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Experimental control of *Phlebotomus papatasi* by spraying attractive toxic sugar bait (ATSB) on vegetation

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ABSTRACT

The effect of attractive toxic sugar bait (ATSB) solution including fruit juice, sucrose and oral insecticides on populations of *Phlebotomus papatasi* (Scopoli), was studied in the central Jordan Valley, in a typical area with sparse desert vegetation. Three similar plots of land, each 35 hectares, were chosen for experiments: two for applications of ATSB and one as a control. Sand fly populations in all plots were monitored weekly from May to December. Experimental area I was sprayed three times between June and October, in patches covering about 10% of the vegetation. Experimental area II was sprayed twice with toxic baits, in August and again in October. The control area was also sprayed every second month with solution containing food dye marker instead of insecticide. After early toxin treatment, the population in area I dropped from ~80 sand flies to ~3 sand flies per trap in one month. In area II, the population declined about a month after treatment from ~110 to ~5 sand flies per trap. The control population was bimodal with peaks in July (~135 flies per trap) and October (~130 flies per trap). The food dye of the control bait marked an average of 65% to 79% of the sampled flies.

1. Introduction

Control of sand flies is relatively problematic largely because adult sand flies often rest in subterranean animal burrows, especially of rodents, and in frameworks of deep cracks in the ground.[1] Moreover the developmental sites of immature stages are mostly unknown and are probably inaccessible to control activities. Nevertheless, leishmaniasis poses significant health problems and so strategies that use insecticide treatment[2–4] or environmental modification[5] to eliminate sand flies are used by civilian and military authorities in many countries. Insecticide based control methods include residual indoor spraying[2,6] with DDT[4,7–10] or synthetic Pyrethroids,[11–13] use of insecticide impregnated bed nets,[14–16] and establishment of insecticide treated barrier zones that prevent the passage of sand flies.[4,17,18] Environmental modifications used to reduce sand flies include destruction of breeding sites by removing garbage and debris near houses, eradication of rodent reservoirs, destruction of their burrow systems and spraying of herbicides to destroy the food plants of rodents.[5] However, the drawbacks and often unsatisfying results of each of these methods has led to a search for methods that can impact sand flies in their hard-to-reach habitats without impractical, widescale environmental modification or potentially damaging insecticide treatments. Therefore, we hypothesized that a method that first attracts the sand flies to a specific area then kills them, would be

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a very useful tool to add to the sand fly control arsenal.

Like other groups of blood sucking flies, phlebotomine sand flies require sugar for survival. In nature, sand flies obtain essential sugars by feeding on plant tissues[19,20] on honeydews excreted by aphids and coccids,[21–23] and on floral nectars when available.[24] Like mosquitoes, *Phlebotomus papatasi* (Scopoli) apparently selects sugar sources according to olfactory cues[25] and we hypothesized that attractive toxic sugar baits (ATSB), previously found to be effective against mosquitoes[26–28] might also be effective in attracting and killing sugar questing sand flies. We tested this hypothesis by monitoring populations of *Ph. papatasi* after spraying patches of vegetation with ATSB or non-toxic, food dye marked control solution on three plots of land in the arid, central Jordan Valley.

2. Materials and methods

2.1. Study area

The study was carried out in the central Jordan Valley between early May and December 2008. In this region, large populations of *Ph. papatasi* are found in the colonies of the prevalent sand rats *Psammomys obesus* (Cretzschmar) which are the rodent reservoir of *Leishmania major*.[29,30] In this area, vegetation is typically restricted to water catchments such as depressions and dry river beds. These island-like patches of vegetation are separated by almost barren desert.[31] For our experiments, we chose three such vegetation islands, each about 35 hectares, that were about 1 km from each other and other similar vegetation islands. At the time of the experiments the annual winter vegetation was already dry and most of the remaining vegetation consisted of chenopod bushes of the species *Atriplex halimus* (L), *Suaeda asphaltica* (Boissier) and *S. fruticosa* (Gmelin). Typically, about 20 to 30% of the area is covered with these shrubs. In between these dominant chenopod bushes were scattered groups of large *Tamarix nilotica* trees (Bunge), *Suaeda aegyptiaca* (Zohary) and *Prosopis farcta* (Macbride) shrubs. The burrows of the *P. obesus* colonies were found in the shade of the chenopod bushes at an estimated density of two to three burrows per 1000 m^2.

