paper clips prevented the paper from falling off into the termite hill shaft or animal burrow.

When the traps were inserted into the termite hill shaft or animal burrow, any spaces between the sides of the traps and the surfaces of the shaft or burrow were sealed off with spongy materials and paper towels (see Plates 2.3 and 2.4). This ensured that sandflies could not get out or into the termite hills and animal burrows through the sides of the trap.

The traps were designed in such a way that air can flow through the screen situated at the centre of the trap but prevent sandflies from going through. Sandflies bouncing back after hitting the screen got stuck on the sticky paper lining the sides of the trap.

2.3 Trapping and sorting of sandflies

At 1800 hours, three traps were set in the termite hills and three others in animal burrows at each of the sampling sites. At midnight, a corresponding number of traps were set at Perkerra and Rabai in fresh termite hills and animal burrows. Thus, in a single night's trapping 12 traps were set in termite hills and 12 others in animal burrows. All the traps were removed 6 hours after being set and taken to the field laboratory at the campsite. Traps set at 1800 hours were collected at 2400 hours, while those set at midnight were collected at 0600h and taken to the campsite laboratory. No trapping was done during the day because sandflies avoid movement during the day due to high temperatures that can cause dessication (Perfiliev 1968). The sandflies were trapped for six nights in a month for a period of seven months. At the campsite the sandflies were grouped according to the time (before or after midnight) and location of capture. They were sorted, then removed carefully from the sticky papers with fine camel hair brushes and put into 2 % detergent in saline for 10 minutes to remove the oil from their bodies. After 10 minutes they were transferred into normal saline to remove the detergent and oil. Then the flies were counted and put in a plastic vial (1.8 ml), containing a solution of Schneider's *Drosophila* medium supplemented with 10 % dimethyl sulfoxide (DMSO). Information (date, trapsite, time of catch, number of sandflies in the vial, direction of movement and the area where caught) was recorded on the vial and also in the recordbook.

Plate 2.1

The entry-exit trap which was used during the project to trap sandflies.

2.4 Preservation of sandflies in liquid nitrogen

The sandflies in the plastic vials were vapour-cooled for 30 min. before being inserted into liquid nitrogen. The sandflies were then kept in the liquid nitrogen and transported to the entomology laboratory at Kenya Medical Research Institute (KEMRI) where they were transferred into a freezer (- 80^{0} C) while awaiting final processing.

Processing of sandflies at KEMRI involved thawing, washing in 2% detergent and normal saline, dissecting, testing for sugar meals using cold anthrone, sexing, classification of gonotrophic status of females and grading of males using their genitalia, mounting, identification and recording.

2.5 Dissection procedures

Vials containing sandflies were retrieved from the freezer, thawed and the contents put in washing dishes. The flies were first put in 2 % detergent to wash away DMSO and excess hairs on their bodies, then transferred to normal saline which was also used as the dissection solution. Important physiological features which were examined during dissection included: sex, bloodfed females, accessory glands to determine whether the sandfly was parous, nulliparous or gravid (Perfiliev 1968).

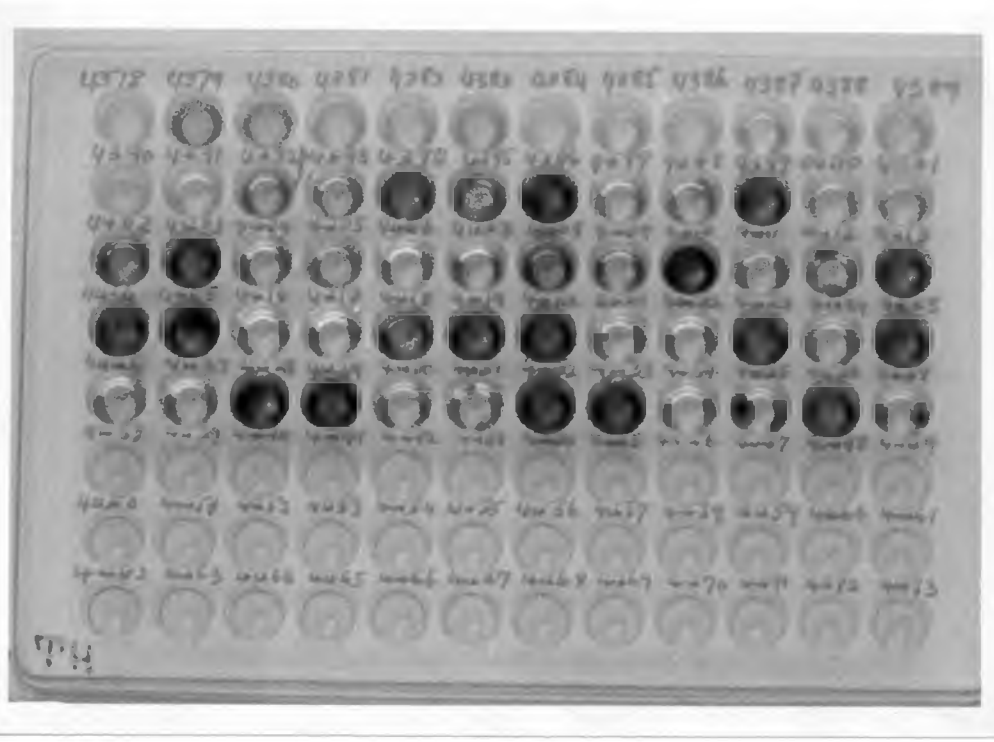
2.6 Testing of sugar meals in the sandfly guts

Detection of sugar in the guts of sandflies was done by removing the whole gut and putting it in an ELISA plate well (Dynatech.laboratories Inc. 900 Slaters Lane, Alexandria, Virginia 22314, USA). Three to four drops of cold anthrone were added into the wells containing the sandfly guts (Van Handel 1972). Each gut was ground with a different plastic grinder to expose sugar contents in the gut and make sure no contamination of wells with materials from another well (see Appendix 1). The cold anthrone, in the presence of sugar changed from yellow or orange colour to deep greenish blue Plate 2.2. The colour change, depending on the amount of sugar available, took about 10-30 minutes to show. The test

was applied to all females and males because both sexes take sugar from plants (Yuval *et al.* 1986).

Plate 2.2

Colour changes of anthrone solution due to presence of sugar in the sandfly guts. The plate wells which have greenish or deep greenish-blue colour are positive for sugar. The yellow looking wells are negative for sugar. The heavier the bluish-green colour, the more sugar there is in the sandfly gut. Sandfly numbers are written in red ink for each well used.

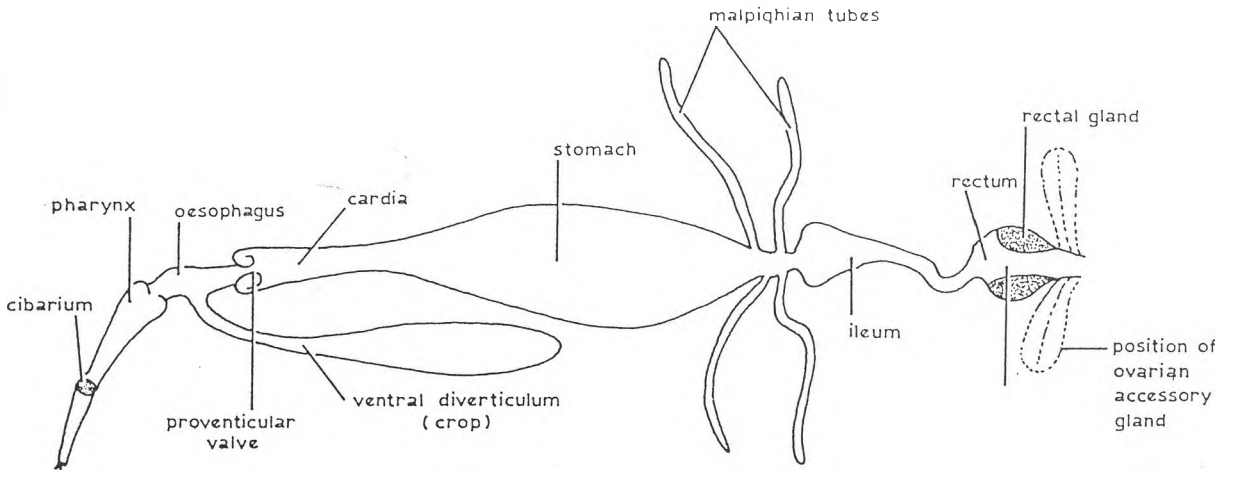


2.7 Age determination of sandflies

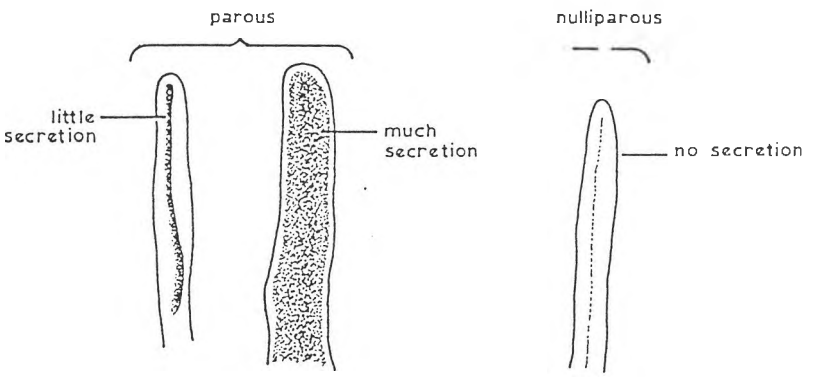
Age grading of male sandflies was done by examining during dissection the genitalia to ascertain whether they were rotated or not. The rotation of the genitalia occurs 0-24 hours after emergence. The genitalia rotates through 180 ^{o} (Beach *et al.* 1983). Males which bore unrotated genitalia indicated that they were less than 24 hours old meaning that they were newly emerged from their breeding sites. This feature was therefore important in determining the breeding sites of sandflies because newly emerged male sandflies would be caught immediately after hatching (see Plate 2.5). For the females, important features in determining their ages are accessory glands which determined whether the sandfly is parous, nulliparous or gravid. Unfed-nulliparous sandflies are considered the youngest females whereas parous are the oldest (Figure 2.2).

Fig. 2.2

A diagram of sandfly alimentary canal showing the position of ovarian accessory glands and how they are used to determine the age of female sandflies.



Sandfly alimentary canal.



Status of ovarian accessory glands.

Plate 2.3

A picture of a termite hill used by sandflies in Baringo District.

A pinnacled and eroded termite hill built by *Macrotermes subhyalinus* typical of Baringo area.

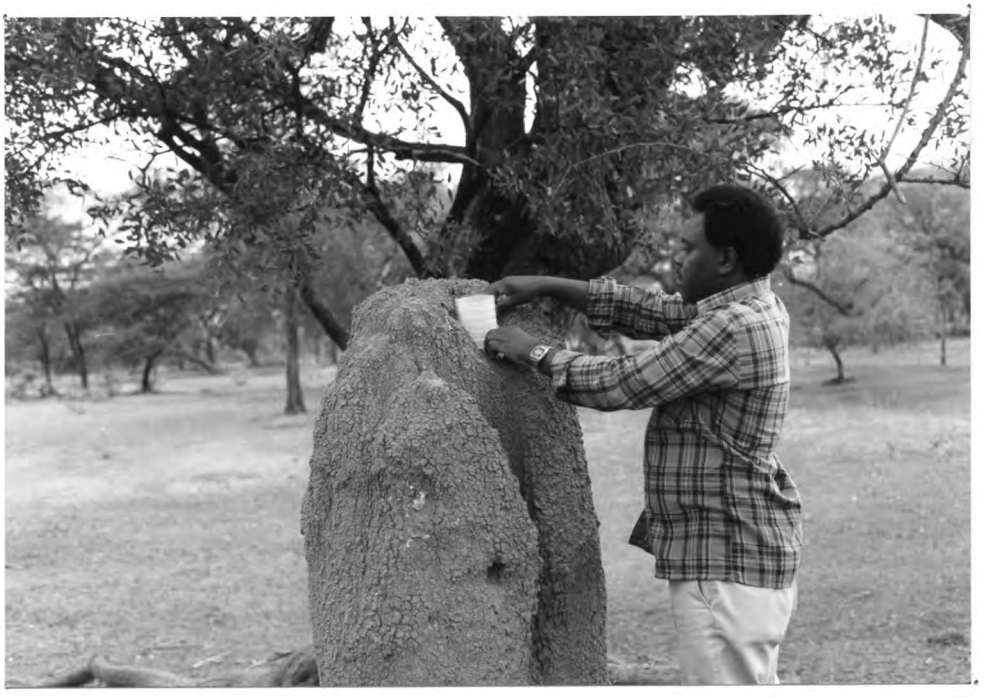


Plate 2.4

A picture of an animal burrow where sandflies were trapped during the project. This was an example of animal burrows dug by ground squirrels (*Xerus rutilus*.)

