Nocturnal activities of phlebotomine sand flies (*Diptera: Psychodidae*) in Baringo County, Kenya.

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SUMMARY

Leishmaniasis is a disease of both humans and animals. It is transmitted by the bite of sand flies of the genus *Phlebotomus* in the Old World and *Lutzomyia* in the New World. Nocturnal activities of phlebotomine sand flies were monitored by trapping flies exiting and entering termite mounds and animal burrows in Baringo County. Exit-entrance traps were set from dusk to midnight and from midnight to dawn in the termite mound and animal burrow openings. The study which lasted seven months (November 1993-May 1994), was designed to reveal sand fly behavior in their natural habitats. A total of 11,787 sand flies was trapped and their nocturnal activities studied. Significantly more sand flies(85.6%) were caught exiting than entering animal burrows and termite mounds in the 1st half of the night p<0.05. This trend was reversed by sand flies (61.4%) caught entering the burrows termite mounds during the 2nd half of the night p<0.05. Most sugar-positive sand flies were collected after midnight in both animal burrows and termite mounds while more blood-fed sand flies were caught in the 1st than in the 2nd half of the night p<0.05. At Perkerra, 87.8% of the blood-fed female sand flies were trapped in the 1st half of the night compared with 12.2% caught in the 2nd half. At Rabai, 72.6% of the total number of those caught blood fed were in the 1st half and 27.4% in the 2nd half of the night. *P. martini* which is the vector of *L. donovani* which causes visceral leishmaniasis, was predominantly trapped in termite mounds whereas *P duboscqi* (vector of *L. major*) that causes cutaneous leishmaniasis, was trapped in large numbers in animal burrows. These habitats pre-dispose themselves as ideal targets for control measures of the vectors.

Key words: Sugar, blood feeding, sand flies, animal burrows, termite mounds, Kenya

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Introduction

Sand flies from the Old World countries are active during the night and rest during the day in their habitats [1]. It is also during this period that a lot of their behavioral activities take place [2]. Knowledge of the biology of sand flies is of great importance in

controlling leishmaniasis [3]. The first step is to determine the target species and the second is to discover its biology in order to mount an economical and effective control program. This knowledge is however, fragmentary and based mainly on observations on a few well studied species such as *Phlebotomus papatasi* [3, 4]. It is some times tempting to assume that the biology of the less well studied, but taxonomically similar sand flies is the same and this assumption at times can be erroneous. Attention is therefore being turned towards the biology of sand flies to gain sufficient background information to control leishmaniasis through vector control [4]. Special attention is being paid to aspects of their bionomics such as blood and sugar feeding, dependence on specialized larval breeding or adult resting sites which can make them vulnerable to control strategies [3]. Specialized factors studied in this paper were sugar and blood feeding patterns of phlebotomine sand flies in their natural habitats.

Materials and methods

Study sites

This study was carried out in a semi-arid region in Baringo district, Kenya. The two sites (Rabai and Perkerra Primary Schools) covered a radius of 5km each (Fig 1). Rabai is 6km south east of Marigat town and Perkerra is 8km east of Marigat town, 0 30' N, 36 0' E. and are between 1000 and 1100m asl. The area is overgrazed with *Acacia* trees and shrubs that form the basic fodder for large flocks of sheep and goats. Perkerra site is characterized by irrigation canals that feed water to plots of the National Irrigation Board. The Rabai site has no crops but there are homesteads with large herds of livestock. Both areas have tall weathered *Macrotermes subhyalinus* mounds and animal burrows of the un-striped ground squirrel (*Xerus rutillus*) and other small rodents.

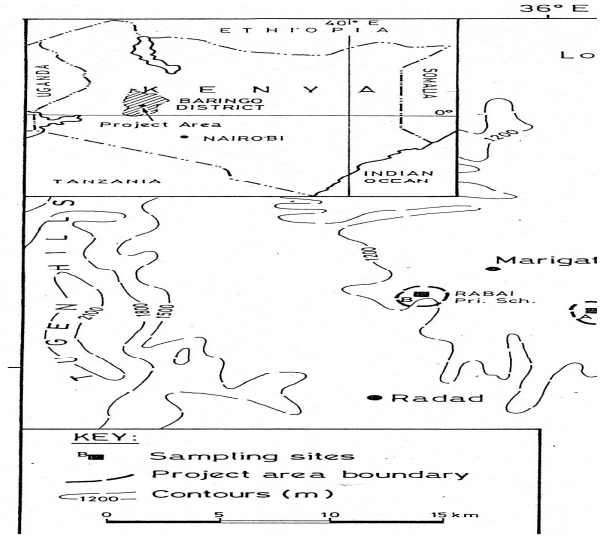


Fig 1: Map showing the study sites in Baringo County, Kenya

The entry-exit trap

The entry- exit trap was made of a plastic cylinder 42cm long 8cm in diameter, open at both ends and divided in the middle by partitioning with netting material of 20 mesh / cm [2, 5].Sticky papers coated with castor oil (to trap sand flies), were rolled into