2.2. Experimental procedures

Three similar plots of ~35 ha were chosen based on several factors: similar vegetation and vegetation cover, similar density of sand rat burrows and relative isolation of each plot from the others. Sand flies were monitored weekly by counting the catches of six CDC-like miniature light traps (Model 512, John W. Hock, Gainesville, FL, USA) from early May to early December (29 weeks).The traps were set in fixed places in each site in the open spaces between the vegetation at least 5 m from bushes and burrows and at least 20 m from each other.

Bait solutions for the experimental sites consisted of the following: ~95% juice of over-ripe nectarines (*Prunus persica* var. *nectarina*: Rosacea), 5% red wine (Cabernet, Tishbi Estate Winery, Binyamina, Israel) and to this solution 10% W/V brown sugar ('Nature Sugar' brown, Louis Dreyfus, Israel), 10% W/V of a mixture of slow release substances and preservatives (BaitStab™, Westham Ltd., Israel), 1.0% W/V oral insecticide (Boric Acid), and 0.04% w/v spinosad (Tracer®; Dow Agrosciences, Toronto, Ontario, Canada) was added. A similarly prepared solution with all components except insecticides but with the addition of 0.5% W/V orange food dye (Carmoisine E122, Stern, Natanya, Israel) was used for the control site. These solutions were ripened for 48 hrs, in covered buckets, outdoors in the sun with daily temperatures reaching up to ~30 ^{0}C. Two low risk insecticides were used in the experimental site recipe because in previous laboratory experiments, both toxins killed sand flies but spinosad killed more rapidly than boric acid while boric acid was more stable in the environment (unpublished data of the authors).

At each site, these solutions were sprayed as patches on a small proportion of the vegetation (~10%) using a 7-liter hand sprayer (Killaspray, Model 4005, Hozelock-ASL, Birmingham, England). This amounted to spraying about one third of every third thicket or bush. Experimental area I was sprayed with toxic solution on the first day of June, August and October. Experimental area II was sprayed in August and October while the control area was similarly sprayed (without insecticide) in June, August, and October. For the observation of food dye in the abdomen, sand flies were immersed in a saline solution with a few drops of detergent and were examined under a dissection microscope.

2.3. Statistical Analysis

Statistical analysis was carried out using the GraphPad Prism 5.0 (GraphPad Software Inc., La Jolla, CA, USA) statistical package. The numbers of female and male sand flies caught in the control site versus the two treated sites were analyzed using the unpaired one-tailed Student's t-test. Significance was taken at $P < 0.05$.

3. Results

The control site population had two peaks between the initial spring growth and the decline towards the winter (Figure 1). The first peak occurred at the beginning of July (week 9) when the average number of sand flies reached 68.3 ± 10.9 females and 70.17 ± 9.82 males per trap. The second peak occurred at the beginning of October (week 22) when the average reached 61.8 ± 15.5 females and 40.33 ± 8.21 males per trap. Between the peaks, the lowest catches occurred in August (week 16; 21.3 ± 4.85 females and 21.5 ± 6.61 males per trap). Thus, quantitative evaluation of the effect of ATSB compared to the control depends on the time of season. After spraying at the control site, the food dye marked 69.6% females and 75.4% of the males on the first night. The average of the whole experimental period was similar, 65.5% marked females and 70.3% males.

The beginning of the sand fly season is in April/May and at the first site, where three treatments were applied, their number had increased to 31.83 ± 5.952 males, 47.66 ± 9.524 females per trap at the time of the first toxin treatment (week 5). After a gradual decline in June (week 5 onwards), it dropped to an average of 0.837 ± 0.148

