SANDFLY PHEROMONES
THEIR BIOLOGY AND POTENTIAL FOR USE IN CONTROL PROGRAMS

HAMILTON J.G.C.*

Summary:

Lutzomyia longipalpis (Diptera: Psychodidae) is the vector of *Leishmania chagasi* the causative agent of visceral leishmaniasis (VL) in South and Central America, particularly Brazil, where the greatest incidence occurs. The disease is fatal if untreated. Although huge efforts have been made to control VL the incidence is increasing. Vector control remains an important element of disease control but residual spraying and other strategies have failed to make any lasting impact. Manipulation of sandfly chemical communication offers the opportunity to add new techniques and tools to reduce sandfly populations and thereby reduce Leishmania transmission. This paper reports the current understanding of several areas of sandfly chemical ecology and their prospects for application.

KEY WORDS : *Lutzomyia longipalpis*, visceral leishmaniasis, sex pheromone, 9-methylgermacrene-B, 3-methyl-a-himachalene, cembrene, control.

Sandflies (Diptera: Psychodidae) are found around the world in tropical, sub tropical and temperate zones. They are small, hairy insects the adults a few millimeters in length. Worldwide there are approximately 1,000 species of sandflies and these are divided into three major groups the most important in terms of numbers of species are the Genus *Lutzomyia* (New World sandflies) *Phlebotomus* (Old World sandflies) and *Sergentomyia* (found in both the Old and New World). Approximately 70 species are considered to be important as vectors of human disease.

Sandflies are obligatory haematophages; although both males and females take a sugar meal, the female must obtain a blood meal prior to successful oviposition. Sandflies are important to human health because they transmit different species of *Leishmania* (Protozoa: Trypanosomatidae) between animals and humans (and in some species from humans to humans). These parasites cause a wide spectrum of diseases, Leishmaniasis, which in the different visceral, cutaneous and mucocutaneous forms, can be fatal, disfiguring and debilitating if untreated. The WHO estimates that the global burden of disease expressed as DALYs (Disability Adjusted Life Years) is 2nd only to the disease burden of Malaria amongst insect transmitted diseases and estimates 50,000 deaths per annum worldwide (WHO, 2008). This burden is carried predominantly by the urban and rural poor. Only *Lutzomyia* and *Phlebotomus* are important vectors of Leishmaniases.

Leishmaniasis is also a significant veterinary problem in South and Central America, Europe as well as N. Africa and the Middle East. Dogs which become infected when bitten by infected sandflies can act as and may supplant local rodent populations as the reservoir of the disease (Lainson & Rangel, 2005)

Sandfly control is largely based on vector control although reservoir control strategies have also been followed. Both strategies have met with varying degrees of success but Leishmaniasis is a growing problem because of urbanization of VL, population disruption, the increase in peridomestic habitation and possibly changing climate patterns.

LUTZOMYIA LONGIPALPIS

Lutzomyia longipalpis (Lutz & Neiva, 1912) is the New World vector of *Leishmania infantum* and has received considerable attention because of its role as a vector. It is also the vector of cutaneous and atypical cutaneous leishmaniasis in Brazil, the rest of South America and Central America (Lainson & Rangel, 2005). In South America *L. longipalpis* has a wide but discontinuous distribution from Southern Mexico to Northern Argentina and inhabits a wide range of biotypes from the Andes to sea level (Young & Duncan, 1994) and from semi-arid to the lower Amazon basin (Lainson *et al.*, 1983) within the rural, peri-urban and urban environment. It feeds on a wide range of host domesticated animals including dogs, pigs, chickens, goats, cattle as well native animals including the presumed natural host *Cerdocyon thous*, the South American fox. Although it is considered to be a crepuscular feeder it is active through the night.

In Brazil *L. longipalpis* is widely distributed throughout the country however VL appears to be predominantly limited to the NE states of the country. This hetero-

* Centre for Applied Entomology and Parasitology, Institute for Science and Technology in Medicine, Keele University, Keele, Staffordshire, ST5 5BG, UK.
Tel: +44 (0)1782 583026 – Fax: +44 (0)1782 583417.
E-mail: j.g.c.hamilton@biol.keele.ac.uk

geneous pattern of disease distribution as well as phenotypic and genotypic variation within *L. longipalpis* have led some researchers to conclude that *L. longipalpis* in Brazil is a species complex (Mangabeira, 1969; Ward *et al.*, 1983; Conn & Mirabello, 2007; Maingon *et al.*, 2008). However it is unclear how many members of the complex there are (Bauzer *et al.*, 2007) although at least five have been suggested (Hamilton *et al.*, 2005; Bauzer *et al.*, 2007).