cylindrical shapes of 6–7 cm diameter so that they could fit into the traps. When inserted into a burrow or termite mound shaft, sand flies were prevented from leaving or entering by the fine mesh placed in the middle of the trap, but air was able to flow through the fine mesh. Spongy materials were used to seal all the spaces between the trap and the soil surfaces around the trap. Sand flies were trapped when they attempted to go in or come out of the habitats and thereby coming into contact with the oily paper.

Three traps were set in animal burrows and three in termite mounds at each of the study sites (Perkerra and Rabai) at 1800 hr and these traps were collected at 2400 hr and new ones set, which were picked up at 0600 hr and taken to the field laboratory for processing. Sand flies were trapped for 6 nights a month from November 1993 to May 1994. Collection of the traps was done at midnight and similar number of traps set in fresh animal burrows and termite mounds from 2400 hr to 0600 hr in both areas. All the sand flies were sorted out at the camp using a fine camel brush to pick them from the sticky papers and washed with 2% detergent. They were then put in 2ml vials containing DMSO to preserve them. Records were kept on the time of catch, type of habitat, date and number of flies. The vials were then cooled down in liquid nitrogen vapor for 30 minutes before being inserted into the liquid nitrogen for preservation and transportation to our entomology laboratory in KEMRI Headquarters, Nairobi.

Dissection and identification of sand flies

In the Laboratory, the sand flies were first put in 2% detergent to wash away dimethyl sulfoxide (DMSO) which was used to protect specimens during freezing down and removal of excess hairs on their bodies. The specimens were then transferred to normal saline which was also used as the dissection solution. Important physiological features examined during dissection were: sex, blood- fed females, accessory glands to determine parity (parous, nulliparous or gravid) [1]. Taxonomic features of sand flies are usually found in the head (cibarium, and pharynx) and the last three segments of the abdomen (genitalia) which contain the spermatheca in females and penis sheath, spines and hair tufts in males. These two parts of each sand fly were mounted on a micro slide using gum chloral as the mountant and covered with a cover slip and then allowed to air dry within a day or two before being identified using the identification keys of Abonnenc and Minter [6].

Detection of sugar meals

Fructose in both males and females was detected by cold anthrone method [7] with the degree of colour change recorded at 10–30 minutes. The detection of colour change was visually observed.

Detection of blood meals

Females were recorded as blood fed if fresh or old blood meals were visible in the dissected gut. Recently acquired blood meals (<24 hours old) were red in colour, whereas the older blood meals were black.

Gonotrophic and parous states of female sand flies

All female sand flies were dissected under a dissecting microscope and examined under a compound microscope to determine parity (reproductive history) and gonotrophic states. The accessory glands contained some granules if the fly was parous, but contained no granules if nulliparous [2]. Gravid females contained maturing eggs in the ovaries. Parous females are older after having survived one or two gonotrophic cycles, whereas nulliparous are young and have not laid eggs.

Age determination of male sand flies

Age determination of males was done by examining the degree of the rotation of the terminalia [8]. Males bearing un-rotated genitalia were <24 hrs old. This feature was important in determining the mature and immature stages of male sand flies.

Results

Nocturnal activities

A total of 11,787 sand flies were collected during the 7 month period. Nine thousand two and nine sand flies were trapped from 1800-2400 hrs and 2,578 from 2400-0600 hrs (Table1). Significantly more sand flies were caught exiting in the first half of the night than those entering (χ^2 =199.8, df = 3, p <0.05), and this proportion was reversed in the second half of the night (χ^2 =205.4, df =3, p <0.05).

Table 1: 2x2 contingent table showing sand fly movement throughout the night from 30th November, 1993 to 11th May, 1994, the variables and the numbers that were tested

