Seasonal and spatial changes of sand fly species in a canyon in the Carmel Mountains

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ABSTRACT: Altogether, 4,008 sand flies belonging to seven species were collected over a period of one year in the microhabitats of a single canyon in the Carmel Mountain ridge. The three most abundant were P. arabis, P. tobbi, and P. simici. Our results suggest that none of the seven sand fly species was indifferent to the heterogeneity of the microenvironment inside the canyon. Apart from the rare P. perfiliewi, which was only collected on the upper part of the south-facing slope, and P. tobbi, which clustered on the north-facing slope, the bulk of the other sand flies were caught on the bottom of the canyon. During the summer, the catches of all sand fly species increased to reach their maximum number in August and September. In April and May, there was lush vegetation and humidity, so species were distributed evenly throughout their habitats. With the onset of summer dryness, the sand flies concentrated in the humid habitats. The rate of concentration was essentially higher for males than for females, and this variation may result from differences in the behavior of the two sexes. During our study, none of the 2,318 dissected female sand flies were positive for Leishmania promastigotes. Journal of Vector Ecology 36 (Supplement 1): S118-S127. 2011.

Keyword Index: Phlebotomine sand flies, Israel, Carmel Mts., P. arabis, P. sergenti, microhabitat, distribution.

INTRODUCTION

Leishmaniasis, both the cutaneous (CL) and visceral (VL) forms, are endemic in large parts of Israel and the West Bank (Wasserburg et al. 2003, Al-Jawabreh et al. 2004, Jaffe et al. 2004). The sand flies that carry Leishmania parasites, when not infective, are also regarded as serious nuisance pests. The control of sand flies is necessary to prevent diseases and to curb nuisance biting, but it is complicated and often of little success (Alexander and Maroli 2003). Unlike other biting flies, little is known about sand fly larval habitats and adult resting sites (Feliciangeli 2004). Accordingly, most control attempts concentrate on the “biting population” in and around human habitations (Alexander and Maroli 2003). The customary methods for outdoor sand fly control involve multiple applications of residual insecticides or fogging. In the last few years, it was shown that these treatments had little or no effect on biting sand fly populations (Coleman et al. 2006, 2007, Orshan et al. 2006).

There are about 14 phlebotomus sand fly species in Israel and three are vectors for cutaneous Leishmaniasis (oral communication with Dr. L. Orshan, entomological laboratory of the Ministry of Health, Israel). Several studies investigated the ecology of P. papatasi, the vector of L. major in desert habitats (Schlein et al. 1984, Wasserberg et al. 2002); however, the few reports on the ecology of the other species are from domestic and peridomestic habitats in the foci of leishmaniasis (Kravchenko et al. 2004, Orshan et al. 2010).

Little is known of the ecology and habitat preferences of the two vectors of Leishmania tropica, P. arabis and P. sergenti, in natural habitats. For more than a decade, cutaneous leishmaniasis caused by L. tropica has been spreading in Israel and at the same time, the number of clinical cases is also increasing (Singer et al. 2008, Schnur et al. 2004). Hyraxes (Procavia capensis Pallas) are considered the main reservoir (Jacobson et al. 2003, Svobodova et al. 2006). Only two decades ago, they used to be shy, were uncommon, and were restricted to natural sites. Recently, hyraxes are spreading within the country and are penetrating human habitations in increasing numbers. We felt it is important to identify and study natural sand fly populations in areas where reservoir animals are present in order to estimate future risks for transmission.

The Carmel Mountain ridge, located south of Haifa, is one of the best studied and described ecological niches in Israel thanks to the efforts of the Department of Evolution, Haifa University. In previous studies, it was noticed that sand flies and their potential reservoirs were both present in the area, yet transmission of Leishmania does not occur here (Müller unpublished data, L. Orshan personal communication). To investigate this, we chose sites on both slopes and the bottom of a canyon in the Carmel Ridge to conduct a survey of sand fly distribution and species composition from January to December, 2005. Though no human cases of leishmaniasis have been reported from this area since the 1940s, sand flies in this study were also checked for the presence of Leishmania parasites.
MATERIALS AND METHODS

Habitat description

Lower Nahal Oren (14 km south of Haifa) is a deep canyon draining the Carmel Mountain ridge westward into the Mediterranean Sea (Figure 1). The distance between slopes is 100 m at the bottom and 400 m at the top. The bottom of the canyon is about 40 m above sea level (a.s.l.) and the south-facing slope is at a 35° angle while the north-facing slope is at a 25° angle (Nevo 1995).