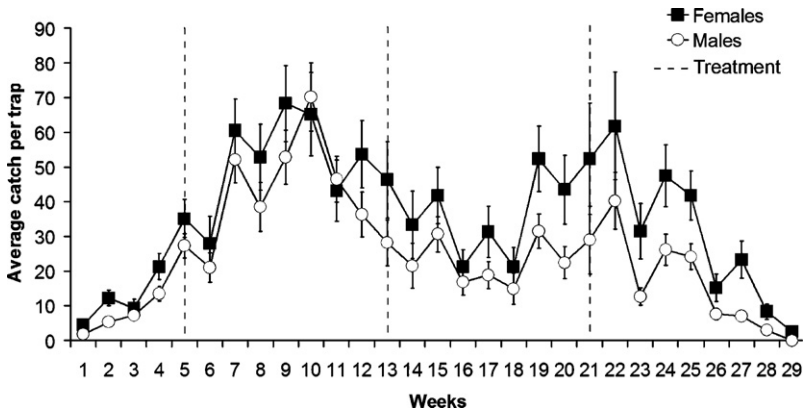

Figure 1. *Phlebotomus papatasi* average catch per trap during the sand fly season in the control site. Attractive sugar bait with food dye but without toxin was sprayed three times on ~10% of the vegetation. Monitoring began on 1 May (week 1) and concluded on 2 December (week 29). Treatments with control food-dye solution occurred on 1 June (week 5), 1 August (week 13) and 1 October (week 21).

males and 1.92 ± 0.430 females per trap in the remaining six months (Figure 2). Compared to the control site, the number of females and the number of males were both significantly reduced after the first ATSB treatment ($t = 6.987$, $df = 56$, $P < 0.05$ for females; $t = 6.085$, $df = 56$, $P < 0.05$ for males).

At the second experimental site, the treatment was given in mid-season and again, during about a month (weeks 13–16), there was a decrease from a daily average of 41.0 ± 9.153 males and 70.66 ± 13.72 females per trap. This decrease persisted to give an average of 1.89 ± 0.787 males and 3.57 ± 1.208 females per trap four months after the initial decrease (Figure 3). Compared to the control site, the number of females and males were significantly reduced after the initial ATSB treatment ($t = 1.969$, $df = 56$, $P < 0.05$ for females; $t = 1.716$, $df = 56$, $P < 0.05$ for males).

4. Discussion

At the control site, the population peaked twice during the season, at the beginning of July (week 9) and again at the beginning of October (week 22). This bi-modal population density pattern is characteristic of *Ph. papatasi* in arid Middle Eastern habitats especially in Israel[4,32,33] and has to be taken into account when determining the best application schedule of ATSB. By using a dye marker in the non-toxic bait solution applied to the control site, we demonstrated that a high proportion of the local sand fly population fed on the bait. The observed marking rates for females (69.6%) and males (75.4%), however, represent only minimal rates of contact as the dye marker persists for only about two days due to digestion processes while the sand flies can sugar-feed throughout their lifespan, which can last up to several weeks under laboratory conditions (unpublished data of the authors).

In our trials, we wanted to verify that we were applying ATSB in locations with large sand fly populations and this is why we started relatively late with the ATSB application after the sand fly population had already built up. The effect of the ATSB was manifested gradually in both experiments (Figure 2, 3). With the first treatment in early June at site I (week 5), the population needed four weeks for a reduction of 95.2%, while at site II, with the first treatment in August (week 13), the population needed six weeks for a similar reduction of 92.3%. The observed delay in population reduction at both sites might have been due to the fact that larval development is slow and uneven. Therefore, the emergence of young adults that belonged to the same batch of eggs may

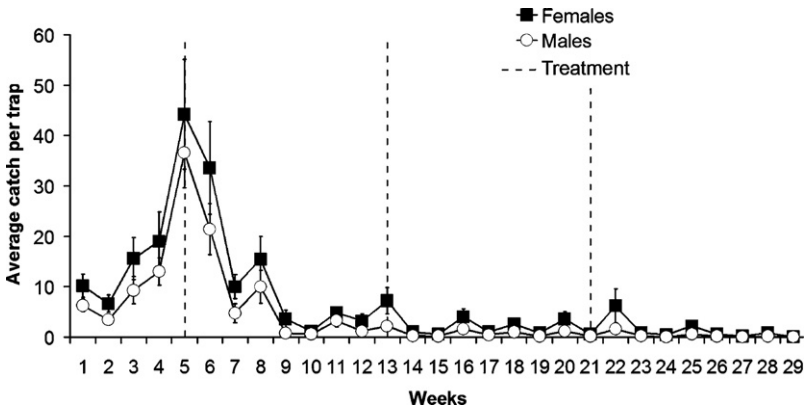

Figure 2. *Phlebotomus papatasi* average catch per trap during the sand fly season in Experimental Site I. Attractive toxic sugar bait was sprayed three times on ~10% of the vegetation. Treatments occurred on 1 June (week 5), 1 August (week 13) and 1 October (week 21).

