

## Activity Patterns of Sand Fly (Diptera: Psychodidae) Species and Comparative Performance of Different Traps in an Endemic Cutaneous Leishmaniasis Focus in Cukurova Plain, Southern Anatolia, Turkey

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### Abstract

An entomological survey for sand flies was conducted from May to October 2006 in a village near an endemic focus of cutaneous leishmaniasis in Cukurova Plain, south Anatolia, Turkey. Standard CDC light traps, CO<sub>2</sub> traps, sticky traps, mouth aspirators, animal-baited traps and human landing collection were used to determine species composition, density and nocturnal activity of sand fly species. BG-Sentinel Trap, a novel monitoring trap originally developed to attract mosquitoes, was also tested to investigate its efficiency for sand flies. Overall, 4 048 specimens belonging to four species of genus *Phlebotomus* Rondani et Berté 1840 and two of genus *Sergentomyia* França et Parrot 1920 were collected. *Phlebotomus tobbi* Adler, Theodor et Lourie 1930, the proven vector of *Leishmania infantum* Nicolle 1908 was found to be the most abundant (65.6%) species while *P. sergenti* Parrot 1917, the proven vector of *L. tropica* (Wright, 1903) in Turkey accounted for 0.1% of the sand flies that were identified. Other species, *P. perfiliewi galilaeus* (Theodor 1958), *P. papatasi* (Scopoli, 1786), *Sergentomyia dentata* (Sinton, 1933) and *Sergentomyia theodori* (Parrot, 1942) represented 31%, 2%, 1.5% and 0.3 % of the sand fly fauna, respectively. Aggregate population of sand flies was found to be the lowest in May. Population size rose through June and July, with the highest peak in August, and decreased through September and October. Among the traps used, CO<sub>2</sub> traps were found to offer a more suitable and productive method than others for both estimating the species composition and the population density of sand flies in the study area. Studies on the nocturnal activity indicated that even the number of captures declined rapidly during dusk period, between 04.00 and 06.00 h, in general, no significant hourly pattern was determined neither the species prevalence nor the nocturnal activity of the species. According to statistical analysis the variation in hourly nighttime temperature did not influence the nocturnal activity of the species whereas the number of collected sand flies during nocturnal period was strongly associated with relative humidity.

*Leishmania, Phlebotomus, Sergentomyia, blood sucking insects, nocturnal activity*

The prevalence of anthroponotic cutaneous leishmaniasis (CL) which is caused mostly by *Leishmania tropica* (Wright, 1903) was high before 1950's in the southeastern Anatolia region of Turkey. After the activation of the Malaria Control Programmes all over the country, the incidence of CL has decreased. The reduced number of infected reservoirs, low density of vector populations and improvement of socio-economic life of the communities resulted in limiting the distribution of CL cases within the southeastern Anatolia region. Although leishmaniasis has been sporadically observed in other parts of the country, over the past 10 years there has been a significant increase in the incidence of CL cases in Cukurova Basin, and this region has become the second endemic focus of CL in Turkey. More than 4 500 CL cases have been recorded during the past eight years by the Cukurova University Tropical Diseases Research Centre and Department of Dermatology, School of Medicine (Anonymous 2007).

Previous studies aiming at determining the species composition and distribution patterns of sand flies in Cukurova Basin revealed nine *Phlebotomus* Rondani et Berté 1840 and two

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*Sergentomyia* França et Parrot 1920 species. In this region, *Phlebotomus tobbi* Adler, Theodor et Lourie 1930 was found to be the most abundant (71.3%) species while *P. sergenti* Parrot 1917, the proven vector of *L. tropica* in Turkey represented only 3% of the sand fly fauna (Simsek et al. 2007). According to the results of recent investigations on the identification of vector species in this region, *Leishmania infantum* Nicolle 1908 was isolated in a CL focus from both *P. tobbi* specimens and local human patients for the first time (Svobodová et al. 2009). All these data show that extensive studies on the population density, seasonal population dynamics and circadian activity of sand fly species including *P. tobbi* are needed to improve our knowledge of leishmaniasis transmission dynamics in this area.

