



Multi-modal Analysis of Courtship Behaviour in the Old World Leishmaniasis Vector *Phlebotomus argentipes*

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Abstract

Background: The sand fly *Phlebotomus argentipes* is arguably the most important vector of leishmaniasis worldwide. As there is no vaccine against the parasites that cause leishmaniasis, disease prevention focuses on control of the insect vector. Understanding reproductive behaviour will be essential to controlling populations of *P. argentipes*, and developing new strategies for reducing leishmaniasis transmission. Through statistical analysis of male-female interactions, this study provides a detailed description of *P. argentipes* courtship, and behaviours critical to mating success are highlighted. The potential for a role of cuticular hydrocarbons in *P. argentipes* courtship is also investigated, by comparing chemicals extracted from the surface of male and female flies.

Principal Findings: *P. argentipes* courtship shared many similarities with that of both *Phlebotomus papatasi* and the New World leishmaniasis vector *Lutzomyia longipalpis*. Male wing-flapping while approaching the female during courtship predicted mating success, and touching between males and females was a common and frequent occurrence. Both sexes were able to reject a potential partner. Significant differences were found in the profile of chemicals extracted from the surface of males and females. Results of GC analysis indicate that female extracts contained a number of peaks with relatively short retention times not present in males. Extracts from males had higher peaks for chemicals with relatively long retention times.

Conclusions: The importance of male approach flapping suggests that production of audio signals through wing beating, or dispersal of sex pheromones, are important to mating in this species. Frequent touching as a means of communication, and the differences in the chemical profiles extracted from males and females, may also indicate a role for cuticular hydrocarbons in *P. argentipes* courtship. Comparing characteristics of successful and unsuccessful mates could aid in identifying the modality of signals involved in *P. argentipes* courtship, and their potential for use in developing new strategies for vector control.

Citation: Bray DP, Yaman K, Underhill BA, Mitchell F, Carter V, et al. (2014) Multi-modal Analysis of Courtship Behaviour in the Old World Leishmaniasis Vector *Phlebotomus argentipes*. PLoS Negl Trop Dis 8(12): e3316. doi:10.1371/journal.pntd.0003316

Editor: Paulo Filemon Pimenta, Fundação Oswaldo Cruz, Brazil

Received: July 25, 2014; **Accepted:** October 3, 2014; **Published:** December 4, 2014

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Data Availability: The authors confirm that all data underlying the findings are fully available without restriction. All relevant data are within the paper and its Supporting Information files.

Funding: DPB, JGCH, VC and BAU were supported by the Wellcome Trust (grant 091689); FM and the GC/MS facility were supported by the Wellcome Trust (grant 089342); <http://www.wellcome.ac.uk>. KY was supported by a Malaysian Government PhD studentship. The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

Competing Interests: The authors have declared that no competing interests exist.

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Introduction

Visceral leishmaniasis (VL) is a debilitating disease estimated to cause 20,000–40,000 deaths worldwide each year [1]. The Indian subcontinent is one of the areas most affected by VL, with over 140,000 cases per year estimated to occur in India alone [1]. The etiologic agent in this region is the protozoan parasite *Leishmania donovani* (Kinetoplastida: Trypanosomatidae), with the sand fly *Phlebotomus argentipes* (Diptera: Psychodidae) the proven or suspected vector in Bangladesh, India, Nepal and Sri Lanka [2]. As there is no vaccine against VL, and cost and drug resistance limit effectiveness of treatment in India [3], control of the sand fly vector remains a priority for reducing transmission [4]. To be

successful these programmes require a thorough understanding of the behaviour of the insect vector [5], not least because many human activities can significantly alter sand fly behaviour and potential risk of transmission. Agricultural practices, for example, may lead to creation of new habitats for sand flies [6]. Insecticide spraying for control can lead to unintentional diversion of sand flies away from normal resting sites in animal houses, potentially increasing the biting risk to humans [7,8].

Studies of insect vector mating behaviour facilitate development of novel tools for control. For example, a new approach for controlling the South American vector of VL, *Lutzomyia longipalpis*, exploits attraction to male-produced sex pheromones. A synthetic version of this chemical attracts both females and males