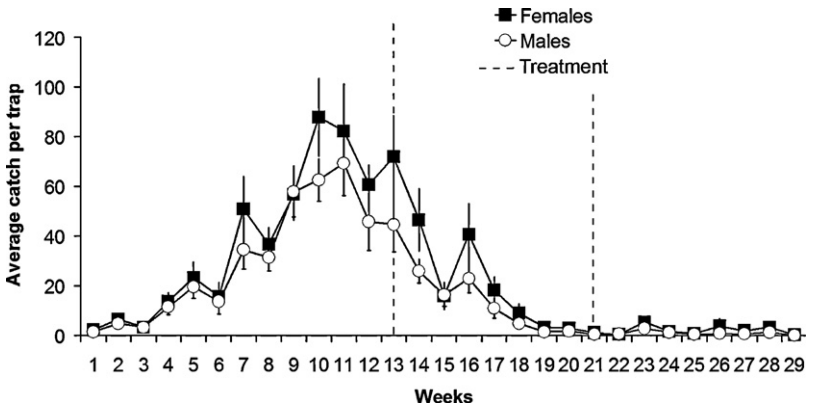

Figure 3. *Phlebotomus papatasi* average catch per trap during the sand fly season in Experimental Site II. Attractive toxic sugar bait was sprayed twice on ~10% of the vegetation. Treatments occurred on 1 August (week 13) and 1 October (week 21).

extend over a long period.[34] Even under optimal insectary conditions in our experience the developmental time for the bulk of the flies is at least one month. This developmental cycle apparently also occurs in the field. In the ATSB treated area, the continuous emergence of adults probably replenished the population for quite a while after exposure of the present adults to the toxin. This assumption is supported by previous observations[35] in the same habitats in the Jordan Valley, in which *Ph. papatasi* lived for six to eight days and 48.6%[35] to 85% of the populations were young flies only one or two days old.[36–38] The intensive emergence of young flies, combined with a long developmental period, suggests that it is difficult to achieve massive control in a short time. We presume that this is the reason for the slow decline of the sand fly population after application of ATSB.

Phlebotomine sand flies are susceptible to insecticides[2–4] provided they come into contact with them, and in the past, some projects achieved effective sand fly control especially if DDT[4,7–10] was used. Nevertheless, the US army studied a variety of pesticides in Iraq that had been applied using several different methods.[39,40] All treatments had only a minimal impact on sand fly abundance and there was never a dramatic reduction in abundance after a pesticide application. Different personal protection methods were also ineffective for various reasons, such as non-compliance with instructions for proper use.[39,40] In general, contact insecticides have to be non-repellent and tangible in the normal course of sand fly movements and even then the sand fly–toxin contact may be insufficient. However, when sand flies are attracted to land on ATSB, their tarsal response to contact with the sugar leads to feeding[41,42] and thereby to ingestion of the oral toxin. In the current study, we chose to treat only around 10% of the vegetation at the sites. This was done to attract the sand flies to concentrated areas where they feed on toxic bait and are killed. This is advantageous because the sand flies do not need to land on, or be sprayed with, a specific contact-insecticide to be killed.

Additionally, like other biting flies, sand flies are developing resistance to pesticides.[43,44] In addition to being highly effective, technologically simple and low-cost, the ATSB method is based on the use of oral toxins as opposed to contact insecticides used in insecticide residual spraying (IRS) or insecticide treated nets (ITNs). As such, this new approach circumvents many of the traditional problems relating to excito-repellency and the development of insecticide resistance in mosquitoes.[45,46] Longer-term, we foresee operational strategies using ATSB solutions with mixtures of two or more different low risk insecticides to minimize the emergence of resistance in local sand fly populations, which is, of course, already a concern for the insecticides associated with ITN and IRS use for malaria vector control in Africa.[45-48]

Physical measures to control sand flies are also problematic. Measures suitable for urban areas include destruction of breeding sites by removing garbage and debris near houses. In rural areas, environmental modification on a large scale involves the eradication of rodent reservoirs, destruction of their burrow systems and spraying of herbicides to destroy the food plants of these rodents. These approaches have been employed to control cutaneous leishmaniasis in Asian countries of the former USSR[5] but obviously drastic methods that require periodic repetition are harmful to the environment and unsuitable for temporary settlements such as military camps. Also, eradication of the reservoirs does not necessarily reduce the sand fly population significantly since they are not the only breeding and resting sites.[39,40] Application of ATSB may help to overcome these problems.