This paper gives the results of an investigation to determine the species composition, seasonal fluctuations of given populations and nocturnal activity patterns of different sand fly species in a village located at the centre of an endemic focus of CL in Cukurova Plain, Southern Anatolia, Turkey. Effects of variations in hourly nighttime temperature and relative humidity on the nocturnal activity patterns of the species were also studied. Besides, in comparison to other trap types, BG-Sentinel Trap, a novel-monitoring trap originally developed to attract mosquitoes, was also tested to determine its efficiency for the monitoring of sand fly species.

### Materials and Methods

#### Study area

Field studies were conducted in the Camili village (37°20'05"N, 35°36'42"E) in Cukurova Plain, South Anatolia, from May to October 2006. This village is located at the centre of an endemic focus of CL and has a high sand fly density throughout the season (Simsek et al. 2007). Camili is bound by West Taurus Mountains to the West; the mountain ranges of Taurus and Anti-Taurus to the North, and the Amanos Mountains to the East. The village is about 55 km northeast of the Adana city at the altitude of 205 m above sea level (a.s.l.). Most of the area is fertile ("mollisol" soil) and used for agricultural activities, however, young *Pinus* and *Abies* forests are also cultivated. Citrus orchards and cotton fields are common around the village. The climate in the study area is classified as temperate, with a short wet season. The mean annual precipitation is 636.8 mm and with 66% relative humidity, and the mean annual temperature is 18.7 °C. The maximum and minimum average monthly temperatures are 34.4 °C and 5.0 °C, respectively. The population of the village is approximately 220 inhabitants. Residents live in single-family houses built from briquette, adobe, stone and cement, surrounded by gardens with henhouses and sheep or cowsheds. Although the animal shelters are closed with four walls and a roof, most villagers prefer to keep their animals in simple open sheds consisting of a roof made of bamboo supported by pillars during the summer season.

#### Collection methods

Sand flies were collected monthly from May to October 2006 using a Centre for Disease Control (CDC) light trap (John W. Hock, USA) and a CO<sub>2</sub> light trap (0.5 kg dry ice) placed in yards and set up about 1.5 m above ground. Four sticky paper traps (20 × 30 cm papers coated with castor oil) were rolled up and placed in the holes of the wall that surrounds the chicken coop. The BGS-Trap has been recently developed by BioGents GmbH (Regensburg, Germany) and utilizes patent-pending technology from the University of Regensburg. Two BG-Sentinel traps containing two different attractants (the BG-Lure® and Octenol lure) were also placed on the ground. Each trap used in the study was placed outside the same house and animal shelter between 18.00 and 06.00 h. The traps were set up at least 15 m from each other and were changed every two hours. As the traps were changed, new ones were placed in the same location. Human landing collection and collections using a cattle baited trap (3 × 3 × 2 m) were also performed over the same 12 h period. All sand flies landing on the exposed legs of human collectors and entering into cattle baited trap were captured during 10 min in every two hours. Indoor collections were performed using mouth aspirators.

#### Sand fly identification

Specimens collected from each trap were stored in 96% alcohol for morphological identification. Identifications were based on the morphology of the male genitalia and female spermatechae and pharynges using the keys of Theodor (1958), Artemiev (1980), Lewis (1982) and Killick-Kendrick et al. (1991).

#### Recording climatic conditions

To determine whether the local variations in hourly temperature and relative humidity (RH) might affect abundance or activity of sand flies in the study area, we placed two data loggers (iButton Hygrochron, DS 1923) associated with each trap. One of them was programmed to record temperature and humidity every 6 h and left at the study area during the whole year. Every month, we retrieved temperature and humidity data from the data logger and obtained daily and monthly temperature and humidity averages. The other one was programmed to record temperature and humidity every half hour and used only during one night in September when the traps were set to investigate if there is any relationship between the nightly variations in climatic conditions and the nocturnal activity of sand flies.

### Statistical Analyses

In order to ensure normality and homogeneity of variances before subjecting to statistical analysis, data on the number of flies collected were square-root transformed. The significance of the effect of species, trap and sex on the number of animals that come to the trap was analyzed using a mixed model analysis of variance (ANOVA), in which species and sex constituted the random factors, while trap constituted the fixed factor. When significant effects of traps were established ( $p < 0.05$ ), differences among traps were tested using Student-Newman-Keuls multiple comparison test. A stepwise multiple regression was conducted to assess the effects of average night-time temperature, average night-time humidity and collection period on the number of flies caught during one study night in September. Basic correlation matrices used to investigate the existence of a correlation between the abundance of the sand flies, average monthly temperature and average monthly relative humidity in the study area.