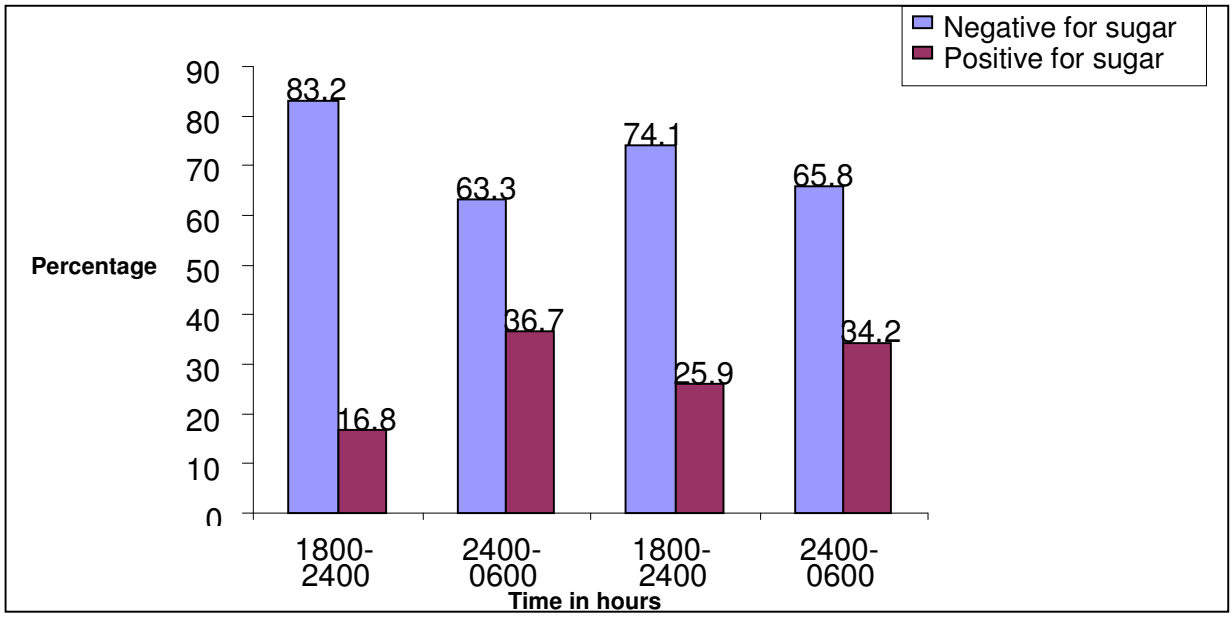
Time	No. of flies caught entering	No. of flies caught exiting	Chi square
1800-2400 h	1,329(14.4)*	7880(85.6)*	199.3, 3df. p<0.05
2400-0600 h	1,583(61.4)*	995(38.6)*	205.4, 3df. p<0.05

* Numbers in parentheses represent total collection in either the first or second half of the night during the study period.

Sugar feeding periods

The number of sugar positive sandflies is shown in Fig 2. Animal burrows produced 16.8% of sand flies positive for sugar between 1800- 2400 hrs and 36.7% for the collection done between 2400-0600 hrs. Sugar positive sand flies trapped in termite mounds were represented by 25.9% between 1800- 2400 hrs and 34.2% between 2400-0600 hrs.

Figure 2: Sugar feeding by phlebotomine sand flies before and after midnight



Blood feeding periods of sand fly species

The number of blood fed female sand flies caught in termite mounds according to species before and after midnight is shown on Table 2. Most of these blood fed female sand flies (72.6%) were caught before midnight. 8 blood-fed *P. martini* were trapped before midnight and 3 more after that. Most of the blood-fed sand flies (89.2%) in animal burrows were caught before midnight and only 10.8% after midnight. Out of 74 blood-fed female sand flies caught in animal burrows *S. schwetzi* were 43 (58.1%) and were trapped before midnight.

Table 2: Blood fed female sand fly species caught in termite mounds and animal burrows before and after midnight

Species	No. fed before midnight 1800-2400 hr		No. fed after midnight 2400-0600 hr	
	Termite mound	Animal burrow	Termite mound	Animal burrow
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)	-	-
Total	53(72.6)*	66(89.2)*	20(27.4)*	8(10.8)*

* Numbers in parentheses represent % of total number of blood fed flies per habitat before and after midnight.

Resting sites of blood fed females

Gonotrophic and parity status of females collected is shown in Table 3. Gravid female sand flies were collected more in animal burrows than in termite mounds χ^2 = 42.4, 1df, and 14.9, 1df ($p<0.05$) respectively. Majority of them were caught while exiting these habitats. There was a significant difference between the numbers exiting and entering these two habitats χ^2 =200.5, 1 df and 67.9 1 df ($p<0.05$) respectively. The proportions of parous and blood-fed females were not significantly different in animal burrows and termite mounds. Nulliparous females were caught more in termite mounds with a representation of 2,132 (82.3%) than animal burrows with1,091 (65.6%).

Table 3: Gonotrophic status of female sand flies trapped in their habitats and their movements.

Status of Females	Animal burrows	Entering	Exiting	Termite mound	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(82.3)	618	1514
Blood-fed	74(4.5)	9	65	73(2.8)	20	53
Total	1661(100)	350	1311	2593(100)	791	1802

*Numbers in parentheses represent % of total number of female sand flies trapped at each habitat.