Both slopes are of the same type of Upper Cenomanian hard limestone with terra rossa as the dominant soil (Karz 1959). The often shallow and rocky soil is well-aerated but at the same time poor in nutrients. In depressions and cracks, the soil can be several meters deep.

The south-facing slope is covered by open xeric forested grassland with Ceratonia siliqua, Pistacia lentiscus, and Pinus halepensis as the dominant trees with rock plants such as Micromeria fruticosa, M. nervosa, Chilaidenus iphonoides, and Stachys palaeatinus, and sudanian perennial grasses like Hyparrhenia hirta, Andropogon distachios, Pennisetum orientale, P. ciliare, and Tricholaena tenerifae as their companions (Danin, 1988; Nevo et al., 1999).

The north-facing slope, apart from some small clearings, is covered with dense maquis dominated by evergreen Quercus calliprinos and deciduous Pistacia palaestina and their mesophytic companions like Crataegus azarolus and Rhamnus sp. In the shade of the dense maquis, the undergrowth is sparse with only few species adapted to the shade. In the clearings, numerous herbaceous plants can be found. The total vegetation cover (including multiple layers measured according to Whittaker (1972), ranges from 35% on the south-facing slope to 150% on the north-facing slope (Nevo et al. 1999). The bottom of the canyon is rather flat and seasonally flooded; it is covered in thick herbaceous vegetation with a few large olive trees (Olea europaea), oak trees (Q. calliprinos), and groups of cactus (Opuntia ficus-indica). The canyon is in the midst of a nature reserve and therefore no grazing by domestic animals is permitted. Most of the nearby coastal plain consists of agriculture areas and human habitation (Orni and Efrat 1980).

The coastal plain near Haifa is located in the 20° C isotherm of annual temperature (Beaumont et al. 1976). As a rule, temperatures drop abruptly in November and reach a minimum in January or February. Days that reach the freezing point occur almost every winter in the hills but are rare on the coastal plain. The warming in April and May is more gradual than the drop of the temperature in autumn. In summer, peak temperatures fluctuate around 40° C (Ashbel 1951). The winter is short and almost 70% of the annual rainfall, which is in the experimental area about 600 mm, occurs between November and February. Rain from May to September is rare and negligible and the dry season is from June to August (Orni and Efrat 1980).

The Institute of Evolution, Haifa University, established a long-term research program to evaluate biodiversity patterns in lower Nahal Oren (Nevo, 1995, 1997, 2001). A microclimatic survey at the canyon manifested drastic interslope differences between the north-facing slope and the dryer, warmer, south-facing slope. Differences between the two slopes in air temperature (1 m above the ground) reach up to 3° C and the soil/stone temperature difference is about 10° C. Relative humidity differences can vary from 4.4 to 7% (Pavliček et al. 2003).

Study sites within the canyon

Seven study sites (types of micro-habitats) were chosen in the canyon, three on the north-facing slope, three on the south-facing slope at heights of 60, 90, 120 m a.s.l., and one on the valley bottom (40 m a.s.l.).