## Results

A total of 4 048 sand flies belonging to four species of the genus *Phlebotomus* and two of the genus *Sergentomyia* were collected. Females comprised 20% of catches during the study period. Of the collected total, *Phlebotomus tobbi* was found to be the most abundant (65.6%) species while *P. sergenti*, the proven vector of *L. tropica* in Turkey accounted for 0.1% of the sand flies that were identified. Other species, *P. perfiliewi galilaeus* (Theodor 1958), *P. papatasi* (Scopoli, 1786), *Sergentomyia dentata* (Sinton, 1933) and *S. theodori* (Parrot, 1942) represented 31%, 2%, 1.5% and 0.3% of the sand fly fauna, respectively (Table 1). During the study period, aggregate population of sand flies was found to be the lowest in May. Population size rose during June and July, with the highest peak in August, and decreased during September and October.

Table 1. Relative abundance of *P. tobbi*, *P. perfiliewi galilaeus*, *P. sergenti* and *Sergentomyia* spp. by month in Camili from May to October

Species \ Month	<i>P. tobbi</i> (%)	<i>P. papatasi</i> (%)	<i>P. sergenti</i> (%)	<i>P. perf. gal.</i> (%)	<i>Sergentomyia</i> spp. (%)
May	68.75	18.75	0	0	12.5
June	43.95	8.07	0	44.39	3.58
July	71.09	2.89	1.15	15.6	9.24
August	59.49	0.63	0	38.76	1.11
September	83.04	3.3	0.2	12.71	0.72
October	80.82	1.37	0	17.8	0

The recorded average monthly temperature and RH values ranged between 23.7–31.2 °C and 46.6–66.8%, respectively. In August, when the maximum number of sand flies (2 523) was collected, the average temperature and RH were found to be 30.5 °C and 56.8%, respectively; while the minimum number of sand flies was sampled in May (16) with an average temperature of 23.7 °C and 66.8% relative humidity (Fig. 1). There were no significant correlations between the abundance of sand flies, average monthly temperature ( $r = 0.5, p = 0.3$ ) and average monthly relative humidity ( $r = -0.19, p = 0.7$ ).

The relative abundance of each species by month is shown in Table 1. *Phlebotomus tobbi* was the predominant species identified in May, July, August, September and October, accounting for > 50% of the collection each month, whereas in June it accounted for ~ 44% of the total collection. Although no specimen of *P. perfiliewi galilaeus* was identified in May, it became the predominant species in June (~ 45%). Later in the season, although it became less abundant, it was the second predominant species during the study period. *Phlebotomus papatasi* and *Sergentomyia* spp. were found to be rare throughout the study period. Neither *P. papatasi* nor *Sergentomyia* spp. accounted for more than 20% of the sand flies collected each month. *Phlebotomus sergenti* was collected only in July and September and accounted for ~ 1.2 and 0.2% of the total collection respectively.

The number of sand flies collected using different types of traps during the study period is presented in Table 2. Almost all sand fly species were commonly captured by CO<sub>2</sub> light traps and standard CDC light traps. Approximately 71.2% of the total collection was made

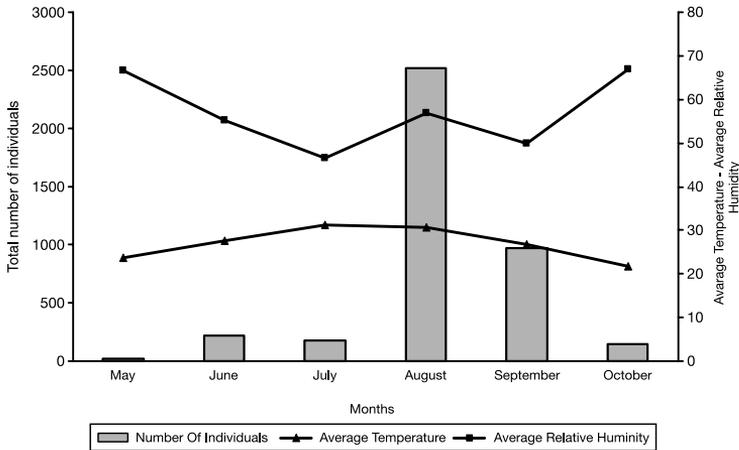


Fig. 1. Total number of sand flies collected each month and variation of average monthly temperature and relative humidity in the study area from May to October 2006