The chemical ecology of *L. longipalpis* has received particular attention because sex pheromones produced by males appear to be good markers of members of the species complex (Ward *et al.*, 1983; Hamilton *et al.*, 2005; Watts *et al.*, 2005) and because of the possibility that the sex pheromones may be used in more selective and efficient monitoring and/or control traps (Hamilton & Krishnakumari, 2004).

L. longipalpis chemical ecology

L. longipalpis use chemical cues in a range of functions *e.g.* host odour kairomones to locate blood meal source, oviposition site kairomones and pheromones to locate oviposition sites. However *L. longipalpis* and some other New World sandfly species appear to be unique amongst blood feeding insects in that males produce a sex pheromone.

Oviposition kairomones and pheromones

Female *L. longipalpis* deposit, dodecanoic acid, from their accessory gland, on freshly oviposited eggs, the pheromone is attractive to other gravid females (Dougherty *et al.*, 1994; Dougherty & Hamilton, 1997). In laboratory studies we found that gravid females were attracted by hexenal and 2-methyl-2-butanol isolated from rabbit faeces (Dougherty *et al.*, 1995) and that these environmental and pheromonal oviposition cues together were highly attractive (Dougherty *et al.*, 1993). Environmental oviposition cues together with the oviposition pheromone may help females to locate a suitable oviposition site (Dougerty *et al.*, 1993: McCall & Cameron, 1995). Other species of *Lutzomyia* also deposit fatty acids on their eggs and it is likely that these also act as oviposition pheromones (Alves *et al.*, 2003).

Host odour kairomones

Laboratory studies have demonstrated that female sandflies use host odour kairomones to locate a suitable host (Nigam & Ward, 1991; Oshaghi *et al.*, 1994; Dougherty *et al.*, 1999; Rebollar-Tellez *et al.*, 1999). It is also clear from both laboratory and field work that female sandflies are able to discriminate between a wide range of different types of hosts that may be available and females tend to feed on one host selected from many possible (Morrison *et al.*, 1995). Although this is partly because of the interaction of host odour components and the male sex pheromone (see below) it is likely also that choice is based on odour components present. Although it has been suggested that host size is an important feature in determining host attractiveness (Quinnell & Dye, 1994) this may be more related to the quality and quantity of host odour kairomones that are produced. Attraction to a host animal then is likely to be based on the balance between attractive and repellent elements in the animal's odour profile. In herds of Danish Holstein-Friesian cattle some are more attractive than others to *Haematobia irritans* (Hornfly) (Jensen *et al.*, 2004), amongst humans there is considerable variation in attractiveness to female *L. longipalpis* based solely on the odours produced (Hamilton & Ramsoondar, 1994) and the variation in individual human attractiveness to mosquitoes has been well documented (Qiu *et al.*, 2006).

Carbon dioxide, plays a significant role in host finding, it is a major component of host breath, and activates and attracts several mosquito species (Gillies, 1980; Takken, 1991; Gibson & Torr, 1999). It also synergises the attractiveness of other host odour components e.g. in *Aedes aegypti* (Eiras & Jepson, 1991, Dekker *et al.*, 2005).

The role of carbon dioxide in host-seeking behaviour of *L. longipalpis* is unclear. In field experiments in Panama and Brazil several species of sandfly were captured using carbon dioxide baited traps (Chaniotis, 1983; Pinto *et al.*, 2001). However in laboratory experiments, removing carbon dioxide from preparations of host compounds and sex pheromone did not significantly reduce their attractiveness (Nigam & Ward, 1991). More recently in laboratory bioassays we found that CO_2 (1 %) in air was attractive to females but its presence did not synergise the attractiveness of sex pheromone. The same study also showed that although males were not attracted to either CO_2 or sex pheromone by themselves the combination was attractive (Bray & Hamilton, unpublished). By comparison an earlier study using whole hamster odour indicated that females were synergistically attracted but males were not (Bray & Hamilton, 2007). These confounding results suggest that the interaction between host odour and sex pheromone is complex and likely to be depended on concentrations of the components of the odour.