Station SFS1 (south-facing slope) is on the upper part of this slope on a narrow terrace which is situated between almost vertical lime stone cliffs and an inclined erosional surface (pediment). On this terrace, the terra rossa soil is relatively deep and partially (20%) covered loosely with eroded rocks. The ground is densely covered by grasses and small shrubs. Station SFS2 is on the middle of the pediment with the slope dipping about 35°. Here the shallow rocky terra rossa soil is covered to a large extent (65%) with mostly consolidated rocks. The patchy vegetation is dominated by grasses, but 10 to 20% of the ground is bare. Station SFS3 is on the lower part of the pediment and is geologically similar to SFS2 but completely covered with grasses, shrubs, and semi-shrubs. Station VB4 (valley bottom) is on the flat bottom of the canyon in tall grassland and lush herbaceous vegetation. Station NFS5 (north-facing slope) is on the lower part of the slope and dips at a 25° angle. This area is characterized by a shady closed forest with lush herbaceous undergrowth and parts of the ground are covered with leaf litter. Less than 10% of the soil is covered with eroded rocks and the soil is relatively rich in nutrients and deep. Here, even during the dry summer period, fresh green vegetation can be found. Station NFS6 is located in the middle of the north-facing slope. The tree-cover is much less dense here, resulting in numerous small clearings covered with grasses and annuals. The soil is rocky and shallow with about 30 to 40% stone cover. Station NFS7 is located on the shoulder of the upper north-facing slope with scattered trees and bushes, grassy undergrowth, shrubs, and semi-shrubs. More than two-thirds of the soil is covered by large consolidated limestone boulders.

Collection and processing of sand flies

Sand flies were collected from January to December, 2005. Collection took place once a month during the dark phase of the moon with CDC miniature UV light traps (Model 1212 John W. Hock Co., Gainesville, FL, U.S.A.). At each station, ten traps were placed on the same contour line a.s.l. with distances of about 10 to 15 m from each other. The traps were suspended from tripods with the fan ~50 cm above the ground. Traps were set 1 h before sunset and trap nets were recovered at 06:00 each morning. Sand flies were transported in cooling bags (5° C) within 2 h to the laboratory where they were then killed by CO₂. Dissection was carried out under a stereo microscope and the guts of females were examined with a phase contrast microscope.
Figure 1. Overview of Nahal Oren and the seven stations.
for *Leishmania* promastigotes while the head and genitalia of both sexes were mounted in either Hoyer’s or Berlese’s medium for species identification (Kravchenko et al. 2004). For identification we used the keys of Artemiev (1980), Lewis and Buttiker (1980, 1982), Lewis (1982), and Lane (1986).

### Calculations and presentation of data

In order to estimate the uniformity of distribution of the sand flies, we calculated the coefficient of variation (CV). It is a normalized measure of dispersion of the probability of distribution. It is defined as the ratio of the standard deviation $\sigma$ to the mean $\mu$:

$$CV = \frac{\sigma}{\mu}$$

A higher CV indicates greater variability in distribution. Coefficients with a value greater than 1 suggest that the distribution is aggregated (Zhou et al. 2004).

### Distribution of different sand fly species within the canyon

Altogether, 4,008 sand flies (2,321 females and 1,687 males) belonging to seven species were collected. The most common was *Phlebotomus (Adlerius) arabicus* Theodor, 1958, which accounted for 43.1% of the total catch (1,015 females/711 males). This was followed by *Phlebotomus (Larrousis) tobbi* Adler & Theodor, 1930, with 32.0% of the catch (733 females and 546 males), *Phlebotomus (Adlerius) simici* Nitzulescu, 1931, with 14.1% (311 females and 255 males), *Phlebotomus (Paraphlebotomus) sergenti* Parrot, 1917, with 4.6% (109 females and 74 males), *Phlebotomus (Larrousia) syriacus* Adler & Theodor, 1931, with 3.9% (97 females and 59 males), *Phlebotomus (Phlebotomus) papatasi* (Scopoli), 1786, with 2.3% (53 females and 40 males), and *P. perfiliewi* Parrot, 1930, with 0.1% (three females and two males) of the catch. The total number of individuals of each species collected at the seven sites can be seen in Table 1 where the cells highlighted in gray depict cases with more than 20 individuals for the three most abundant species: *P. arabicus*, *P. tobbi*, and *P. simici*.

#### Phlebotomus arabicus

This species could be found at all south-facing slope stations, the bottom of the valley (VB4), and the lower part of the north-facing slope (NFS5) from March to October, with the exception of the lower part of the south-facing slope (SFS3) where it was absent during July and August (Table 1). No specimens were caught among the bushes on the mid- and upper part of the north-facing slope (NFS6 and 7). From July to September, the total number of specimens collected increased, reaching the highest numbers in August and September, but the spatial distribution of the sand fly became disjunctive.