up using CO<sub>2</sub> light traps. Twenty-one percent and 5.2% of sand flies were collected using CDC light traps and animal baited traps, respectively. Collections using BG-Sentinel trap with octenol Lure and BG-Lure constituted 0.98 and 1.01% of the total collection. Neither human landing collections (0.3%) nor sticky traps (0.2%) showed a high efficiency during the study. Results of three-way analysis of variance (ANOVA) revealed a significant interaction between the collection methods and the number of individuals collected ( $p = 0.032$ ), whereas there was no significant interaction between the trapping methods and the proportion of males and females collected ( $p = 0.107$ ). When significant effects of traps were established ( $p < 0.05$ ), differences among traps were tested using Student-Newman-Keuls multiple comparison test. Student-Newman-Keuls test exposed that sticky papers, animal baited trap, BG-S with octenol, BG-S with BG lure, aspirator, and human landing collection displayed similar capturing performance, whereas CO<sub>2</sub> and standard CDC light traps differed from the others ( $p < 0.05$ ) and they were found to be more effective in terms of the total number of individuals collected.

A total of 1 042 phlebotomine sand flies were collected throughout one night in September. Males were found to be 4.7-fold more abundant than females. Results with respect to the nocturnal activity indicated that even though the number of captures declined rapidly between 04.00 and 06.00 h, there was no significant hourly pattern either in the species prevalence or in the activity of the species (Fig. 2). *Phlebotomus tobbi* (84.3%) was found to be predominant throughout the night, followed by *P. perfiliewi galilaeus* (11.7%), *P. papatasi* (3.1%) and *Sergentomyia* spp. (0.9%). Between 04.00–06.00 h, only *P. tobbi* and *P. perfiliewi galilaeus* were collected. The nocturnal activity pattern and variation in temperature and relative humidity in the study station is represented in Fig. 3. Multiple regressions testing effects of collection intervals and hourly nighttime temperature on the number of collected sand flies revealed no significant relationships. However, the number of sand flies was strongly and negatively associated with hourly nighttime humidity ( $R^2 = 0.729$ ,  $df = 11$ ,  $p = 0.034$ ).

## Discussion

This study is the first detailed research in terms of species composition, density and nocturnal activity of sand flies in an endemic focus of cutaneous leishmaniasis in Cukurova

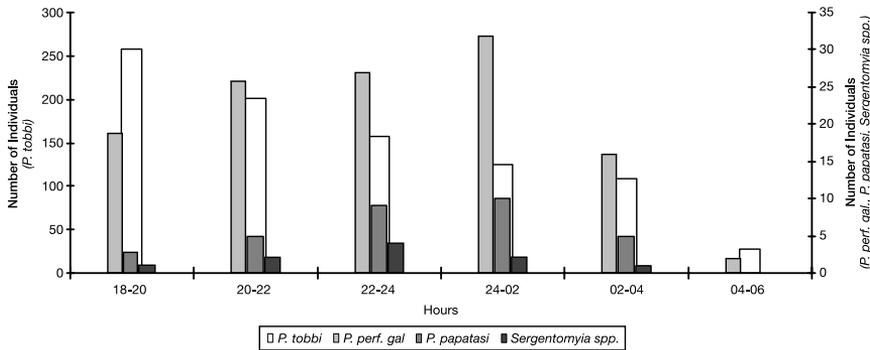


Fig. 2. Hourly activity pattern of the sand fly species in September in Camili

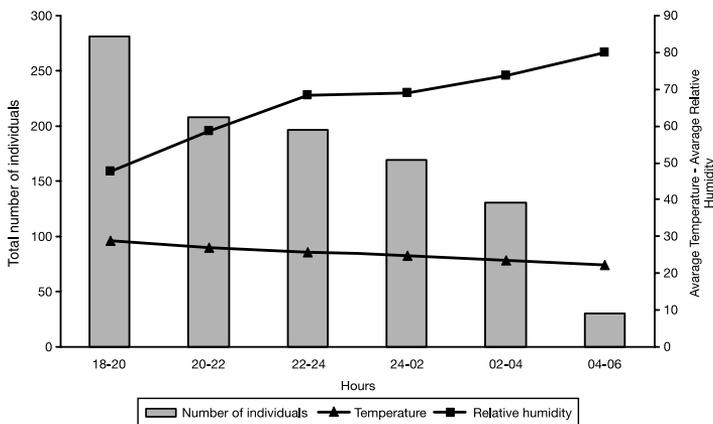


Fig. 3. Nocturnal activity pattern of the sand flies and variation in temperature and relative humidity in September in Camili