Plate 2.5

A picture showing rotated (R) and unrotated (U) male genitalia of a sandfly.

In the rotated genitalia/picture sternites (S) are shown in the normal position and in the unrotated genitalia/picture sternites are upside down.

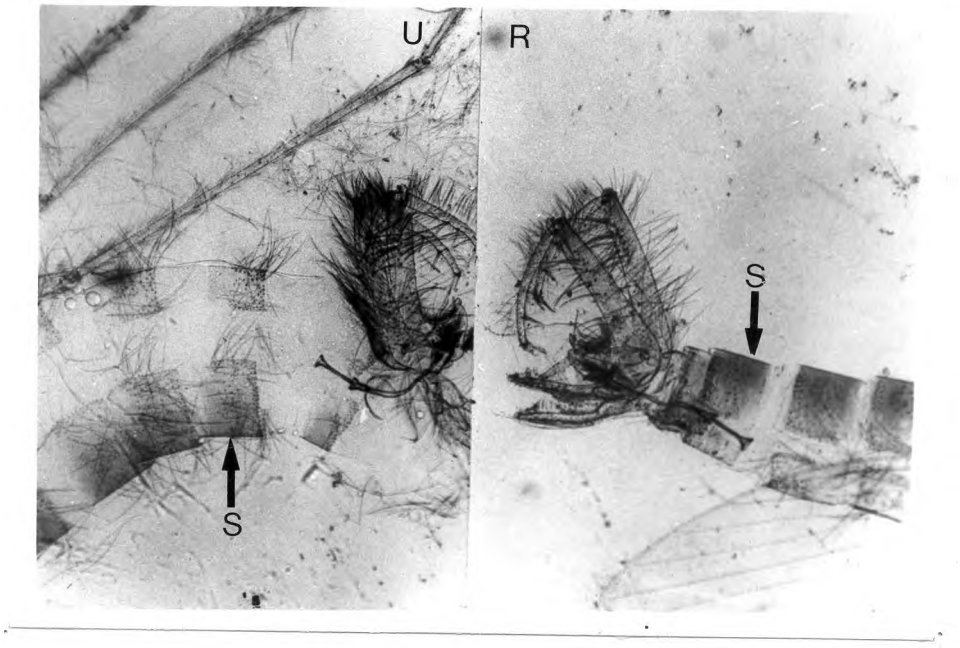
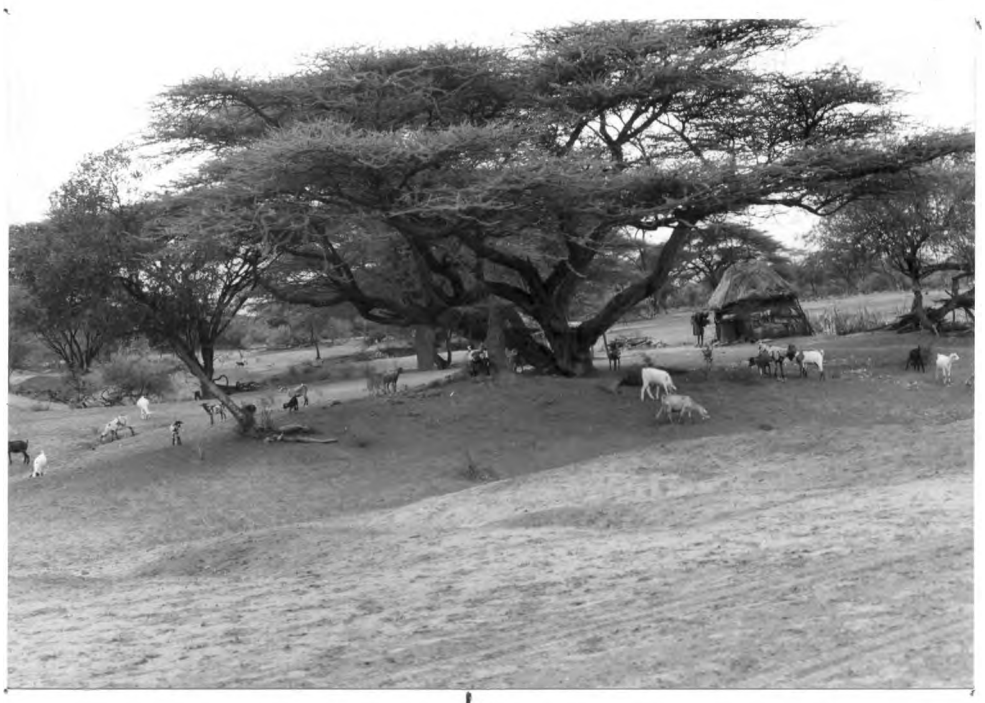


Plate 2.6

Shows two pictures of the two trapping sites a) Perkerra b) Rabai.

Notice the difference in vegetation cover at the two sites. Perkerra offered better sandfly breeding conditions than Rabai.

a



b

2.8 Identification of sandfly species

The taxonomic characters for identification of sandflies are usually found in the head (cibarium, and pharynx) and the last three segments of the abdomen (genitalia) which contains the spermatheca in females and penis sheath, spines and hair tufts in males. These two parts of each sandfly were mounted on a slide using gum chloral (see Appendix 2) as the mountant and covered with a coverslip (22 mm^2) and allowed to airdry within a day or two before being identified using the identification keys of Abonnenc (1972). All the information about each sandfly was entered on data sheets ready for use in the computer for analysis.

CHAPTER III

RESULTS

3.1 Climate (Weather conditions)

During the period of the study (November 1993-May 1994) the short rains (November-December) and part of the long rains (March-August) (Figure 3.1) were covered. Some rain was recorded in November and December but January and February were dry months. The long rains started in March and showed a peak in April.

Relative humidity was highest during the rains and lowest in the dry season during January and February. January and February were the hottest months covered by the study period with average temperatures of about $35^{o}C$.

3.2 Effects of rain on sandfly populations

The level of significance between variables was calculated using the Chi-square test (X^2). To test whether there was any relationship existing between two variables, Pearson's Correlation Coefficient was applied. The value of correlation that was computed to test if there existed any association between the amount of rainfall that fell during the study period and the number of sandflies collected was r= -0.12. This showed that there was no association. Meaningful association exists between two variables when $r \geq 0.5$.

The monthly catches of sandflies from November 1993 to May 1994 and the amount of rainfall that fell during this period are shown on Fig.3.1 An increasing trend of sandfly catches for the two trapping sites of Perkerra and Rabai was observed. There was however, a decline in the number of sandflies caught in March and April at Perkerra. The decline in sandfly catches was caused by heavy rainfall that fell in March and April, flooding some of the animal burrows. The sandfly catches at Rabai were less affected by the heavy downpours during this period. When it is windy or cold sandflies do not move from their resting places. The dry period between January and February did not result in a decrease in the numbers of sandflies.

Fig. 3 .1

Rainfall and monthly catches of sandflies at Perkerra and Rabai.

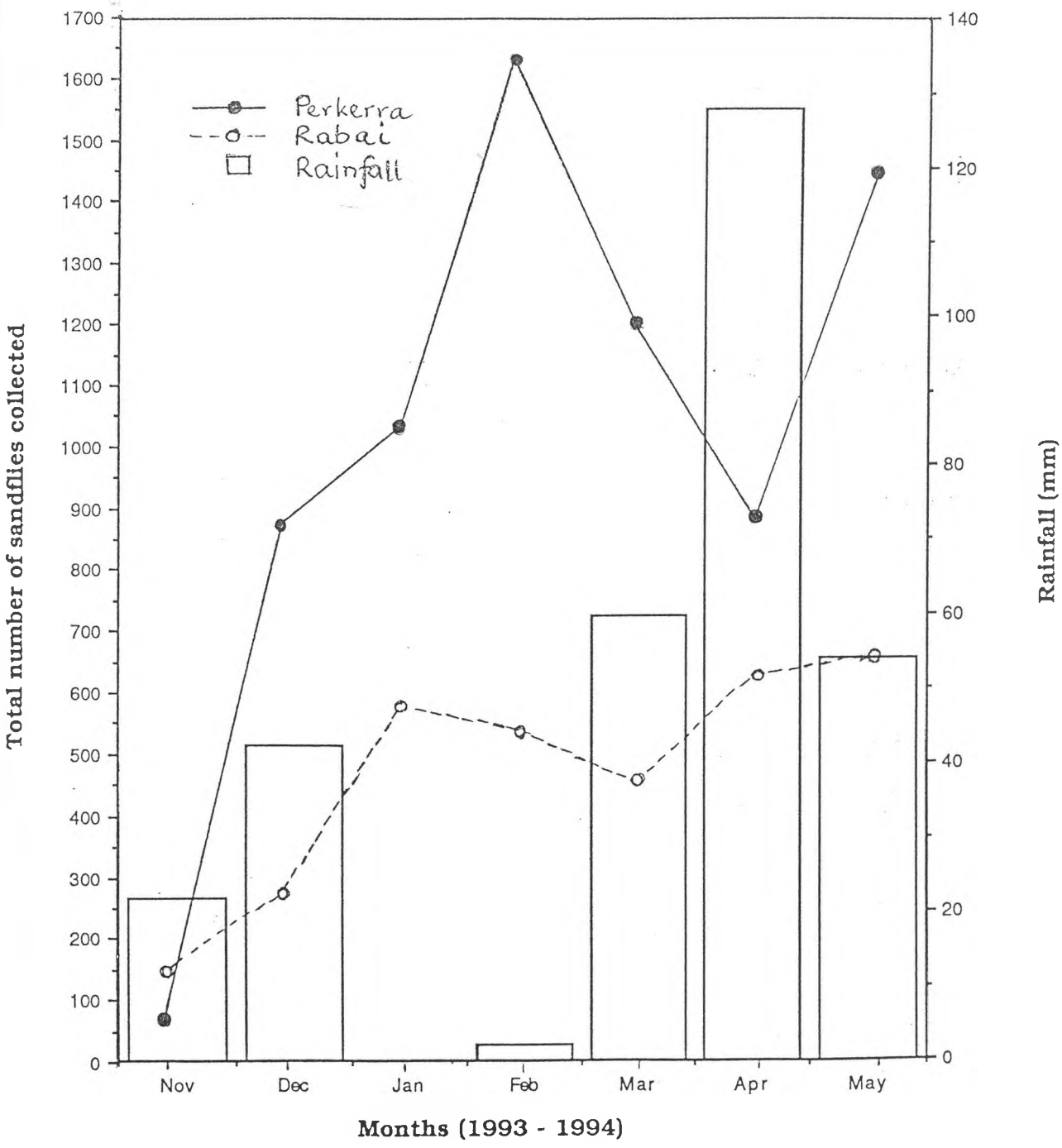


Fig. 3.2

Proportion of males to females trapped at Perkerra from November 1993 to May 1994.