*P. arabicus* was found predominantly on the terrace along the cliffs (SFS1) and on the bottom of the canyon (VB4). On the terrace of SFS1, it was found throughout the year but on the valley bottom they were absent from December to February. On the middle and lowest part of the south-facing slope (SFS 2 and SFS 3), the species occurred with a bimodal seasonal dynamic – in spring, from March to June, and again in autumn, in September and October.

#### Phlebotomus tobbi

This species was not observed during the cold months from December to March and most of the individuals were collected during the summer from July to September (Table 1). The species occurred mainly on the north-facing slope and to a much smaller extent on the bottom of the canyon. Almost two-thirds (790/1,279) of the total catch was collected on the lower part of the NFS at station NFS5 and no *P. tobbi* were collected on the south-facing slopes.

#### Phlebotomus simici

This species showed a disjunctive distribution type like *P. arabicus*. The bulk of the specimens (94%) were collected at the bottom of the canyon (VB4), on the adjacent part of the north-facing slope (NFS5) and on the upper part of the south-facing slope (SFS1). This species was caught from April to November with the highest rate of occurrence from June to September (Table 1).

#### Phlebotomus sergenti

Females of this species were found in all stations but in low numbers (Table 1). Most of the specimens (54%) were collected on the bottom of the canyon (VB4). This species also showed a disjunctive distribution type, clearly preferring the bottom of the canyon (VB4) and the upper part of the south-facing slope (SFS1). This species was caught from May to November, with the highest rate of occurrence from July to September.

#### Phlebotomus syriacus

Both males and females of this species accounted for 98.1% of the total catch on the bottom of the canyon (VB4) and in the forest of the adjacent part of the north-facing slope (NFS 5), with the period of highest numbers occurring from July to August (Table 1).

#### Phlebotomus papatasi

Though small numbers of *P. papatasi* were collected on all stations apart from the SFS2 and NFS7, this species occurred mainly on the bottom of the canyon and the adjacent parts of the slopes (VB4, SFS3, and NFS5) and again on the upper part of the south-facing slope (SFS1). The highest number of specimens was collected from June to September (Table 1).

#### Phlebotomus perfiliewi

Only three females and two males were collected on the upper part of the south-facing slope (SFS1) in July and August.
Table 1. Seasonal distribution of sand flies at the seven stations in Nahal Oren.

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Seasonal-spatial variability of males and females

In order to estimate the variability in the spatial distribution of males and females within the stations, we calculated the CV (coefficient of variation) for the three most abundant species (P. arabicus, P. tobbi, and P. simici) whenever more than 20 female or male individuals of one species were caught per night. From April to October all together 31 such cases were found for females and 18 for males (Figure 3).

Regardless of species and sex, in all stations the spatial variability of the three species tended to increase towards summer, but the slope of the regression line was different for females and males (Figure 3). During the summer, the CV of females ranged from 0.51 (P. tobbi in June) to 1.82 (P. arabicus in September), while the variability of males ranged from 0.51 (P. tobbi) to 2.73 (P. arabicus) in the same time period. Parameters of the regression lines were calculated in multiply regression analysis after arcsine transformation. Differences in the lines were significant (P < 0.05).

Cluster analysis

In order to reveal the rate of similarity/dissimilarity in the distribution pattern of the six common sand fly species we clusterized the distribution of females. The data were standardized, for analysis the method of clustering—single linkage was used and the metric distances were Euclidian. The highest rate of similarity was for P. arabicus, P. simici, and P. sergenti, i.e., for species that occurred predominantly on the bottom of the canyon (VB 4) and on the upper part of the north-facing slope (SFS 1). The other three species with distinct types of distribution differ from this group and from each other. The highest rate of dissimilarity from all other species showed for P. tobbi that occurred mainly in the lower part of the north-facing slope (NFS 5) (Figure 3).