The basic goal of this study was to demonstrate proof of the concept that sand flies can be controlled, like mosquitoes,[26–28] with ATSB and herein, we successfully demonstrated this concept. We also think that in further studies, our methods need adjustment for the control of sand flies in other types of habitats where suitable vegetation is not available. Our hypothesis is that it is possible to improve the bait and the application method, which was originally developed for mosquitoes, by using more durable attractant formulations with a longer range of effect, and oral insecticides that, in outdoor conditions, remain active for a long time after application.

In future studies, the sand fly populations that survive ATSB applications for several weeks should be checked for their age. We assume that there might be a similar phenomenon with sand flies, as previously observed with mosquitoes,[26-28] that sand flies caught shortly after ATSB

treatment, and as long as the bait is effective, will be mostly adults that are too young to carry and transmit *Leishmania* infections. We conclude that ATSB treatment for sand fly control should begin early in the season rather than at the first peak of sand fly populations in order to kill the maximum number of sand flies throughout the whole season.

Authors' contributions: GCM and YS were jointly responsible for the design and carrying out of the experiments. Most of the fieldwork was designed and conducted by GCM, whilst YS was responsible for the interpretation and presentation of the results. Both authors read, approved and revised the final manuscript. YS is guarantor of the paper.

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Conflicts of interest: None declared.

Ethical approval: Not required.