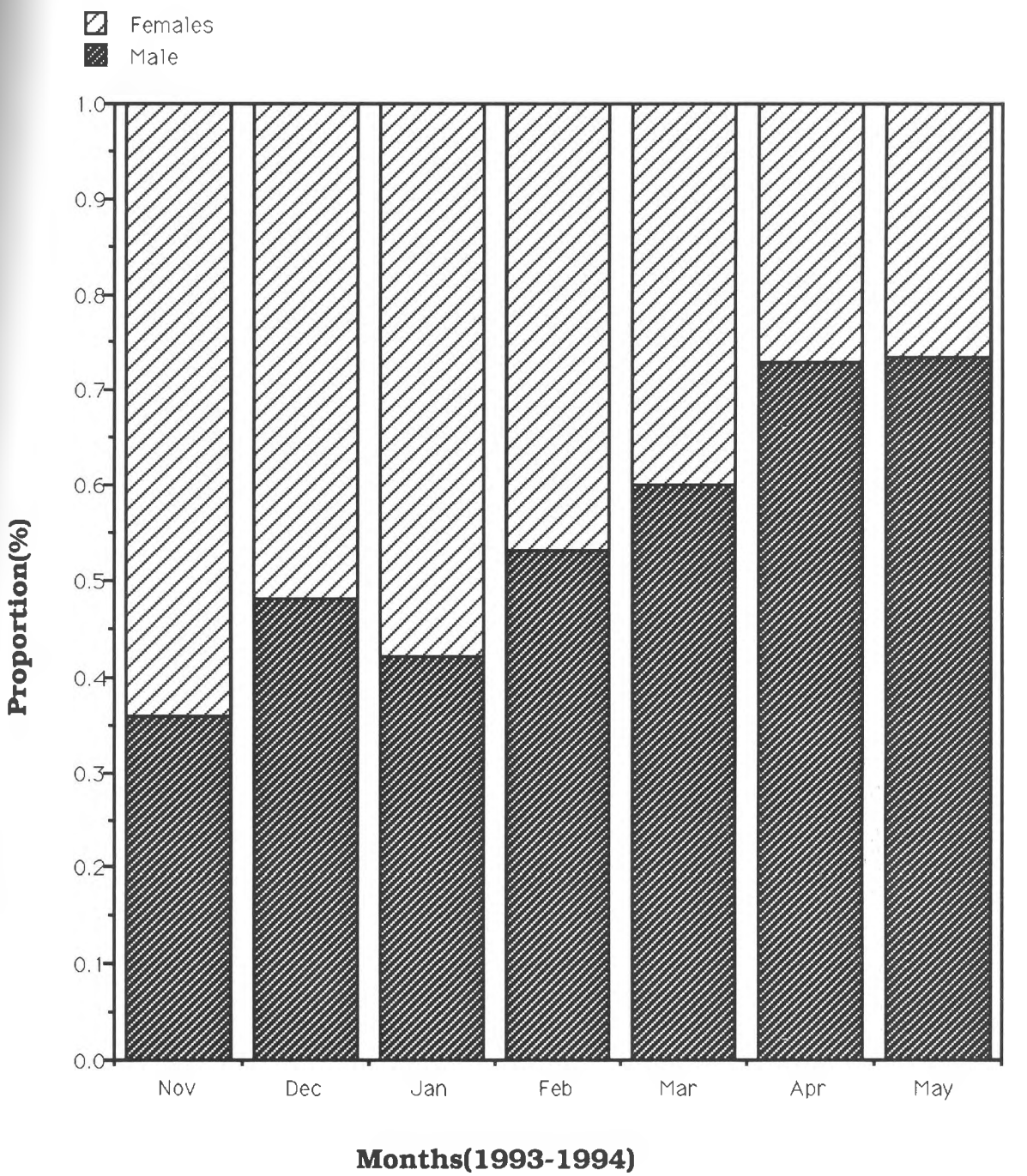
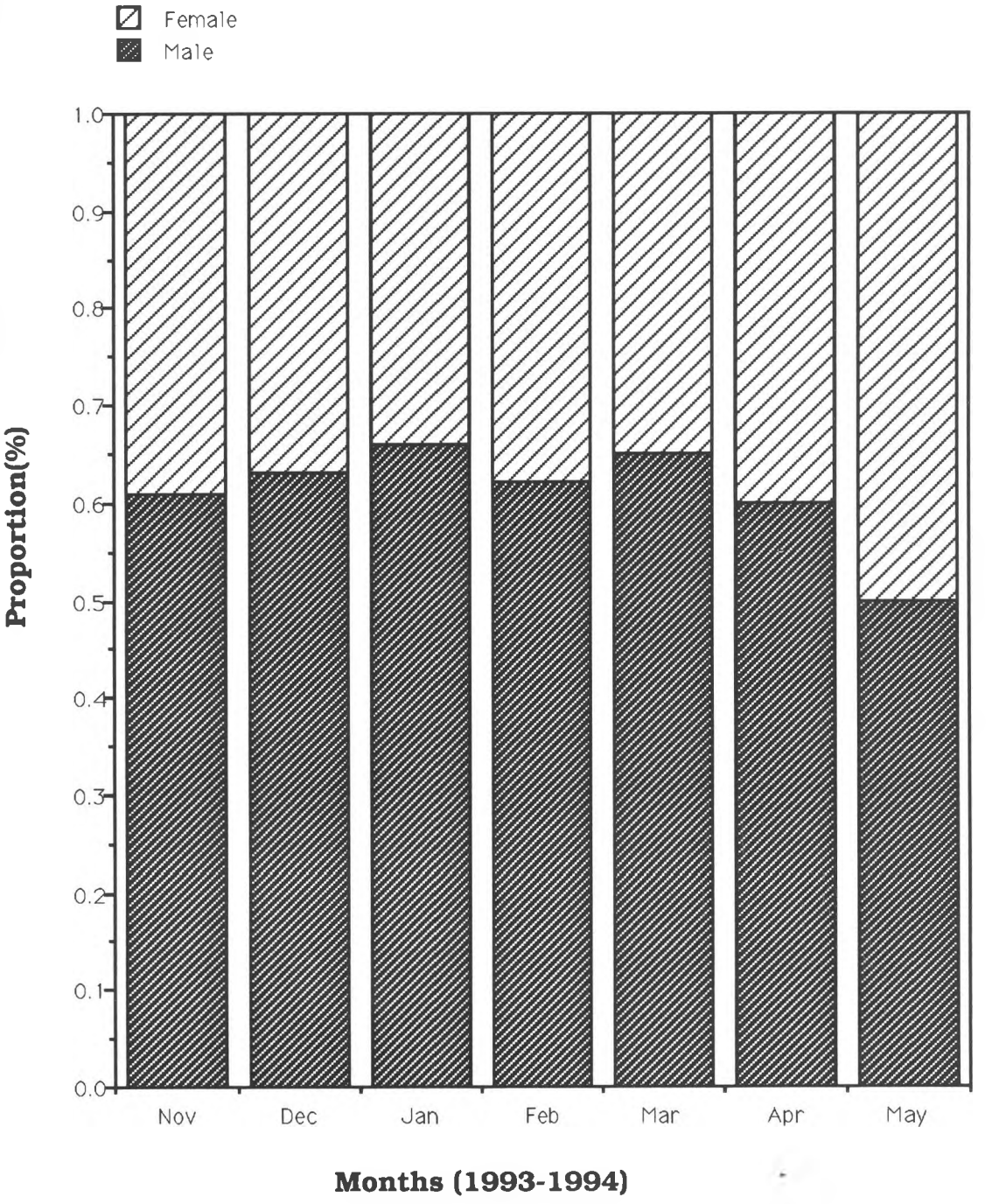


Fig. 3.3

Proportion of males to females trapped at Rabai from November 1993 to May 1994.



3.3 Peaks of activity in sandfly populations at night

Sandfly movement throughout the night had a pattern as shown on Table 3.1. From 1800 to 2400 hours the movement of sandflies was out of the termite hills and animal burrows. 85.6% of the sandflies trapped during this period were going out, whereas only 14.4% were entering the termite hills and animal burrows. From 2400 to 0600 hours, the majority of sandflies (61.4%) were caught returning into the termite hills and animal burrows. Those trapped going out of the termite hills and animal burrows were represented by 38.6%. during the last half of the night.

3.4 Sugar feeding periods

All sandflies trapped during the study period were tested for sugar in their guts (Table 3.2). In animal burrows the number of sandflies showing traces of sugar in the gut was represented by 16.8% for those trapped between 1800 and 2400 hours while the group trapped between 2400 and 0600 hours was represented by 36.7%. More sandfly species were fed on sugar meals between 2400 and 0600 hours than 1800 to 2400 hours relative to their negative numbers for the sandflies that visited animal burrows.

Twenty-five point nine percent of sandflies with sugar in their guts were collected in termite hills between 1800 and 2400 hours. The period between 2400 and 0600 hours was represented by 34.2%. Animal burrows had more sandflies negative for sugar between 1800 and 2400 hours than between 2400 and 0600 hours, both categories being represented by 83.2% and 63.3% respectively. In termite hills, sandflies without sugar in their guts were represented by 74.1% between 1800 and 2400 hours and 65.8% between 2400 and 0600 hours. Sandflies caught between 1800 and 2400 hours had less sugar than those caught between 2400 and 0600 hours.

Table 3.1

Sandfly movements throughout the night from 30th November, 1993 upto 11th May, 1994.

Time	Area	Trap site	Number going in	Number going out	Total
1800h-2400h	PK	TH	480	3,155	3,635
		AB	567	2,011	2,578
	RB	TH	149	1,055	1,204
		AB	133	1,659	1,792
Totals			1,329 (14.4)	7,880 (85.6)	9,209
2400h-0600h	PK	TH	746	606	1,352
		AB	125	169	294
	RB	TH	558	110	668
		AB	154	110	264
Totals			1,583 (61.4)	995 (38.6)	2,578

% in parentheses are based on total collections after six hours trapping duration.

PK= Perkerra
RB= Rabai
TH= Termite hill
AB= Animal burrow

Table 3.2

Sugar feeding of phlebotomine sandflies before and after midnight.

Sandfly species	Animal burrows				Termite hills			
	1800- 2400h		2400- 0600h		1800- 2400h		2400- 0600h	
	-ve	+ve	-ve	+ve	-ve	+ve	-ve	+ve
P. martini	31	27	4	2	153	110	72	43
P. duboscqi	32	11	20	14	-	-	1	1
P. rodhaini	11	4	-	-	3	-	-	-
P. saevus	1	-	-	-	-	-	-	-
S. schwetzi	1461	267	130	69	1235	333	682	300
S. antennata	1037	197	107	44	949	322	301	175
S. clydei	202	65	20	36	45	17	9	10
S. africana	211	26	9	6	152	39	29	18
S. bedfordi	129	30	21	8	682	305	80	62
S. adleri	16	6	1	1	9	1	-	1
S. squamipleuris	3	-	-	1	-	-	-	-
Sub-Total	3134	632	312	181	3228	1127	1174	610
Total	3766		493		4355		1784	
%	83.22	16.78	63.3	36.7	74.12	25.88	65.8	34.2

-ve -number of sandflies negative for sugar.
+ve -number of sandflies positive for sugar.

3.5 Bloodfeeding patterns

Monthly collections of bloodfed female sandflies before and after midnight at Perkerra and Rabai can be seen on Table 3.3. The majority of the bloodfed females were trapped between 1800-2400 hours at both sites. Out of a total of 74 bloodfed females trapped at Perkerra, 65 (87.8%) were caught before midnight while only 9 (12.2%) were caught after midnight. Statistically this was significant $P<0.05$. Out of a total of 73 bloodfed females trapped at Rabai, 53 (72.6%) were caught before midnight and 20 (27.4%) after midnight. There was also a significant difference between catches before and after midnight $P< 0.05$.