Dissection for parasites

No Leishmania parasites were found in the 2,318 dissected guts of female sand flies, including 1,015 P. arabicus, 733 P. tobbi, 311 P. simici, 109 P. sergenti, 97 P. syriacus, 53 P. papatasi, and three P. perfiliewi.

DISCUSSION

Within a single year, once a month, ten CDC traps were operated in Nahal Oren at each of the seven stations amounting to 840 trapping nights. From the roughly 4,000 sand flies caught during the study, the most common species was P. arabicus (1,726) followed by P. tobbi (1,279), P. simici (564), P. sergenti (183), P. syriacus (156) and P. papatasi (93). Only five specimens of P. perfiliewi were collected. This is about half of the known Israeli Phlebotomus species represented in an area of about 10 hectares. It is worth mentioning that this is the first record for P. arabicus, a vector of L. tropica, outside its known distribution area north of the Sea of Galilee.

The area is known for its high biodiversity and about a third of the known Israeli scorpion species (Raz et al. 2009), about 20% of the Lepidoptera (Kravchenko et al. 2007a,b), and about 15% of the known Israeli Coleoptera (Chikatunov et al. 2000) are found in this canyon.

Our results suggest that none of the sand fly species were indifferent to the heterogeneity of the microenvironment inside the canyon. Apart from the rare P. perfiliewi, which was only collected on the upper part of the south-facing slope, and P. tobbi, which clustered on the north-facing slope and the adjacent part of the valley bottom, the bulk of the other sand flies were caught on the bottom of the canyon (BV4) and on the terrace of the upper part of the south-facing slope (SFS1). The highest rate of similarity was found for P. arabicus, P. simici, and P. sergenti, species that occurred predominantly on the bottom of the canyon (VB 4) and on the upper part of the north-facing slope (SFS 1). P. tobbi showed the highest rate of dissimilarity from all other species by occurring mainly on the lower part of the north-facing slope (NFS During the summer, the catches of all sand fly species increased, reaching a maximum in August and September. In April and May, in lush vegetation and with adequate humidity, all species were distributed evenly throughout their habitats. With the onset of summer dryness, the sand flies concentrated in the humid habitats (SFS1 and VB4). Those sites, together with the lower part of the north-facing slope, are the most humid in the canyon.
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The rate of concentration (CV) was essentially higher for males than for females (Figure 2). The variation may result from differences in the behavior of the two sexes (Yuval 1991, Lane et al. 1990). Male sand flies are not attracted by the same stimuli as are females (Dye et al. 1987, Gibb et al. 1988, Schlein et al. 1989), and they also tend to disperse much less than females (Yuval et al. 1988). Adult sand flies of both sexes need sugars obtained from honeydew and nectar and by probing leaves (Schlein 1987, Schlein and Müller 1995, Müller and Schlein 2004), but females also need blood meals in order to develop their eggs (Yuval 1991). We found that during all seasons, the availability of sugar was not a limiting factor in all micro-habitats, while the scarcity of suitable blood sources might have forced the females to disperse. *P. arabicus* and *P. sergenti* females were found on the south-facing slopes (SFS2 and SFS3) about ten times more often than males, probably pursuing the hyraxes that concentrate there.

It is a common assumption that many sand fly species are associated with certain soil types with enough moisture for breeding and with nearby suitable, diurnal resting sites (Feliciangeli 2004). In previous studies in the Galilee and in southern Israel, sand fly distribution in anthropogenic habitats was attributed mainly to soil moisture caused by human activity (Kravchenko et al. 2004, Wasserberg et al. 2003). Soil humidity is an important factor for the local sand fly fauna in Nahal Oren, but we do not think that the highest overall catches of sand flies necessarily have to correspond with breeding sites.