Plain, South Anatolia, Turkey. Trapping over the summer season revealed that *P. tobbi* is the most abundant species (65.6%) in this region. Our finding of high population density for *P. tobbi* compares favourably with our previously published data for which this species is the predominant sand fly species in the lowlands (0-300 m a.s.l.) of the Cukurova Region (Simsek et al. 2007). However, according to previous results, *P. perfliewi* was very rare (0.02%) in the Adana Province, whereas we found this species as the second most abundant (31%) species. Both the previous and recent findings showed that *P. sergenti*, the proven vector of *L. tropica* in Turkey, was represented with small populations in the study area. The village in which we conducted our study is located at an altitude of 180 m a.s.l. and *P. sergenti* accounted for only 0.1% of the total collection. The observed low abundance of *P. sergenti* may be another indicator that this species is most common at 500–700 m a. s. l. (Büttiker and Lewis 1983), and described as a mountainous species (Seyedi Rashti and Nadim 1992).

Although sand fly abundance was not strongly and significantly influenced by the variations in average monthly temperature and relative humidity during the six months of survey, the maximum number of sand flies was recorded in the hottest and driest season between June and September when the average temperature was comparatively high (26.9 to 31.2 °C), the relative humidity was low (46.6 to 56.8%) and the rainy days ranged

Table 2. Numbers of sand flies collected using different types of traps in Camili during May-October 2006

Species	CO <sub>2</sub> baited light traps		CDC light traps		Sticky traps		Animal baited traps		BG-Sentinel (octenol)		BG-Sentinel (biogent)		Human landing collections		Aspirator	
	Females	Males	Females	Males	Females	Males	Females	Males	Females	Males	Females	Males	Females	Males	Females	Males
<i>Phlebotomus tobbi</i>	402	1375	68	573	0	5	45	107	2	34	0	38	4	1	0	0
<i>P. papatasi</i>	12	29	6	12	0	0	1	5	0	1	0	0	6	0	2	2
<i>P. sergenti</i>	0	0	3	0	0	0	1	0	0	0	0	0	0	0	0	0
<i>P. perfiliewi galiliacus</i>	174	858	33	140	0	0	12	36	0	0	0	0	0	0	0	0
<i>Sergentomyia dentata</i>	28	0	11	1	1	0	2	0	2	0	2	0	0	0	0	0
<i>S. theodori</i>	1	4	0	3	2	0	0	2	0	1	0	1	0	0	0	0
All species	617	2266	121	729	3	5	61	150	4	36	2	39	10	1	2	2

between 1.5 to 3.5 days. Singh (1990) studied the role of climatic factors in the seasonal distribution of sand flies in the arid areas of India and revealed that the majority of species preferred comparatively higher temperatures and low RH%, a prerequisite for survival in arid and semi-arid conditions. The abundance of *Lutzomyia vexator* (Coquillett, 1907) in New York was reported not to be influenced by local variation over time in temperature and humidity, but the number of captures of sand flies was strongly and positively associated with the percent slope, suggesting some feature of topography to be a good predictor of sand fly abundance (Ostfeld et al. 2004). Although our findings contribute to our understanding of the factors affecting sand fly abundance and dynamics in the study area, further studies of topographic characteristics of the area, distribution and the dynamics of the reservoir hosts are required to better describe these patterns.