L. longipalpis sex pheromone

Male *L. longipalpis* have large spots of lighter coloured cuticle on tergite 4 or tergites 3 and 4. These pale patches of cuticle, first observed by Mangabeira (1969), were subsequently found through chemical and behavioural analysis, to be the site of sex pheromone production (Lane & Ward, 1984; Lane *et al.*, 1985; Morton & Ward, 1989). The most abundant chemicals within these glandular areas have been shown to be responsible for the observed pheromonal activity (Hamilton *et al.*, 1994) and have been characterised as belonging to a class of natural products known as terpenes *i.e.* the molecules are constructed from three or four five carbon (C5) isoprene units. The sex pheromone of one member of the complex has been shown to be the novel C16 (3 × C5) monocyclic terpene (S)-9-methylgermacrene-B (Hamilton *et al.*, 1996a, 1999a) and another the novel C16 bicyclic terpene, 3 methyl-α-himachalene (Hamilton *et al.*, 1996b, 1999b). These two *L. longipalpis* methyl sesequiterpenes are unusual in that they have an additional – CH_3 (methyl) group, probably added in the early biosynthesis of the molecule (Hamilton *et al.*, 1999c). A further two members of the *L. longipalpis* species complex have different cembrene isomers i.e. they are C20 monocyclic diterpenes (4 × C5). The chemical structure of these cembrenes remains to be fully elucidated.

EXPLOITATION OF SANDFLY PHEROMONES

Oviposition pheromones and stimulants may offer potential for exploitation through monitoring or control traps however little interest has been shown in their development.

Male sex pheromones equally offer the possibility for the development of monitoring and control traps. Interestingly, because male pheromones attract females, potentially a significantly greater impact could be made on the fecundity of the population than if a female pheromone, attractive to males, was used. We have synthesized (S)-9-methylgermacrene-B, and its analogue (±)-9-methylgermacrene (Krishnakumari *et al.*, 2004). These can be synthesized from a readily available plant intermediate. The analogue is more stable than the natural sex pheromone and has similar activity in laboratory bioassays (Hamilton & Krishnakumari, unpublished). Our recent field studies in an emerging VL focus in Brazil have shown that synthetic sex pheromone is highly attractive to female *L. longipalpis*. This offers the possibility for the development of pheromone based strategies to exert a longterm negative pressure on population fecundity (Bray, Brandi, Brazil & Hamilton, unpublished).

We have also found in laboratory studies that although sex pheromone is attractive to female sandflies, sex pheromone + host odour is significantly more attractive. This strong synergistic interaction between host odour and male pheromone suggests that synthetic pheromone lures should be positioned near host animals until the attractive elements of host odour have been identified and incorporated into the sex pheromone lure.

REFERENCES

Alves J.C.M., Hamilton J.G.C. & Brazil R.P. Oviposition response of *Lutzomyia* (*Lutzomyia*) *renei* (Martins, Falcão & Silva) (Diptera: Psychodidae) to extracts of conspecific eggs in laboratory bioassays. *Entomotropica*, 2003, *18* (2), 121-126.

Bauzer L.G.S.R., Souza N.A., Maingon R.D. & Ward R.D. *Lutzomyia longipalpis* in Brazil: a complex or a single species? A mini-review. *Memorias do Instituto Oswaldo Cruz*, 2007, *102* (1), 1-12.

Bray D. & Hamilton J.G.C. Host odour synergises the attraction of virgin female *Lutzomyia longipalpis* (Diptera: Psychodidae) to male sex pheromone. *Journal of Medical Entomology*, 2007, *44* (5), 779-787.

Chaniotis B.N. Improved trapping of Phlebotomine sand flies (Diptera: Psychodidae) in light traps supplemented with dry ice in a Neotropical rain forest. *Journal of Medical Entomolgy*, 1983, *20*, 222-223.

Conn J.E. & Mirabello L. The biogeography and population genetics of neotropical vector species. *Heredity*, 2007, 1-12.

Dekker T., Geier M. & Cardé R.T. Carbon dioxide instantly sensitizes female yellow fever mosquitoes to human skin odors. *Journal of Experimental Biology*, 2005, 208, 2963-2972.

Dougherty M.J., Guerin P., Ward R.D. & Hamilton J.G.C. Behavioural and electrophysiological responses of the phlebotomine sandfly *Lutzomyia longipalpis* (Diptera: Psychodidae) when exposed to Canid host odour kairomones. *Physiological Entomology*, 1999, *24*, 251-262.