The overall sex ratio (F:M) of sand flies in Nahal Oren was similar for all collected species and varied from 1.23 (*P. simici*) to 1.64 (*P. syriacus*). Nevertheless, the observed sex ratios at different stations varied considerably for most species. Males generally disperse less than females (Yuval et al. 1988, Morillas-Marquez 1983) and they often accumulate near emergence sites (Ready et al. 1986, Bettini 1988, Yuval 1991). The overall sex ratio of all the collected *P. arabicus* was 1.43, but at SFS1 the ratio was 0.99 and at VB4 it was 0.96. Similar observations were made with *P. tobbi* (overall: 1.34, NFS6: 0.83), *P. simici* (overall: 1.23, NFS5: 0.51), *P. sergenti* (overall: 1.47, VB4: 0.91), and *P. papatasi* (overall: 1.33, SFS1 0.73), while no shift in sex ratio was observed for *P. syriacus*, though males clearly clustered at VB4 and NFS5. The higher concentrations of males observed might indeed mean that these are breeding sites, but further work is needed especially concerning the association of sand flies with soil properties. Solid data derived from emergence traps is also needed (Feliciangeli 2004, Müller et al. 2011).

In Nahal Oren, we did not analyze variations in the soil as was done in previous studies (Nevo et al. 1998, Dan 1988), but in the future, we feel that a collaboration with soil ecologists would be beneficial. In further studies, it would also be interesting to identify the exact breeding sites of the two vectors *P. arabicus* and *P. sergenti* in Nahal Oren, as well as in other regions of Israel.

The local sand fly fauna can be clustered according to their habitat preference. These clusters include species which are common only on the bottom of the canyon and the adjacent part of the north-facing slope (*P. tobbi* and *P. syriacus*), and species with a disjunctive distribution which are common on the bottom of the slopes and on the upper part of the south-facing slope (*P. arabicus*, *P. simici*, and *P. sergenti*). Only *P. papatasi* did not show a clear pattern, though it seems to avoid the driest habitat within the canyon (SFS2) and the top of the north-facing slope. These results are not surprising because a similar kind of spatial distribution was also observed for other insect groups, namely Lepidoptera and Coleoptera as well as some dipteran species (Kravchenko et al. 2002, Chikatunov et al. 2000, Harry et al. 1999).

During our study, none of the 2,318 dissected female sand flies was positive for *Leishmania* promastigotes. In a recent study less than 60 km northeast of Nahal Oren, five out of 97 *P. arabicus* and two out of 162 *P. sergenti* females were found to be infected with *L. tropica*, and three out of 29 hyraxes were positive for *Leishmania* ribosomal DNA.
About 120 km to the south, in settlements in the Judean Desert just a few km east of Jerusalem, hundreds of cutaneous leishmaniasis cases caused by *L. tropica* were recorded (Schnur et al. 2004, Singer et al. 2008) and numerous *P. sergenti* were positive for *L. tropica* (Schnur et al. 2004).

In the relatively small area of Nahal Oren, all three known Israeli vectors of cutaneous leishmaniasis (*P. arabicus*, *P. sergenti*, and *P. papatasi*) were found to be common. Interestingly, from the Haifa area and the Carmel Mountain ridge, no local infections at present have been reported, though the reservoir of *L. tropica*, the hyrax (*Procavia capensis*), is also common in peri-urban habitats. The last reported cases of active cutaneous Leishmaniasis in Haifa are from the mid-1930s to the mid-1940s, and at the time, *P. papatasi* was suspected of being the vector for the many hundreds of cases (Sternfeld 1944, Gill 1952). The assumption was based on the fact that *P. papatasi* was, at the time, the only known vector for this disease and accordingly, the local sand fly fauna was not explored (Gill 1946). The high density of the two *L. tropica* vectors in Nahal Oren and their wide distribution within the canyon, combined with the highly mobile hyraxes and their proximity to Haifa, the third largest city in Israel, seem to be a serious risk for public health. The situation in Nahal Oren needs to be observed, and further investigations in Haifa and surrounding villages are necessary to see if *P. arabicus* and *P. sergenti* are also found in peri-urban and urban settings.

The ultimate goals of this research are to understand the association of sand fly vectors with their habitats, to identify areas with a risk for future *Leishmania* outbreaks and to understand the requirements of sand flies for their breeding habitats in order to control sand flies by treating specific areas, rather than using area-wide applications of pesticides.

REFERENCES CITED