Although CDC light traps and CO<sub>2</sub> traps are used extensively in field studies of sand flies (Alexander 2000), sticky traps have no known attractiveness and have generally been used for determining species composition of an area as they randomly sample the species where they are set. However, while unbiased sticky traps provide more realistic results than CDC light traps that could attract additional phototropic sand flies, previous studies revealed that the effective range of CDC light traps were less than 5 m (Wheeler et al. 1996). Therefore, although the results obtained from both CDC light traps and CO<sub>2</sub> traps may not be reliable with respect to human-vector contact, in terms of the efficiency of the capturing method, these two trapping methods appeared to be the most productive for both estimating the number of sand flies and the species composition in the study area, in agreement with previous studies. Veronesi et al. (2007) reported that when compared to sticky traps, CO<sub>2</sub> traps were found to be more effective in collecting sand flies in northern Italy and addition of a light source improved the catches. The BG-Sentinel trap was originally developed to capture adult *Aedes aegypti* (Linnaeus 1762) as a monitoring tool and as an attempt to reduce adult mosquito density. The BG-Lure<sup>®</sup> is an effective mosquito attractant used in the BG-Sentinel #2880 and other BioQuip mosquito traps. It is packaged in a mesh dispenser that releases a combination of non-toxic substances found on human skin (lactic acid, ammonia, and fatty acids). Octenol lure, the other attractant used in this study, is reported to have pheromone-like properties. It is used by vector control professionals and researchers to enhance the effectiveness of mosquito traps. Even though this novel trap proved to be a reliable and standardized tool for collecting *A. aegypti* in urban areas (Maciel-de-Freitas et al. 2006) and its attractiveness was also recorded

for a range of other anthropophagic mosquitoes, the low efficiency of this method in our study showed that BG-Sentinel trap may not be a useful alternative for monitoring sand flies. Beavers et al. (2004) evaluated the efficiency of 1-octen-3-ol and CO<sub>2</sub> for catching sand flies in southern Egypt and showed that *P. papatasi*, the most abundant species in the study area was more attracted to CO<sub>2</sub> baited traps than to those baited with octenol. They also did not find any synergistic interaction between CO<sub>2</sub> and octenol in terms of attracting sand flies. Similar results were recorded by Bernier et al. (2008). According to the results of trapping experiments in Bahrin, Egypt using CO<sub>2</sub> (alone), CO<sub>2</sub> + octenol and CO<sub>2</sub> + human hair baited traps, addition of chemical lures or odors did not enhance the trap collections.

The nocturnal activity patterns of sand flies have been recorded for Old World species in many studies (Roberts 1994; Guernaoui et al. 2006; Coleman et al. 2007). According to our results obtained in the late summer season, sand fly activity was not significantly different over time even though total counts decreased between 04.00–06.00 h. No significant difference was found in the species prevalence throughout the nights. *Phlebotomus tobbi* seemed to be active and predominant until sunrise. The most important factor affecting sand fly nocturnal activity in this study was found to be the relative humidity. Activity started just before the sunset and increased rapidly when the average RH was 47.7%. As the humidity increased to 58.6 and 69.1%, activity remained almost constant until midnight and decreased rapidly when the recorded average humidity values were over 70%. Our results agree with those of Roberts (1994) who found that there was no significant hourly activity pattern for the sand flies in Oman and the most important factor affecting sand fly activity was low humidity, followed by low wind velocity and high temperature. It is clear that activity of sand flies is continuous through the night and relative humidity has a significant effect on the nocturnal activity in our study area. However, many investigations demonstrated the existence of seasonal variations in the nocturnal activity of sand flies. Coleman et al. (2007) showed that in Southern Iraq, the peak activity period shifted from early evening (20.00–22.00 h) in April and October to late night (22.00–24.00 h) in May and June. Similarly, in Morocco the sand fly activity was found to be continuous all night in August, whereas in October the activity declined rapidly after midnight (Guernaoui et al. 2006). Therefore, more detailed studies with respect to the seasonal variations and the effects of abiotic conditions, other than temperature and relative humidity, such as cloud cover, wind velocity and lunar cycle on the nocturnal activity of sand flies may improve our knowledge of the behaviour in this epidemiologically important area.