References

1. Lewis DJ. Phlebotomid sand flies. *Bull World Health Organ* 1971;**44**: 535–51.
2. Alexander B, Jaramillo C, Cadena H, Usma MC, Roa W, Travi BL. An attempt to control phlebotomine sandflies (Diptera: Psychodidae) by residual spraying with deltamethrin in a Colombian village. *Mem Inst Oswaldo Cruz* 1995;**90**:421–4.
3. Alexander B, Maroli M. Control of phlebotomine sandflies. *Med Vet Entomol* 2003;**17**:1–18.
4. Orshan L, Szekely D, Schnur H, Wilamowski A, Galer Y, Bitton S, et al. Attempt to control sand flies by insecticide-sprayed strips along the periphery of a village. *J Vector Ecol* 2006;**31**:114–8.
5. Vioukov VN. Control of transmission. In: Peters W, Killick-Kendrick R, editors. *The Leishmaniases in biology and medicine*, Vol. 2. London: Academic Press; 1987. p. 909–28.
6. Davies CR, Llanos-Cuentas EA, Campos P, Monge J, Leon E, Canales J. Spraying houses in the Peruvian Andes with lambdacyhalothrin protects residents against cutaneous leishmaniasis. *Trans R Soc Trop Med Hyg* 2000;**94**:631–6.
7. Hertig M, Fisher LR. Control of sandflies with DDT. *Bull US Army Med Dept* 1945;**88**:97.
8. Jacusiel F. Sandfly control with DDT residual spray. Field experiments in Palestine. *Bull Entomol Res* 1947;**38**:479–88.
9. Hertig M, Fairchild GB. The control of *Phlebotomus* in Peru with DDT. *Amer J Trop Med Hyg* 1948;**28**:207–30.
10. Hertig M. *Phlebotomus* and residual DDT in Greece and Italy. *Am J Trop Med Hyg* 1949;**29**:773–809.
11. Le Pont F, Padilla JM, Desjeux P, Richard A, Mouchet J. Impact de pulverisations de deltamethrine dans un foyer de leishmaniose de Bolivie. *Annal Soc Belge Med Trop* 1989;**69**:223–32.
12. Falcao AL, Falcao AR, Pinto CT, Gontijo CMF, Falqueto A. Effect of deltamethrin spraying on the sandfly populations in a focus of American cutaneous leishmaniasis. *Mem Inst Oswaldo Cruz* 1991;**86**:399–404.
13. Marcondes CB, Nascimento JA. Avaliacao da eficiencia de deltametrina (K-Othrine CE) no controle de *Lutzomyia longipalpis* (Diptera: Psychodidae), no municipio de Santa Rita, Paraiba, Brasil. *Rev Soc Brasil de Med Trop* 1993;**26**:15–8.
14. Alexander B. Sampling methods for phlebotomine sandflies (Diptera: Psychodidae). *Med Vet Entomol* 2000;**14**:109–22.
15. Rayburn H, Ashford R, Moshen M, Hewitt S, Rowland M. A randomized controlled trial of insecticide-treated bednets and chaddars or top sheets, and residual spraying of interior rooms for prevention of cutaneous leishmaniasis in Kabul, Afghanistan. *Trans R Soc Trop Med Hyg* 2000;**94**:361–6.
16. Ritmeijer K, Davies C, van Zorge R, Wang SJ, Schorscher J, Dongu'du SI, et al. Evaluation of a mass distribution programme for fine mesh impregnated bednets against visceral leishmaniasis in eastern Sudan. *Trop Med Int Health* 2007;**12**:404–14.
17. Esterre P, Chippaux JP, Lefait JF, Dedet JP. Evaluation d'un programme de lutte contre la leishmaniose cutanee dans un village forestier de Guyane francaise. *Bull World Health Organ* 1986;**64**:559–65.
18. Perich MJ, Hoch AL, Rizzo N, Rowton ED. Insecticide barrier spraying for the control of sandfly vectors of cutaneous leishmaniasis in rural Guatemala. *Am J Trop Med Hyg* 1995;**52**:485–8.
19. Schlein Y, Jacobson RL. Mortality of *Leishmania major* in *Phlebotomus papatasi* caused by plant feeding of the sand flies. *Am J Trop Med Hyg* 1994;**50**:20–7.
20. Schlein Y, Muller G. Assessment of plant tissue feeding by sand flies (Diptera: Psychodidae) and mosquitoes (Diptera: Culicidae). *J Med Entomol* 1995;**32**:882–7.
21. Moore JS, Kelly TB, Killick-Kendrick R, Killick-Kendrick M, Wallbanks KR, Molyneux DH. Honeydew sugars in wild caught *Phlebotomus ariasi* detected by high performance liquid chromatography (HPLC) and gas chromatography. *Med Vet Entomol* 1987;**1**:427–34.
22. MacVicker JAK, Moore JS, Molyneux DH, Maroli M. Honeydew sugars in wild caught Italian phlebotomine sandflies (Diptera:Psychodidae) as detected by high performance liquid chromatography. *Bull Entomol Res* 1990;**80**:339–44.
23. Wallbanks KR, Moore JS, Bennet LR, Soren R, Molyneux DH, Carlin JM, et al. Aphid derived sugars in the neotropical sand fly *Lutzomyia peruensis*. *Trop Med Parasit* 1991;**42**:60–2.
24. Müller G, Schlein Y. Nectar and honeydew feeding of *Phlebotomus papatasi* in a focus of *Leishmania major* in Neot–Hakikar oasis. *J Vector Ecol* 2004;**29**:154–8.
25. Schlein Y, Yuval B. Leishmaniasis in the Jordan Valley. IV. Attraction of *Phlebotomus papatasi* (Diptera: Psychodidae) to plants in the field. *J Med Entomol* 1987;**24**:87–90.
26. Müller GC, Kravchenko VD, Schlein Y. Decline of *Anopheles sergentii* and *Aedes caspius* populations following presentation of attractive, toxic (Spinosad), sugar bait stations in an oasis. *J Am Mosq Control Assoc* 2008;**24**:147–9.
27. Müller GC, Junnila A, Schlein Y. Effective control of adult *Culex pipiens* by spraying an attractive toxic sugar bait solution in the vegetation near larval developmental sites. *J Med Entomol* 2010;**47**:63–6.
28. Müller GC, Junnila A, Qualls W, Revay EE, Kline DL., Allan S, et al. Control of *Culex quinquefasciatus* in a storm drain system in Florida with attractive toxic sugar baits (ATSB). Forthcoming 2010.
29. Schlein Y, Warburg A, Schnur LF, Gunders AE. Leishmaniasis in the Jordan Valley II. Sand flies and transmission in the central endemic area. *Trans R Soc Trop Med Hyg* 1982;**76**:582–6.
30. Schlein Y, Warburg A, Schnur LF, Le Blancq SM, Gunders AE. Leishmaniasis in Israel: reservoir hosts, sand fly vectors and leishmanial strains in the Negev, Central Arava and along the Dead Sea. *Trans R Soc Trop Med Hyg* 1984;**78**:480–4.
31. Danin A. *Desert vegetation of Israel and Sinai*. Jerusalem: Cana Publishing; 1983.
32. Kravchenko V, Wasserburg G, Warburg A. Bionomics of phlebotomine sandflies in the Galilee focus of cutaneous leishmaniasis in northern Israel. *Med Vet Entomol* 2004;**1**:418–28.
33. Wasserberg G, Yarom I, Warburg A. Seasonal abundance patterns of the sandfly *Phlebotomus papatasi* in climatically distinct foci of cutaneous leishmaniasis in Israeli deserts. *Med Vet Entomol* 2003;**17**:452–6.
34. Schmidt ML. Labority culture of two *Phlebotomus* species, *P. papatasi* and *P. orientalis*. *Bull World Health Organ* 1964;**31**:577–8.
35. Schlein Y, Jacobson RL. Sugar meals and longevity of the sandfly *Phlebotomus papatasi* in an arid focus of *Leishmania major* in the Jordan Valley. *Med Vet Entomol* 1999;**13**:65–71.
36. Magnarelli LA, Modi GB, Tesh RB. Follicular development and parity in phlebotomine sand flies (Diptera: Psychodidae). *J Med Entomol* 1984;**21**:681–9.
37. Yuval B. Populations of *Phlebotomus papatasi* (Diptera:Psychodidae) and the risk of *Leishmania major* transmission in three Jordan valley habitats. *J Med Entomol* 1991;**28**:492–5.
38. Yuval B, Warburg A, Schlein Y. Leishmaniasis in the Jordan Valley. V. Dispersal characteristics of the sand fly *Phlebotomus papatasi*. *Med Vet Entomol* 1988;**2**:391–5.
39. Coleman RE, Burkett DA, Putnam JL, Sherwood V, Caci JB, Jennings BT, et al. Impact of Phlebotomine Sand Flies on U.S. Military Operations at Tallil Air Base, Iraq: 1. Background, Military Situation, and Development of a "Leishmaniasis Control Program". *J Med Entomol* 2006;**43**:647–62.