Table 3.4 shows the number of bloodfed female sandflies caught in termite hills and animal burrows according to species before and after midnight. One *Phlebotomus* species and four *Sergentomyia* species were collected with bloodmeals in their guts in termite hills. Most of the bloodfed sandflies were caught before midnight (72.6%). Only 27.4% were caught after midnight in termite hills. Eight bloodfed *Phlebotomus martini* females were captured before midnight and three bloodfed females after midnight. *Sergentomyia schwetzi*, *Sergentomyia antennata*, *Sergentomyia africana*, and *Sergentomyia bedfordi* bloodfed females were captured before midnight, represented by 27.4%, 16.4%, 12.3% and 5.5% respectively of the total number of bloodfed female sandflies caught in THs before midnight. The same species were captured after midnight representing 13.7%, 8.2%, 0% and 1.4% respectively for female sandflies caught in THs after midnight.

Two *Phlebotomus* and six *Sergentomyia* species were caught in animal burrows with bloodmeals in their guts. Only one *P. martini* was caught bloodfed before midnight. One *Phlebotomus duboscqi* was caught bloodfed before and another one after midnight. Most of the bloodfed sandflies in animal burrows were caught before midnight with a total representation of 66 (89.2%) out of a total number of 74 bloodfed females. Only eight specimens (10.8%) were caught bloodfed after midnight. Out of 74 bloodfed females caught in animal burrows, 43 (58.1%) were *S. schwetzi* caught before midnight. *S. antennata*, *S. africana*, *S. bedfordi*, *Sergentomyia clydei* and *Sergentomyia adleri* were represented by 11 (14.9%), 4 (5.4%), 1 (1.5%), 4 (5.4%) and 1 (1.4%) respectively before midnight. These same species were caught in much fewer numbers or not at all after midnight.

Table 3.3

Bloodfed female sandflies caught at Perkerra and Rabai before and after midnight.

Month	Female sandflies caught from 1800- 2400h		Female sandflies caught from 2400- 0600h	
	Perkerra	Rabai	Perkerra	Rabai
November 1993	3	5	-	-
December 1993	10	7	2	-
January 1994	9	11	-	4
February 1994	11	6	3	4
March 1994	17	10	1	5
April 1994	5	10	2	5
May 1994	10	4	1	2
Totals	65(87.8)	53(72.6)	9 (12.2)	20(27.4)

% in parentheses.

Table 3.4

Number of bloodfed female sandflies caught in termite hills and animal burrows before and after midnight.

Species	No. fed before midnight 1800- 2400h		No. fed after midnight 2400- 0600h	
	TH	AB	TH	AB
P. martini	8 (11.0)	1(1.4)	3 (4.1)	-
P. duboscqi	-	1(1.4)	-	1(1.4)
S. schwetzi	20 (27.4)	43(58.1)	10 (13.7)	4(5.4)
S. antennata	12 (16.4)	11(14.9)	6 (8.2)	2(2.7)
S. africana	9 (12.3)	4(5.4)	-	-
S. bedfordi	4 (5.5)	1(1.4)	1 (1.4)	1(1.4)
S. clydei	-	4(5.4)	-	-
S. adleri	-	1(1.4)	-	-
Totals	53 (72.6)	66(89.2)	20 (27.4)	8(10.8)

TH=Termite hill
AB=Animal burrow

% in parentheses

3.6 Resting sites of gravid, parous, nulliparous and bloodfed female sandflies

Table 3.5 shows gonotrophic status of female sandflies which were caught in animal burrows and termite hills at the two study sites. It also shows their direction of movement in these habitats. There were fewer female sandflies trapped in animal burrows (1,661) than in termite hills (2,593). The data show that there were 4 times more gravid females in animal burrows than termite hills as shown by the representative percentages of approximately 20% and 5% of the total numbers respectively. Fewer gravid female sandflies were trapped in both animal burrows and termite hills. Similar trend was observed for nulliparous and bloodfed females at the two resting sites. Parous female sandflies however, showed that more sandflies were caught entering the animal burrows and termite hills. Parous females in animal burrows 164 (9.9% of 1,661) were almost similar to termite hill ones 255 (9.8% of 2,593). Nulliparous females trapped in animal burrows were 1,091 (65.7% of 1,661) and 2,132 (82.2% of 2,593) in termite hills. There were 74 (4.5% of 1,661) bloodfed females in animal burrows as compared to termite hills with 73 (2.8% of 2,593).

Table 3.5

Gonotrophic status of female sandflies collected in animal burrows and termite hills and their direction of movement.

Status of females	Animal burrows.	entering	exiting	Termite hills	entering	exiting
Gravid	332 (20.0)	37	295	133 (5.1)	19	114
Parous	164 (9.9)	83	81	255 (9.8)	134	121
Nulliparous	1091 (65.6)	221	870	2132 (82.2)	618	1514
Bloodfed	74 (4.5)	9	65	73 (2.8)	20	53
Total	**1661 (100)**			**2593 (99.9)**		

% in parentheses are out of the total number of female sandflies caught at each trapping site.

3.7 Sandfly abundances

Table 3.6 shows distribution of male and female sandfly species trapped at Perkerra and Rabai as well as their trap positions. Females were fewer than males in animal burrows and termite hills at both sites. Sandfly species like *S. squamipleuris*, and *P. saevus* only appeared at Perkerra and were only found in animal burrows. Other species found at both sites (Perkerra and Rabai) with notable preferences were *P. martini* and *P. duboscqi*.

Abundances of sandflies in animal burrows and termite hills are shown on Table 3.7. Termite hills were chosen by more sandflies as a resting site than animal burrows 6139 (59.1%) and 4259 (40.9%), respectively $P<0.05$. Although *P. martini* were caught both in animal burrows and termite hills, it is evident that this species is more abundant in termite hills with figure representations of 64 (0.6%) for animal burrows and 378 (3.6%) for termite hills as regards the total number of sandflies caught. Other species which were abundant included *S. schwetzi* which were caught more abundantly in termite hills than in animal burrows. *S. antennata* were captured in large numbers in termite hills whereas *S. clydei* occured in animal burrows. *S. bedfordi* are termite hill dwellers. *P. saevus*, *P. rodhaini* and *S. squamipleuris* were caught living in animal burrows. Sandfly species such as *S. africana* and *S. adleri* are found living in animal burrows and termite hills (Table 3.7).

Table 3.8 shows sexes and monthly distribution of sandfly species around Perkerra Primary School. *P. martini* and *P. duboscqi* were present in every month. *P. martini* were fewer at Perkerra than at Rabai: 66 and 376 respectively. *P. duboscqi* was trapped in larger numbers at Perkerra than at Rabai: 57 and 22 respectively. Its close relative, *P. rodhaini* had a similar pattern of distribution except for the month of December 1993. *Sergentomyia* species were more abundant than *Phlebotomus* species at the two trapping sites. Of the two sites, Perkerra had a higher population of *P. duboscqi* than Rabai. The sex ratio of male to female sandfly population at Perkerra was 1.4 : 1. The propotional distribution of males and females is shown on Fig.3.2.

Table 3.9 shows monthly distribution of species of sandflies according to their sexes around Rabai Primary School. *P. martini* species was caught in every month and also in larger numbers than Perkerra site. The other *Phlebotomus* spp. were caught in much fewer numbers here than at Perkerra. Rabai had more *P. martini* than Perkerra and therefore better suited for its breeding and a better bet for trapping this species. *Sergentomyia adleri* and *S. squamipleuris* were either absent or rarely caught at Rabai area. The male to female sex ratio at Rabai was 1.5 : 1 and its proportional distribution is shown on Fig.3.3.

Table 3.10 shows the status of males trapped both in animal burrows and termite hills during the study period. 92.5% of males trapped in animal burrows had rotated genitalia, while 7.5% had unrotated ones. In termite hills 92.6% had rotated genitalia against 7.4% with unrotated

The two most abundant *Phlebotomus* species were *P. martini* and *P. duboscqi* (Fig.3.4), while the three most abundant *Sergentomyia* species were *S.schwetzi*, *S. antennata* and *S. bedfordi* (Fig.3.5).

Table 3.6

Distribution of males and females trapped at Perkerra and Rabai and their trap positions.

	Perkerra				Rabai			
Species	**AB**		**TH**		**AB**		**TH**	
	F	M	F	M	F	M	F	M
P. martini	1	6	5	54	18	39	42	277
P. duboscqi	23	32	0	2	5	17	0	0
P. rodhaini	4	7	3	0	1	3	0	0
P. saevus	0	1	0	0	0	0	0	0
S. schwetzi	456	753	665	1432	316	402	209	244
S. antennata	322	484	570	643	196	382	277	257
S. clydei	30	101	5	40	70	122	9	27
S. africanna	134	97	112	102	8	13	10	14
S. bedfordi	40	75	593	324	30	43	92	120
S. adleri	2	6	2	2	8	8	2	5
S. squamipleuris	3	1	0	0	0	0	0	0
Total	1015	1563	1955	2599	652	1029	641	944

AB= Animal burrow
TH= Termite hill

F= Females
M= Males

Table 3.7

Abundances of sandflies in animal burrows and termite hills.

	Animal burrows				Termite hills			
Species	**Sex**		**Total**	**%**	**Sex**		**Total**	**%**
	F	M			F	M		
P. martini	19	45	64	0.62	47	331	378	3.64
P. duboscqi	28	49	77	0.74	0	2	2	0.02
P. rodhaini	5	10	15	0.14	3	0	3	0.03
P. saevus	0	1	1	0.01	0	0	0	0
S. schwetzi	772	1155	1927	18.53	874	1676	2550	24.52
S. antennata	518	866	1384	13.31	847	900	1747	16.80
S. clydei	100	223	323	3.11	14	67	81	0.78
S. africana	142	110	252	2.42	122	116	238	2.29
S. bedfordi	70	118	188	1.81	685	444	1129	10.86
S. adleri	10	14	24	0.23	4	7	11	0.11
S. squamipleuris	3	1	4	0.04	0	0	0	0
TOTALS	1667	2592	4259	40.96	2596	3543	6139	59.05

F= Females
M= Males

Table 3.8.

Monthly distribution of sandfly species at Perkerra.

	Nov		Dec.		Jan		Feb		Mar		Apr		May		Total	
Species	M	F	M	F	M	F	M	F	M	F	M	F	M	F	M	F
P. martini	2	1	3	-	16	2	15	-	15	3	5	1	3	-	59	7
P. duboscqi	-	1	7	1	2	2	7	14	9	3	7	1	-	3	32	25
P. saevus	1	-	-	-	-	-	-	-	-	-	-	-	-	-	1	-
P. rodhaini	-	-	-	-	2	2	1	2	1	1	2	1	1	1	7	7
S. schwetzi	16	7	162	82	180	171	370	215	302	239	189	101	892	280	2111	1195
S. antennata	2	10	142	86	143	221	268	289	248	151	200	84	140	35	1143	876
S. bedfordi	1	18	53	242	20	140	109	152	90	32	106	35	27	7	406	626
S. africana	2	9	47	37	49	61	30	67	36	29	16	16	19	27	199	246
S. clydei	2	-	4	2	15	3	70	20	33	7	15	1	3	1	142	34
S. adleri	-	-	4	-	3	-	-	-	1	-	-	1	1	2	9	3
S. squamipleuris	-	-	1	3	-	-	-	-	-	-	-	-	-	-	1	3
Total	26	46	423	453	430	602	870	759	732	465	540	241	1086	356	4110	3022

Sex ratio M:F = 1.4:1

M=Male
F=Female

Table 3.9.