The overall percentage of male sand flies (80%) recorded during the study period is consistent with other published data. Morrison et al. (1995) reported that during the 10 months of their survey in an endemic region of visceral leishmaniasis in Colombia, male *Lutzomyia longipalpis* (Lutz and Neiva, 1912) composed 60% and 83% of the collections conducted at the pigpen and at the cattle corral, respectively. Similar results were obtained by Reza and Mansour (2006) who collected significantly more males than females of both *P. papatasi* (85%) and *P. sergenti* (70%). According to the results of a more recent study conducted in the Indian state of Bihar, males and non-fed females of *Phlebotomus argentipes* (Annandale and Brunetti, 1908) comprised > 60% of the total collections (Dinesh et al. 2008). Previous studies on sand fly abundance revealed that the sex ratio of *L. longipalpis* was more male biased at higher sand fly densities (Quinnell and Dye 1994) and when there were more available hosts (Dye et al. 1991). It is known that male sand flies arrive on their host first, form an aggregation and wait for the females for mating. This “lekking” behaviour of the males is believed to allow them to disperse high levels of sex pheromone to attract females and increase their chances to mate (Morrison et al. 1995; Killick-Kendrick 1999). During the summer season, the villagers in our study area sleep outside of their houses and keep their animals in open shelters where we set our traps. Therefore, potential hosts for sand fly species during the night are available. The

significant male biased sex ratio we observed during our study may be explained by this adaptive behaviour.

Our data demonstrate the existence of important vector species of sand flies in the study area. *Phlebotomus tobbi* is the predominant species and abundant from May to October with a continuous nocturnal activity. Although the abundance of this sand fly species is not by itself sufficient to incriminate it as a vector, it is clear that *P. tobbi* could potentially transmit leishmaniasis in this endemic focus. Further studies should focus on *P. tobbi* to confirm the sand fly species transmitting leishmaniasis in this region.

### **Aktivita některých druhů dvoukřídleho hmyzu z čeledi Psychodidae a porovnání účinnosti různých typů pastí v endemických ohniscích kožní leishmaniózy soustředěných v oblasti Cukurova Plain, jižní Anatólie, Turecko**

Entomologický průzkum dvoukřídleho hmyzu z čeledi Psychodidae byl prováděn v období od května do října 2006 v jedné z vesnic nedaleko endemického ohniska kožní leishmaniózy v oblasti Cukurova Plain, jižní Anatólie v Turecku. Pro sběr hmyzu byly použity standardní metody odchyty pomocí CDC světelných pastí, CO<sub>2</sub> pastí, lepů, ručních aspirátorů, lapačů na návnadu a pomocí metody „human landing collection“ pro určení druhového zastoupení, hustoty a noční aktivity těchto druhů. Dále byla testována efektivita a využití nové monitorovací pasti BG-Sentinel Trap pro druhy čeledi Psychodidae, původně určené k odchyty komárů. Celkem bylo získáno 4 048 exemplářů patřících do čtyř druhů rodu *Phlebotomus* Rondani et Berte 1840 a dvou druhů rodu *Sergentomyia* Franca et Parrot 1920. Nejvíce zastoupeným (65.6%) druhem byl *Phlebotomus tobbi* Adler, Theodor et Lourie 1930, prokázán vektor *Leishmania infantum* Nicolle 1908, zatímco *P. sergenti* Parrot 1917, prokázán vektor *L. tropica* (Wright 1903) v Turecku, tvořil pouze 0.1% identifikovaných jedinců této skupiny hmyzu. Další druhy, *P. perfiliewi galilaeus* (Theodor 1958), *P. papatasi* (Scopoli 1786), *Sergentomyia dentata* (Sinton 1933) a *Sergentomyia theodori* (Parrot 1942) reprezentovaly 31 %, 2 %, 1.5 % a 0.3 % exemplářů této skupiny hmyzu, v tomto pořadí. Četnost populací dvoukřídleho hmyzu z čeledi Psychodidae bylo nejslabší v květnu. Velikost populací narůstala během června a července, kulminovala v srpnu, a redukovala se během září a října. Za nejvhodnější a nejproduktivnější metodu odchyty hmyzu čeledi Psychodidae lze z použitých metod považovat CO<sub>2</sub> pastí, které se osvědčily jak pro odhad druhové skladby hmyzu, tak pro zjištění populační hustoty těchto druhů v námi zvolené oblasti. Při studiu noční aktivity zmíněných druhů bylo také zaznamenáno snížené množství odchyceného hmyzu během soumravné fáze dne, mezi 04.00 a 06.00 h, jinak nebyly pozorovány žádné změny druhového složení ani noční aktivity v závislosti na časovém úseku během noci. Podle výsledků statistické analýzy bylo zjištěno, že noční aktivita není ovlivňována změnami teploty během noci, ale je silně spjata s relativní vlhkostí vzduchu.

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