Discussion

In this study sand fly behaviors were observed during the first and second halves of the night (1800-2400 hrs and 2400-0600 hrs) respectively. In the first half majority of the sand flies were caught while exiting the habitats (Table1). There were fewer sand flies positive for sugar during this first half of the night because firstly, the exiting population was composed of freshly emerged sand flies which had not fed on any sugar and secondly, the old sand flies had depleted their sugar reserves in their guts (Fig 2). It is important to note here that sugar (fructose) in the sand fly gut gets converted by enzymes into other forms, like energy for flight movement which do not react with the testing reagent (cold anthrone) within a few hours [7]. On the other hand, sand flies caught entering animal burrows and termite mounds in the second half of the night (2400-0600 hrs), had more sugar in their guts and this is because they had imbibed plant sugars and other sources like aphid secretions out there in the field. This explains why a smaller percentage of sugar- fed sand flies, were caught leaving burrows and termite mounds. Yuval and Schlein [2] reported that although sugar starvation may be an important factor in initiating flight activity, it does not necessarily mean that sugar positive females will cease their flight activities because they can move in search of mating partners.

Blood feeding periods of female sand flies at Perkerra and Rabai showed that more blood feeding took place in animal burrows than in termite mounds (Table 2). There were more blood fed females caught exiting in the first half than in the second half of the night, meaning that the animals which reside in these burrows and termite mounds, interact with the sand flies thus offering them the much needed blood meals. Yuval and Schlein [2] noted that blood-fed sand flies tend to remain in the animal burrows digesting their blood meals and maturing their ovaries, a state which induces inactivity. This state however, terminates before oviposition, when flight related activity resumes and more flies are caught leaving or entering these habitats. One of the reasons for renewed activity could be due to search for suitable oviposition sites in nearby burrows and termite mounds while another would be due to desire to replenish their sugar reserves for the old sand flies and the search for fructose by the newly emerged nulliparous sand flies out in the fields [10]. *Phlebotomus martini* was trapped more in termite mounds [12] while *Phlebotomus duboscqi* was shown to prefer animal burrows [9].

The vector of visceral leishmaniasis in Kenya is *P. martini* (5), which in this study was collected more frequently in termite mounds than in animal burrows, hence portraying the behavior that it is a termite mound dweller. A significantly large percentage of *S. schwetzi* (58.1%) was caught blood fed with more fresh than old blood meals in the animal burrows before midnight (Table 2). This shows that, animal burrows apparently offer better blood feeding opportunities than termite mounds. Many blood fed phlebotomine sand fly species (89.2%) were caught before midnight when sand flies are most active. Mutinga *et al* [11] observed a large number of phlebotomine sand flies in animal burrows which was not the case with this study (Table 2). This could have been due to error in reporting their results because studies from other scientists have results that agree with our findings.

Gonotrophic status of female sand flies collected in animal burrows and termite mounds and their direction of movement showed significant differences between sand flies that were caught entering and those caught exiting, with χ^2 = 200.5, p<0.05, 1 df for animal burrows and χ^2 =67.9, p<0.05, 1 df for termite mounds (Table 3). Majority of the gravid female sand flies caught exiting showed that after maturing their ovaries, they left these habitats to look for breeding sites and sugar feeding in the fields. Parous females were evenly distributed with no major differences recorded for the flies caught entering or exiting animal burrows and termite mounds with χ^2 =0.02, p<0.05, 1 df and χ^2 = 0.7, p<0.05, 1 df respectively. The large proportion of nulliparous flies caught exiting animal burrows and termite mounds may mean that these young flies had recently emerged and were leaving their habitats to attend to their physiological needs in the fields and these were significant with χ^2 =386.0, p<0.05, 1df and χ^2 = 376.5, p<0.05, 1df for animal burrows and termite mounds respectively. The differences of numbers of gravid sand flies caught entering and exiting were also significant; χ^2 =42.4, p<0.05, 1 df with χ^2 = 14.9, p<0.05, 1df for both animal burrows and termite mounds respectively.

Our findings in this study have been similar to Yuval *et al* [2] who studied *P papatasi* in Jordan Valley in Israel.

It can therefore, be concluded from the results of this study that animal burrows and termite mounds act as the breeding, resting and feeding sites of phlebotomine sand flies which form part of nocturnal activities. In these habitats, vector control measures can be initiated with great success because the sand flies are concentrated in habitats that are easy to attack with biological control agents like entomopathogenic fungi or *Bacillus* sphaericus. The known vector for visceral leishmaniasis (*P. martini*) lives in the termite mounds and the one for cutaneous leishmaniasis (*P. duboscqi*) in animal burrows, the studied habitats. Future leishmaniasis control measures need to be directed to these habitats in Kenya.

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