Monthly distribution of sandfly species at Rabai

	Nov		Dec.		Jan		Feb		Mar		Apr		May		Total	
Species	M	F	M	F	M	F	M	F	M	F	M	F	M	F	M	F
P. martini	58	6	81	20	54	11	40	9	22	4	33	5	27	6	315	61
P. duboscqi	-	-	-	-	6	2	2	-	2	-	3	1	4	2	17	5
P. saevus	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
P. rodhaini	-	1	-	-	-	-	-	-	-	-	3	-	-	-	3	1
S. schwetzi	12	11	37	24	96	63	81	68	92	58	131	61	178	259	627	544
S. antennata	15	30	31	38	173	87	133	87	93	78	112	107	72	56	629	483
S. bedfordi	4	9	7	15	15	18	35	20	45	28	46	31	8	4	160	125
S. africana	2	1	1	1	8	4	5	1	1	5	1	1	7	7	25	20
S. clydei	1	-	16	4	16	13	42	13	18	5	49	34	7	10	149	79
S. adleri	-	-	-	-	12	-	-	-	-	1	-	5	1	4	13	8
S. squamipleuris	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Total	92	58	173	102	380	198	338	198	273	179	378	245	304	348	1938	1328

Sex ratio M:F = 1.5:1

M= Male
F= Female

Table 3.10.

Status of males trapped in animal burrows and termite hills.

Month	Animal burrows			Termite hills		
	Total No. of Males	Rotated genitalia	Unrotated genitalia	Total No. of Males	Rotated genitalia	Unrotated genitalia
Nov.1993.	69	60	9	47	41	6
Dec.1993.	278	258	20	317	289	28
Jan.1994.	367	334	33	446	404	42
Feb.1994.	561	535	26	639	595	44
Mar.1994	536	521	15	476	448	28
Apr.1994	254	225	29	761	701	60
May.1994.	575.	510	65	813	763	50
Totals.	2640	2443(92.5)	197(7.5)	3499	3241(92.6)	258(7.4)

% in parentheses are out of the total number of males caught at each of the habitats.

Fig.3.4 Monthly abundance of *P. martini* and *P. duboscqi* at the study area.

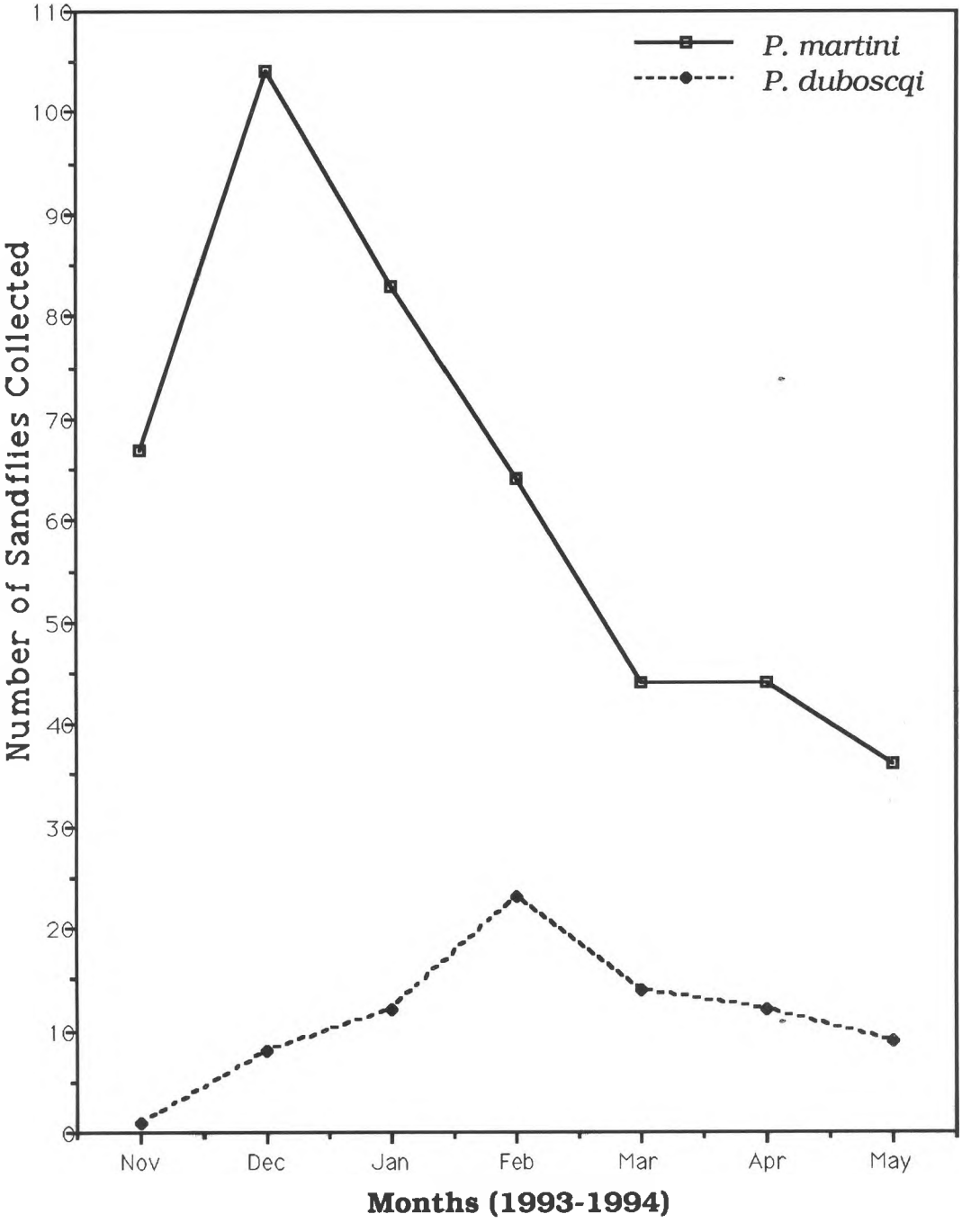
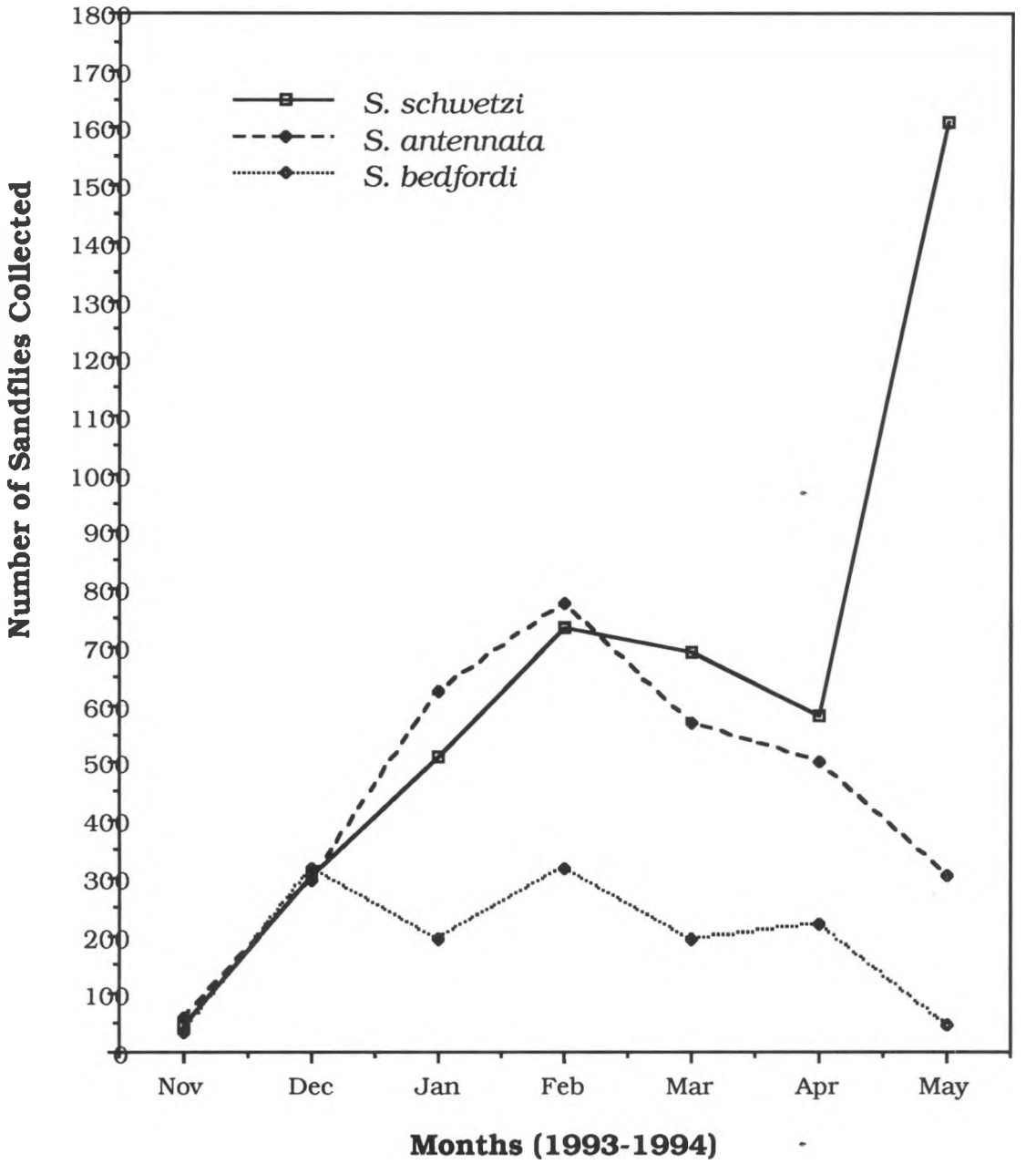


Fig. 3.5 Monthly abundance of the most abundant *Sergentomyia* species at the study area.



CHAPTER IV

DISCUSSION AND RECOMMENDATIONS

4.1 Discussion

Although in the past more phlebotomine sandfly species had been reported from Baringo District (Lawyer *et al.* 1989; Mutinga *et al* ,1989, 1990; Basimike *et al.* 1992), only 11 species amounting to 10,398 sandflies were encountered in this study. Sandfly species encountered by Lawyer *et al.*(1989) which were not collected in this study were *P. pedifer* Lewis Mutinga & Ashford 1972, *S. inermis* Theodor 1958, *S. dureni* Parrot 1934 and *S. affinis* Theodor 1933. Worthy to note was the absence of *S. ingrami* which was reported in large numbers by Basimike *et al.*(1992). Robert *et al.*(1994) also trapped phlebotomine sandflies in the same area and did not collect *S. ingrami*. The presence of this species in Baringo District needs confirmation.

There was a significant difference in all the comparisons between the sandflies caught entering and exiting the traps from 1800-2400 hours, and 2400-0600 hours in both termite hills and animal burrows ($P>0.05$). In the first half of the night the majority of the sandflies were caught leaving the termite hills and animal burrows. 85.6% of the sandflies were caught exiting compared to 14.4% trapped entering animal burrows and termite hills between 1800-2400 hours. Sandflies rest in termite hills and animal burrows during the day and leave these places in the evening when temperatures drop and are equal to those in termite hills and animal burrows. This drop in temperature signals the start of their nocturnal activities of mate searching, feeding and dispersal. In the second half of the night, the direction of movement changed and the majority of the sandflies were caught entering termite hills and animal burrows ($P>0.05$). 61.4% of the sandflies were caught entering and 38.6% exiting between 2400-0600 hours.

Phlebotomus martini trapped in animal burrows and termite hills were represented by 0.6% and 3.6% of the total sandflies collected respectively. This means that *P. martini* can live in animal burrows as well as in the termite hills (Basimike *et al.* 1992; Mutinga *et al.* 1986, 1990; Johnson *et al.* 1993; Ngumbi *et al.* 1992). The observations of Basimike and

Mutinga (1992) in Baringo that *P. martini* were more abundant in animal burrows than termite hills is in disagreement with these findings and more so when one considers that the methods of trapping used were sticky papers or sheets of polythene papers smeared with castor oil for all cases. Basimike *et al.* (1992) found *P. martini* to be four times more abundant in animal burrows than in termite hills. Mutinga *et al.*(1986) suggested that *P. martini* used termite hills as secondary resting places which is in disagreement with these findings which have found *P. martini* to be six times more abundant in termite hills than animal burrows.