40. Coleman RE, Burkett DA, Sherwood V, Caci J, Spradling S, Jennings BT, et al. Impact of Phlebotomine Sand Flies on U.S. Military Operations at Tallil Air Base, Iraq: 2. Temporal and Geographic Distribution of Sand Flies. *J Med Entomol* 2007;**44**:29–41.
41. Frings H, Hamrum CL. The contact chemoreceptors of adult yellow fever mosquitoes *Aedes ægypti*. *J New York Entomol Soc* 1950, **58:** 133–42.
42. Papas LG, Larsen JR. Gustatory mechanisms and sugar-feeding in the mosquito, *Culiseta inornata*. *Physiol Entomol* 1978;**3**:115–9.
43. Kaul S, Wattal B, Bhatnagar V, Mathur K. Preliminary observations on the susceptibility status of *Phlebolomus argentipes* and *P. papatasi* to DDT in two districts of North Bihar State (India). *J Commun Dis* 1978;**14**:208–11.
44. Tetreault GE, Zayed A, Hanfi AH, Beavers GM, Zeichner GM. Susceptibility of sand flies to selected insecticides in North Africa and the Middle East. *J Am Mosq Cont Assoc* 2001;**17**:23–7.
45. Corbel V, N'Guessan R, Brengues C, Chandre F, Djogbenou L, Martin T, et al. Multiple insecticide resistance mechanisms in *Anopheles gambiae* and *Culex quinquefasciatus* from Benin, West Africa. *Acta Trop* 2007;**101**:207–16.
46. Enayati A, Hemingway J. Malaria management: past, present, and future. *Annu Rev Entomol* 2010;**55**:569–91.
47. Read AF, Lynch PA, Thomas MB. How to make evolution-proof insecticides for malaria control. *PLoS Biol* 2009;**7**:e1000058.
48. Davidson G, Zahar AR. The practical implications of resistance of malaria vectors to insecticides. *Bull World Health Organ* 1973;**49**:475–83.