Out of 10,398 sandflies caught during the study period, 0.7% were *P. duboscqi* trapped in animal burrows and only 0.02% in termite hills. This clearly confirms what early researchers found out about this species which lives in animal burrows (Beach *et al.* 1984; Basimike *et al.* 1992; Mutinga *et al.* 1986). 0.1% of *P. rodhaini* out of the total number of sandflies caught were found living in animal burrows and 0.03% in termite hills. *Phlebotomus saevus* had a very low representation of 0.01% of the total number of sandflies caught and were found only in animal burrows. The low numbers of these two *Phlebotomus* species may be so because the species may be one of those that are seasonal or have a different preferred habitat.

Generally, sandflies were significantly more abundant in termite hills than animal burrows ($P< 0.05$). This suggests that termite hills are better resting sites for phlebotomine sandflies than animal burrows. *Phlebotomus duboscqi* which feeds on small mammals (rodents) may find it beneficial to live in animal burrows where it can feed and breed (Beach *et al.* 1984; Githure *et al.* 1986). Perkerra trapping site which had more rodent burrows than Rabai had also more numbers of *P. duboscqi*. The reason behind many sandflies at Perkerra can be due to large numbers of rodents and other fauna. *Phlebotomus martini* which also feeds on mammals may have used these animal burrows as feeding grounds followed by the digestion of the bloodmeal in the same position (Mutinga *et al.* 1989).

Sandflies collected in animal burrows and termite hills and tested for sugar showed a feeding pattern. Most of the sandflies exiting termite hills and animal burrows had little or no sugar in their guts, while those entering them had more sugar. This behavioural pattern

in feeding shows that sandflies use the first half of the night for various activities, one of which is sugar feeding. Similar observations were noted by (Yuval *et al.* 1986; Killick-Kendrick *et al.* 1978) working on different sandfly species. In a study done by Yuval, (1988) on *P. papatasi* to find out which group of sandflies (exiting or entering burrows) had more fructose in their guts, noticed that only 38.4% of the exiting flies were positive, whereas 93.8% of those entering burrows were positive.

Sugar feeding in animal burrows showed that there were fewer sugar-fed sandflies between 1800 and 2400 hours than between 2400 and 0600 hours (16.7%) and (36.7%) respectively. Corresponding collections in termite hills showed a similar pattern, whereby sandflies trapped from 1800-2400 hours were represented by 25.9% and from 2400-0600 hours by 34.2% for the fructose positive ones. This indicates that sandflies feed on sugars during the first half of the night, which was evidenced by higher percentages of sugar fed sandflies that were caught returning into animal burrows and termite hills from 2400-0600 hours. The large numbers of sandflies devoid of sugar can be explained by the fact that the newly hatched sandflies had not had a chance to feed on any sugar inside the burrows and termite hills,and secondly the old sandflies have not had a chance to replenish their already depleted sugar reseves in their guts. During dissection some sandflies lost some of their sugar to bacteria in their guts. This happened when alot of flies were thawed to ambient temperature and the dissection took too long to finish. These factors may account for why many sandflies were negative for sugar in the first half of the night.

The total numbers of both sexes in each trapsite showed that females were less abundant than the males (Table 3.6). This trend of males being more available for trapping than females could be because they engage in activities that make them more mobile than the females. For example, bloodmeal taking by females is followed by a period of resting for egg development. Gravid females are not active and their activity is mostly for sugar feeding (Yuval *et al.* 1988). The data show that monthly catches of sandflies at Perkerra were higher than at Rabai. The difference in numbers could have resulted from environmental differences found in the two areas such as vegetation, animals and water content in the soil.

Animal burrows had significantly more gravid females than termite hills ($P<0.05$). Parous females in both animal burrows and termite hills were not significantly different ($P<0.05$). Nulliparous females indicated that termite hills had more young females (82.2%) than animal burrows (66.5%). There were more bloodfed females in animal burrows than in termite hills and this can be explained by the fact that animals that use the burrows provide ready bloodmeal to the sandflies occupying the same burrow. Bloodfed female sandflies have a tendency of resting, digesting and even developing their eggs at the feeding sites and this is probably why many gravid sandflies were caught in animal burrows. Mutinga *et al.*(1990) also caught a higher number of phlebotomine sandflies bloodfed in animal burrows. In this study more gravid female sandflies were caught exiting ABs and THs than entering. This is so because bloodfed female sandflies confine themselves in these sites digesting and maturing their ovaries in readiness for oviposition. A large number of nulliparous females caught exiting explains why these places are considered to be hatching grounds of sandflies. More bloodfed females were caught exiting than entering the ABs and THs, a trend that means that these places harbor animals which interact with sandflies offering them the needed blood meals.

Both animal burrows and termite hills had almost equal representative percentages of males with rotated genitalia which indicated that they were older than 24-hours and had probably mated. Males with unrotated genitalia showed that they had not mated and had probably recently emerged from their pupal cases. Breeding of sandflies was observed to take place throughout the year as shown by the monthly data collections of males which had unrotated genitalia. The areas where male sandflies with unrotated genitalia were caught could be treated as sandfly breeding sites.

Abundance of sandflies increased from November 1993 to May 1994 with intermitent lower numbers recorded in March and April. This was so because of heavy rains that fell during those two months. January and February are dry months but, a good number of sandflies was caught compared to the wet months during the trapping period. Perkerra seemed to offer better breeding conditions with a collection of 68.6% of the total sandflies trapped as compared to Rabai with 31.4% being trapped during the study period. One

hills in order to engage themselves in their nocturnal activities, which Killick-Kendrick (1978) cites as comprising of many components such as; search for bloodmeals, sugarmeals, mates, and breeding sites as well as dispersal.

Table 3.8 which provides monthly distribution of sandfly species caught at Perkerra showed that *P. martini* and *P. duboscqi* were caught in every month of the study period indicating that transmission of leishmaniasis can take place all the year round. Notable in this table were three most abundant *Sergentomyia* species, of *S. schwetzi, S. antennata* and *S. bedfordi.* Rabai is well suited to breeding of *P. martini* when compared to Perkerra despite its environmental difficulties. Of these two areas, *P. duboscqi* breeds better at Perkerra than at Rabai. It could be suggested that the fewer numbers of *S. schwetzi, S. antennata* and *S. bedfordi* at Rabai were as a result of adverse environmental conditions at that area, where vegetation and favourable soil moisture are less abundant than at Perkerra. The month with the most bloodfed female sandflies was March. This coincided with the onset of the long rains and may be, this triggered off increased feeding by sandflies to prepare them for breeding season. Most of these bloodfed sandflies were caught before midnight when they are most active (Yuval *et al.*1986). It can be suggested that many female sandflies were bloodfed before they left the THs and ABs resting sites. Animal burrows seemed to offer better feeding opportunities for sandflies than termite hills because more sandfly species were caught bloodfed. Small mammals and rodents live in these animal burrows, thus providing easy meals for female sandflies. A number of lizards and snakes live in the same burrows. The termite hills harbor a number of lizards, snakes and small mammals too.

Despite the low numbers of *P. martini P. duboscqi* caught, these two Phlebotomus species are the vectors of VL and CL in Kenya. *Phlebotomus duboscqi* showed low numbers throughout the study period except for February when a collection of more than 20 sandflies was made. This species is an efficient vector for *L. major* despite its low numbers in Baringo district (Basimike *et al.*1992). Rain seemed to affect the catching of sandflies in those months of very heavy rainfall. The factors contributing to low catches of sandflies in those nights when there was a heavy downpour were: damp and chilly temperatures, wet and

soiled paper traps, flooded animal burrows, interference of termite hills by rain water running down the ventilation shafts hence disturbing the sandfly populations.

Sergentomyia clydei are notably animal burrow dwellers (Heisch *et al.* 1956) though they can be found in termite hills. *Sergentomyia schwetzi*, one of the dominant species caught during the study period were caught living in both places but more abundant in termite hills (Fig.2.2.7). *Sergentomyia bedfordi*, which feed on lizards (Mutinga *et al.* 1990) are found in termite hills where they are likely to readily come into contact with their source of food. Due to the low numbers of *P. saevus*, *P. rodhaini* and *S. squamipleuris* trapped a conclusion on their preference may be misleading. However, *P. rodhaini* were more abundant in animal burrows than in termite hills.

The discovery of kala-azar in Baringo and West Pokot districts dates back to 1955 and 1956 respectively (Lawyer *et al.* 1989). Since that time research work had been aimed at finding the vectors and reservoirs of kala-azar. Efforts to determine good control methods have not been extensively studied.

In this study knowledge of sandfly distribution, behaviour, habitat utilization and species preferences were sought. Information gained from the study can be used in designing control methods. Sandflies existed throughout the trapping period (November 1993 to May 1994) with the fewest number of sandflies being caught in November 1993. This could have been so due to a dry spell that preceded the short rains from November to December 1993. Other months covered by the study period showed increasing numbers of sandfly catches except at those times when rain seemed to interfere with sandfly movements in March, April and May at Perkerra.

Since it is has been shown by this study and others that the vectors of VL and CL live either in animals burrows or termite hills or in both places, it is therefore prudent to plan attacking them at such places. Sandfly species inhabiting animal burrows and termite hills may be attacked and controlled through studying their nocturnal behaviour and activities. One of the methods of approach to control sandflies would be to interfere with their sugar meal sources. This can be done by spraying the entrances of ABs and THs and the vegetation around them with poison baited sugar solution. Sugar solutions may be

laced with biological pathogens like *Bacillus thuringensis* or *Bacillus sphaericus* which are harmful to sandflies. Vaccination is another method of protecting healthy individuals from contracting leishmaniasis. Killed leishmania parasites may be injected into the skin of healthy individuals to increase immunity to leishmania attacks.

Termite hills and animal burrows play a key role in the ecology of phlebotomine sandflies in Kenya. Termite hills act as breeding and resting sites for many sandflies and especially for *P. martini*, which has been incriminated as the vector for visceral leishmaniasis in Kenya (Perkins *et al.*,1988). Animal burrows act as resting places for *P. duboscqi*, which transmits *L. major* in Baringo District. Both termite hills and animal burrows are used by sandflies as breeding sites. Feeding on vertebrates takes place also in the same places.

To institute a control programme, these two areas are useful targets to consider. Sugar feeding by sandflies provided information that can be used to give phlebotomine sandflies a poisoned sugar meal that can kill them.

In case of a biological control method like the use of bacteria which can be lethal to sandflies and not harmful to other organisms, they may be put in the sugar solution and offered to sandflies to feed on. Since both sexes of sandflies feed on sugar the bacteria will affect both sexes.

4.2. Recommendations

Since nearly all the areas that have kala-azar tend to have termite hills and animal burrows, it is important to think of simple techniques and methods of sandfly and disease control in these endemic areas. This was arrived at after observing that:

1. Sandflies breed, rest and even feed on animals in these places.
2. Both male and female sandflies take sugar meals from natural sources which can be imitated by making artificial sugar that can be laced with poison or bacteria to kill them.
3. Sandflies return to their resting places after their nocturnal activities every night.
4. Biological as well as chemical control methods can be designed to reduce or eradicate sandfly populations in their natural habitats, and hence control the spread of leishmaniasis.

CHAPTER V

REFERENCES

Abonnenc, E. and Minter, D.M.(1965) Bilingual keys for the identification of the sandflies of the Ethiopian Region (French and English), Cahiers, Office de la Recherche Scientifique et technique d' Outre -*Mer Entomologie medicale*, **5:**1.

Adler, S. (1963). In immunity to Protozoa. A *symposium of the British Society for immunology*, p. 235. *Oxford: Blackwell Scientific Publications.*

Anderson, T. F. (1943). Kala-azar in East African Forces. *East African Medical Journal*, **20:** 172.

Ashford, R.W. (1974). Sandflies (Diptera: Psychodidae) from Ethiopia taxonomic and biological notes. *Journal of Medical Entomology*, **11**: 605-616.

Aussel, J. P. (1993). Ecology of the biting midge *Leptoconops albiventris* in French Polynesia. II. Location of breeding sites and larval micro-distribution. *Medical* and *Veterinary Entomology*, **7**: 80-86.

Basimike, M. and Mutinga, M. J. (1992). The relative abundance of *Phlebotomus martini* (Parrot) and *P. duboscqi* (Neveu-Lemaire), (Diptera: Psychodidae) in animal burrows and termite mounds in Marigat Location, Baringo District, Kenya. I*nsect Science and its Application*, **13:** 173-176.

Basimike, M; Mutinga, M.J. and Kumar, R. (1992). Habitat preference and seasonal variations of phlebotomine sandflies (Diptera: Psychodidae) in Marigat area, Baringo District, Kenya. *Insect Science and its Application*, **3:** 307- 314.

Bates, P. A. (1994). Minireview. The developmental Biology of *Leishmania* promastigotes. *Experimental Parasitology*, **79:** 215- 218.

Beach, R; Kiilu, G; Hendricks, L; Oster, C; and Leeuwenburg, J; (1984). Cutaneous leishmaniasis in Kenya: transmission of *Leishmania major* to man by bite of a naturally infected *Phlebotomus duboscqi. Transactions of the Royal Society of Tropical Medicine and Hygiene*, **78:** 747-751.

Beach, R. F; Mutinga, M. J; Young, D. G. and Kaddu, J. B. (1982). Laboratory colonization of *Phlebotomus martini* Parrot 1936 (Diptera: Psychodidae), a vector of visceral leishmaniasis in Kenya. *Proceedings of the 3rd Annual Medical Scientific Conference*, KEMRI/KETRI pp.189-190.

Binhazim, A.A; Githure, J.I; Muchemi, G. K. and Reid, G.D.F. (1987). Isolation of *Leishmania major* from a naturally infected velvet monkey (*Cercopithecus aethiops)* in Kiambu District, Kenya. *Journal of Parasitology*, **73:** 1278- 1279.

Bray, R. S. (1982). The zoonotic potential of reservoirs of leishmaniasis in the Old World. *Ecology of disease*, **4:** 257- 267.

Chance, M. L; Schnur, L. F; Thomas, S. C.and Peters, W. (1978). The biochemical and serological taxonomy of *Leishmania* from the Aethiopian zoogeographical region of Africa. *Annals of Tropical Medicine and Parasitology*, **72**: 533- 542.

Chance, M.L. (1981). Leishmaniasis. *British Medical Journal*, **2:** 1245-1247.

Chulay, J. D; Odoyo, M. A. and Githure, J. I. (1985). Leishmania parasitemia in Kenya visceral leishmaniasis. *Transactions of the Royal Society of Tropical Medicine and Hygiene*, **79:** 218- 222.

Cole, A. C. E; Coosgrare, P. C. and Robinson, G. (1942). A preliminary report of an outbreak of kala- azar in a battalion of King's African Rifles. *Transactions of the Royal Society of Tropical Medicine and Hygiene*, **36:** 25.

Elnaiem, D. A; and Ward, R. D. (1992). Oviposition attractants and stimulants for the sandfly *Lutzomyia longipalpis* (Diptera: Psychodidae). *Journal of Medical Entomology*, **29**: 5-12.

Forrester, A.T.T. (1966). Treatment of visceral leishmaniasis with Amphotericin B. *East African Med.ical Journal* , **43:** 568. (abstract).

Githure, J. I. Beach, R. F. and Lightner, L. K. (1984). The isolation of *Leishmania major* from rodents in Baringo District, Kenya. *Transactions of the Royal Society of Tropical Medicine and Hygiene*, **78:** 283.

Githure, J. I; Schnur, L.F; Le.Blanq, S. M, and Hendricks, L. D. (1986). Characterization. of Kenyan *Leishmania* spp.and identification of *Mastomys natalensis, Taterillus emini* and *Aethomys kaiseri* as new hosts of *Leishmania major*. *Annals of Tropical Medicine and Hygiene*, **80:** 501- 507.

Gunders, A. E; (1974). Ecology of leishmaniasis in Israel. *Israel Journal of Medical Science*, **12:** 6.

Hargrove, J. W; (1988). Tsetse, the limits to population growth. *Medical and Veterinay Entomology*, **2:** 203- 217.

Harwood, R. F. and James, T. M. (1979). Entomology in human and animal health. Washington State University, Pullman, *Macmillan Publishing Co. Inc.* New York. pp.152- 159.

Heisch, R. B. (1957). The isolation of Leishmania from a ground squirrel in Kenya. *East.African Medical Journal*, **34:** 183.

Heisch, R. B. (1954). Studies in leishmaniasis in East Africa. 1. The epidemiology of an outbreak of kala-azar in Kenya. *Transactions of the Royal Society of Tropical Medicine and Hygiene*, **48:** 449.

Heisch, R. B. (1961). Rodents as reservoirs of arthropod-borne disease in Kenya. *East African Medical Journal*, **38:** 256- 261.

Heisch, R. B; Grainger, W. E. and Harvey, A. E. C. (1959). The isolation of *Leishmania* from gerbils. *Journal. of Tropical Medicine and Hygiene*, **62:** 158- 159.

Heisch, R. B; Guggisberg, C. A. W. and Teesdale, C. (1956). Studies in leishmaniasis in East African. II. The sandflies of the Kitui kala-azar area in Kenya, with description of six new species. *Transactions of* the *Royal Society of Tropical Medicine and Hygiene* , **50:** 209- 226.

Heisch, R. B; Wijers, D. J. and Minter, D. M. (1962). In pursuit of the vector of kala-azar in Kenya. *British Medical Journal*, **1:** 1456- 1458.

Johnson, R. N; Ngumbi, P. M; Gachihi, G. S; Mwanyumba, J. P; Mbugua, J; Mosonik, N; Were, J. B. O; and Roberts, C. R. (1993). A new focus of kala-azar due to *Leishmania donovani* s.l. in Kenya. *Transactions of the Royal Society of Tropical Medicine and Hygiene*, **87:** 142- 144.

Kaddu, J. B. and Mutinga, M. J. (1981). *Leishmania* in Kenyan phlebotomine sandflies 1. *Leishmania aethiopica* in the midgut of naturally infected *Phlebotomus pedifer*. *Insect Science and its Application*, **2:** 245- 250.

Kaddu, J. B; Mutinga, M.J; Nokoe, S. and Musyoki, R. M. (1992). Phytophagy of *Sergentomyia ingrami* - I. Feeding Rates. *Insect.Science and its Application.* **13:** 731-735.

Kaddu, J.B; Mutinga, M.J; Nokoe, S. and Musyoki, R.M. (1992). Phytophagy of *Sergentomyia ingrami*- II. Feeding performance on selected indigenous and exotic plants. *Insect Science and its Application*, **6:** 807-811.

Kager, P.A. and Rees, P. H. (1983). Clinical aspects of kala-azar in Kenya. *Academisch Proefschrift*, Unversiteit van Amsterdam. pp. 1-47.

Killick- Kendrick, R; Rioux, J. A; Bailly, M; Guy, M. W; Wilkens, J. J; Guy, F; Davidson, I; Knechtli, R; Dubois, H; Ward, R. D; Guilvard. E. and Perieres. J. (1984). Ecology of leishmaniasis in the South of France XX. Dispersal of *Phlebotomus ariasi* Tonnoir 1929 as a possible factor in the spread of leishmaniasis of the Cevennes. *Annales de Parasitologie humaine et compar'ee*, **59:** 555- 572.

Killick- Kendrick, R. (1990). Phlebotomine vectors of the leishmaniasis: a review. *Medical and Veterinary Entomology*, **4:** 1-24.

Killick- Kendrick, R. (1978). Recent advances and outstanding problems in the biology of Phlebotomine sandflies. *Acta, Tropica*, **35:** 297- 313.

Killick-Kendrick, R. (1989). The ecology and entomology of the leishmaniasis. Leishmaniasis, Hart, D. T. (editor). New York: *Plenum Publishing Corporation*, pp. 175- 178.

Kirk, R. (1956). Epidemiology of kala-azar in East Africa. *Transactions of the Royal Society of Tropical Medicine and Hygiene*, **50:** 169- 177.

Kirk, R. and Lewis, D. J. (1947). Epidemiology of kala- azar in East Africa. *Transactions of the Royal Society of Tropical Medicine and Hygiene*, **40:** 869.

Kungu, A; Mutinga, M. J; and Ngoka, J. M. (1972). Cutaneous leishmaniasis in Kenya. *East African Medical Journal*, **4:** 459.

Laison, R; (1982). Leishmanial parasites of mammals in relation to human disease. *Symposium of Zoological Society* Lond. No. 137- 179.

Lane, R.P; (1991). The contribution of sandfly control to leishmaniasis control. *Annals of the Society of Belgian Medicine in the Tropics*. 71 Suppl. **1:** 65- 74.

Lawyer, P.G; Ngumbi, P.M; Anjili, C.O; Odongo, S.O; Mebrahtu, Y.B; Githure, J.I; Koech, D. K; and Roberts, C. R. (1990). Development of *Leishmania major* in *Phlebotomus duboscqi* and *Sergentomyia schwetzi* (Diptera: Psychodidae). *American Journal of Tropical Medicine and Hygiene*, **43:** 31- 43.

Lawyer, P.G; Mebrahtu, Y.B; Ngumbi, P. M; Mwanyumba, P. J; Mbugua, J; Kiilu, G; Kipkoech, D; Nzovu, J; and Anjili, C. O. (1991). *Phlebotomus guggisbergi* (Diptera: Psychodidae), a vector of *Leishmania tropica* in Kenya. *American Journal of Tropical Medicine and Hygiene*, **4:** 290- 298.

Lawyer, P.G; Githure, J.I; Mebrahtu, Y.B; Perkins, P.V; Muigai, R. and Leeuwenburg, J. (1989). Leishmaniasis research in Kenya: Parasite- vector-host associations. In: Leishmaniasis, Hart, D.T. (editor). New York: *Plenum Publishing Corporation*, pp. 189-206.

Leeuwenburg, J; Mutinga, M. J. and Koech, D.K. (1981). Report on a Leishmanin skin test survey. *Proceedings of the 2nd Annual Medical Scientiffic Conference KEMRI/KETRI*, pp.141.

Lewis, D.J. (1974). The biology of Phlebotomidae in relation to leishmaniasis. *Annual Review of Entomology*, **19:** 363- 384.

Magnarelli, L.A., Modi, G.B. and Tesh.R.B.(1984). Follicular development and parity in phlebotomine sandflies (Diptera: Psychodidae). *Journal of Medical Entomology*, **21:** 681-689.

Manson- Bahr, P.E.C; Heisch, R.B. (1956). Studies in leishmaniasis in East Africa. III. Clinical features and treatment. *Transactions of the Royal Society of Tropical Medicine and Hygiene*, **50:** 465-471.

Manson-Bahr, P.E.C. (1959). East African kala-azar with special reference to the pathology and prophylaxis and treatment. *Transactions of the Royal Society of Tropical Medicine and Hygiene*, **53:** 123-137.

Marinkelle, C.J. (1980). The control of leishmaniases. *World Health Organization Bulletin*, **58:** 807- 818.

Markell, E.K.and Voge, M.(1976). *Medical parasitology*. 4th Edition.W.B.Saunders Company, Philadelphia. pp.124- 166.

Mckinnon, J.A; and Fendall, N.R.E. (1955). Kala-azar in the Baringo District of Kenya: a preliminary communication. *Journal of Tropical Medicine and Hygiene*, **58:** 205.

Mckinnon,J.A. (1962). Kala-azar in the Upper Rift Valley of Kenya. *Journal of Tropical Medicine and Hygiene*, **65:** 51-63 and 82-90.

Mebrahtu, Y.B; Oster, C.N; Shatry, A.M; Hendricks, L.D; Githure, J.I; Rees, P.H; Perkins, P.V.and Leeuwen, J. (1987). Cutaneous leishmaniasis caused by *Leishmania tropica* in Kenya. *Transactions of the Royal Society of Tropical Medicine and Hygiene*, **81:** 923- 924.

Minter, D.M. (1964). Seasonal changes in populations of phlebotomine sandflies (Diptera: Psychodidae) in Kenya. *Bulletin of Entomological Research*, **55:** 421-435.

Minter,D.M.(1976). Trypanosomes: *In Medical Protozoology*. 4th Edition.W.B. Saunders Company, Philadelphia. pp.650- 759.

Moriaty, F. (1969). The sublethal effects of synthetic insecticides on insects. *Biological Review*, **44:** 321- 357.

Muigai, R; Githure, J.I; Gachihi, G.S; Were, J.B.O; Leeuwenburg, J. and Perkins, P.V. (1987). Cutaneous leishmaniasis caused by *Leishmania major* in Baringo District, Kenya. *Transactions of the Royal Society of Tropical Medicine and Hygiene*, **81:** 600-602.

Mutinga, M.J. (1986). Leishmaniasis in Kenya. *Medicus*, **4:** 11- 22.

Mutinga, M.J; Kamau, C.C; Kyai, F.M; Omogo, D.M. (1989). Epidemiology of leishmaniasis in Kenya. V. Wider search for breeding habitats of phlebotomine sandflies in three kala-azar endemic foci. *East African Medical Journal*, **66:** 173- 182.

Mutinga, M.J; Ngoka, J.M. and Odhiambo, T. R. (1984). Epidemiological investigations of leishmaniasis in the West Pokot District, Kenya. *Insect Science and its Application*, **5:** 521- 525.

Mutinga, M.J; Basimike, M; Kamau, C.C. and Mutero, C.M. (1990). Epidemiology of leishmaniasis in Kenya, natural host preference of wild caught phlebotomine sandflies in Baringo District, Kenya. *East African Medical Journal*, **67:** 319- 327.

Mutinga, M.J. and Ngoka, J.M. (1978). Incrimination of the vector of visceral leishmaniasis in Kenya. *East African Medical Journal*, **55:** 337- 340.

Mutinga, M.J. and Ngoka, J.M. (1981). Suspected vectors of lizard leishmaniasis in Kenya and their possible role in partial immunization of the human population against *Leishmania donovani* in kala-azar endemic areas. *Insect. Science and its Application*, **1:** 207-221.

Mutinga, M.J; Ngoka, J.M; Schnur, L.F.and Chance, M.L. (1980). Isolation and identification of leishmanial parasites from domestic dogs in the Machakos District of Kenya and possible role of dogs as reservoirs of kala-azar in East Africa. *Annals of Tropical Medicine and Parasitology*, **74:** 139.

Mutinga, M.J. (1971). *Phlebotomus. longipes*, a vector of cutaneous leishmaniasis in Kenya. *Transactions of the Royal Society of Tropical Medicine and Hygiene*, **65:** 106.

Mutinga, M.J. (1975). The animal reservoir of cutaneous leishmaniasis on Mount Elgon, Kenya. *East African Medical Journal*, **52:** 142- 151.

Mutinga, M.J. and Kyai, F.M. (1985) A new record of *Sergentomyia garnhami* (Heisch, Guggisberg and Teesdale. 1956), in Kenya. *Transactions of the Royal Society Tropical Medicine and Hygiene*, **79:** 137.

Mutinga, M. J. and Ngoka, J.M. (1970). Culture, isolation and description of cutaneous leishmaniasis in Kenya. A preliminary report. *Proceedings of the East African Medical Research Council of Scientiffic Conference*, **4:** 72.

Naggan, L.A; Gunders, A. V; Dizian, R; Dannon, S; Shibolet, S; Ronen, A; Schneeweis,and Michaeli, D. (1970). Ecology and attempted control of cutaneous leishmaniasis around Jericho in the Jordan Valley. *Journal of Infectious Diseases*, **121:** 427- 432.

Ngoka, J.M; Madel, G. and Mutinga, M.J. (1975). *Phlebotomus* (*Larroussius*) *elgonensis* sp. nov. (Diptera: Phlebotomidae), new sandfly from Kenya. *East African Medical Journal*, **52:** 132-141.

Ngumbi, P.M; Lawyer, P.G; Johnson, R.N; Kiilu, G. and Asiago, C. (1992). Identification of phlebotomine sandfly bloodmeals from BaringoDistrict, Kenya by direct enzyme - linked immunosorbent assay (ELISA) . *Medical and Veterinary Entomology*, **6:** 385-388.

Perfiliev, P.P. (1968). Fauna of the USSR Diptera, Vol. **3** , No. **2** Phlebotomidae (sandflies) *Academy of Sciences USSR New Series*, No. 92. (In Russian; English translation by Israel Program for Scientific Translations, Jerusalem).

Perkins, P.V; Githure, J.I; Mebrahtu, Y.B; Kiilu, G; Anjili, C.O; Ngumbi, P.M; Nzovu, J; Oster, C.N; Whitmire, R.E; Leeuwenburg, J; Hendricks, L.D. and Koech, D.K. (1988). The isolation of *Leishmania donovani* from *Phlebotomus martini* collected in Baringo District, Kenya. *Transactions of the Royal Society of Tropical Medicine and Hygiene,* **82:** 695- 700.

Rogers, D.J. and Randolph. (1984). Local variation in the population dynamics of *Glossina palpalis* (Robineau-Desvoidy) (Diptera: Glossinidae) 1. Natural population regulation. *Bulletin Entomological Research,* **74:** 403- 423.

Robert, L. L; Schaefer, K.U. and Johnson. R.N. (1994). Phlebotomine sandflies associated with households of human visceral leishmaniasis cases in Baringo District, Kenya. *Annals of Tropical Medicine and Parasitology,* **88:** 649-657.

Sang, D.K; Mbugua, G.G.and Arap Siongok, T. K. (1983). Cutaneous leishmaniasis and *Phlebotomus longipes* on Mount Elgon. *East African Medical Journal,* **60:** 826.

Sang, D.K; Okello, G.B.A; Ndegwa, C. W. and Ashford, R. W. (1993). New foci of cutaneous leishmaniasis in Central Kenya and the Rift Valley. *Transactions of the Royal Society of Tropical Medical and Hygiene,* **87:** 629- 632.

Schaefer, K.U.; Kurtzhals, J.A.L; Sherwood,J.A.; Githure, J.I.; Kager, P.A.; and Muller, A.S. (1994). Epidemiology and clinical manifestations of visceral and cutaneous leishmaniasis in Baringo District, Rift Valley, Kenya. *Tropical and Geographical Medicine,* **46:** 129-133

Schaefer, K.U.; Kurtzhals, J.A.L; Kager, P.A.; Gachihi, G.S.;Gramiccia, M; Kagai, J.M.; Sherwood, J.S.; and Muller, A.S. (1994). Studies on the prevalence of leishmanin skin test positivity in the Baringo District,

Rift Valley, Kenya. *American Journal of Tropical Medical Hygiene*, **50:** 78-84

Schlein,Y. & Warburg, A. (1986). Phytophagy and feeding cycle of *Phlebotomus papatasi* (Diptera: Psychodidae) under experimental conditions. *Journal of Medical Entomology*, **23:** 11-15.

Schlein, Y; Yuval, B. and Jacobson, R. L. (1989). Leishmaniasis in the Jordan Valley: differential attraction of dispersing and breeding site populations of *Phlebotomus papatasi* (Diptera: Psychodidae) to manureand water. *Journal of Medical Entomology*, **26:** 411-413.

Southgate, B.A. and Oriedo, B.V.E. (1962). Studies in the epidemiology of East.African. Leishmaniasis. The circumstantial epidemiology of kala-azar in the Kitui District of Kenya. *Transactions of the Royal Society of Tropical Medicine and Hygiene*, **56:** 30-47.

Van.Handel, E. (1972). The detection of nectar in mosquitoes. *Mosquito . News*, **32:** 458.

Weitz, B. (1960). Feeding habits of bloodsucking arthropods. *Experimental Parasitology*, **9:** 63-82.

World Health Organization (1980). Report of a training seminar on Epidemiological methods for the leishmaniases. TDR/LEISH._SEM/**80.3** pp. 1- 58.

World Health Organization (1990). Control of Leishmaniasis. Technical report series No.**793,** pp. 158.

World Health Organization (1993). TDR.News Bulletin. Published by the UNDP/WORLD BANK/WHO special Programme for Research and Training in Tropical Diseases (TDR) No. **42** July 1993, pp 2-6.

World Health Organization (1988). Guidelines for leishmaniasis control. Parasitic Diseases Programme, WHO/LEISH/**88:**25.

Wijers, D.J.B. and Minter, D. M. (1962). Studies on the vector of kala- azar in Kenya 1. Entomological Evidence. *Annals of Tropical Medicine and Parasitology*, **56:** 462- 472.

Wijers, D.J.B, and Minter, D. M. (1966). Studies on the vector of kala- azar in Kenya. V. The outbreak in Meru District. *Annals of Tropical Medicine and Parasitology*, **60:** 11-21.

Wijers, D.J.B, and Kiilu, G. (1984). Studies on the vector of kala-azar. VIII. The outbreak in Machakos District; epidemiological features and a possible way of control. *Annals. of Tropical Medicine and Parasitology*, **78:** 597- 604.

Wijers, D.J.B. and Mwangi, S. (1966). Studies on the vector of kala-azar in Kenya. VI. Environmental epidemiological aspects in Meru District. *Annals of Tropical Medicine and Parasitology*, **60:** 373- 391.

Yuval, B. and Schlein, Y. (1986). Leishmaniasis in the Jordan Valley III. Nocturnal activity of *Phlebotomus papatasi* (Diptera: Psychodidae). In relation to nutrition and ovarian development. *Journal of Medical Entomology*, **23:** 411-415.

Yuval, B. Warburg, A. and Schlein, Y. (1988). Leishmaniasis in the Jordan Valley. V. Dispersal characteristics of the sandfly *Phlebotomus papatasi. Medical and Veterinary Entomology*, **2:** 391- 395.

Appendix 1 Composition and application of anthrone solution.

Procedure:

1) Pour carefully, and while cooling, 380 ml. concentrated sulfuric acid into 150 ml. distilled water (dilute sulfuric acid).

2) Mix 150 mg anthrone powder with 100 ml dilute sulfuric acid (anthrone reagent). The anthrone reagent should be kept in a refrigerator after use and may be used for about one week.

Testing for sugar:

1) Place each mosquito or sandfly gut in a test tube or ELISA plate wells.

2) Add 0.5 ml of anthrone reagent and crush the mosquto / sand fly gut with a glass rod or plastic grinder. Alternatively each mosquito / sandfly gut may be treated with a drop of chloroform-methanol (1:1). This removes the wax to allow easy penetration of anthrone reagent.

3) The reagent will turn green or blue at room temperature depending on the amount of sugar present. Any specimen that will not have changed its yellow colour to green or blue after 1 hr. will be considered negative for sugar.

Appendix 2 Gum-Chloral mountant.

Puri's medium

1) Distilled water .....10 mls

2) Gum acacia (powder) ..... 8 gms

3) Chloral hydrate (crystals) .....70 gms

4) Glycerine .....5 mls

5) Glacial acetic acid .....3 mls

Ingredients should be dissolved in the above order at room temperature. A magnetic stirrer may be used to help mix them well. Filter the fluid through cotton wool.

After Minter; *Bulletin of Entomological Research*, 54: 483 (1963).