Dougherty M.J., Hamilton J.G.C. & Ward R.D. Semiochemical mediation of phlebotomine sandfly (*Lutzomyia longipalpis*) oviposition. *Medical and Veterinary Entomology*, 1993, *7*, 219-224.

Dougherty M.J., Hamilton J.G.C. & Ward R.D. Isolation of oviposition pheromone from the eggs of the sandfly *Lutzomyia longipalpis*. *Medical and Veterinary Entomology*, 1994, *8*, 119-124.

Dougherty M.J., Guerin P. & Ward R.D. Identification of oviposition attractants for the sandfly *Lutzomyia longipalpis* (Diptera: Psychodidae) present in volatiles of faeces from vertebrates. *Physiological Entomology*, 1995, *20*, 23-32.

Dougherty M.J. & Hamilton J.G.C. Dodecanoic acid is the oviposition pheromone of *Lutzomyia longipalpis*. *Journal of Chemical Ecology*, 1997, *23* (12), 2657-2671.

Eiras A.E. & Jepson P.C. Host location by *Aedes aegypti* (Diptera, Culicidae) – a wind-tunnel study of chemical cues. *Bulletin of Entomological Research*, 1991, *81*, 151-160.

Gibson G. & Torr S.J. Visual and olfactory responses of haematophagous Diptera to host stimuli. *Medical and Veterinary Entomology*, 1999, *13*, 2-23.

Gillies M.T. The role of carbon dioxide in host finding by mosquitoes (Diptera: Culicidae): a review. *Bulletin of Entomological Research*, 1980, *70*, 525-532.

Hamilton J.G.C. & Krishnakumari K. Sex pheromone analogue for controlling *Lutzomyia longipalpis*. *BRPI0500799*, 2004.

Hamilton J.G.C., Dawson G.W. & Pickett J.A. 9-Methylgermacrene-B, a novel homosesquiterpene from sex pheromone glands of *Lutzomyia longipalpis* (Diptera: Psychodidae) from Lapinha, Brazil. *Journal of Chemical Ecology*, 1996a, *22* (8), 1477-1491.

Hamilton J.G.C., Dawson G.W. & Pickett J.A. 3-Methyl-α-himachalene; sex pheromone of *Lutzomyia longipalpis* (Diptera: Psychodidae) from Jacobina, Brazil. *Journal of Chemical Ecology*, 1996b, *22* (12), 2331-2340.

Hamilton J.G.C., Dougherty M.J. & Ward R.D. Sex pheromone activity in a single component of tergal gland extract of *Lutzomyia longipalpis* (Diptera: Psychodidae) from Jacobina, Northeastern Brazil. *Journal of Chemical Ecology*, 1994, *20* (1), 141-151.

Hamilton J.G.C., Hooper A.M., Mori K., Pickett J.A., & Sano S. 3-Methyl-α-himachalene confirmed, and the relative stereochemistry defined, by synthesis as the sex pheromone of the sandfly *Lutzomyia longipalpis* from Jacobina, Brazil. *Chemical Communications*, 1999b, 355-356.

Hamilton J.G.C., Ibbotson H.C., Hooper A.M., Mori K., Pickett J.A. & Sano S. 9-Methylgermacrene-B confirmed by synthesis as the sex pheromone of the sandfly *Lutzomyia longipalpis* from Lapinha, Brazil, and the absolute stereochemistry defined as 9*S*. *Chemical Communications*, 1999a, 2335-2336.

Hamilton J.G.C., Maingon R.D.C., Alexander B., Ward R.D. & Brazil R.P. Analysis of the sex pheromone extract of individual male *Lutzomyia longipalpis* sandflies from six regions in Brazil. *Medical and Veterinary Entomology*, 2005, *19*, 480-488.

Hamilton J.G.C., Morgan E.D., Brazil R.P. & Alexander B. Chemical analysis of oxygenated homosesquiterpenes: a putative sex pheromone from *Lutzomyia lichyi* (Floch & Abonnenc) (Diptera: Psychodidae). *Bulletin of Entomological Research*, 1999c, *89*, 139-145.

Hamilton J.G.C. & Ramsoondar T.M.C. Attraction of *Lutzomyia longipalpis* to human skin odours. *Medical and Veterinary Entomology*, 1994, *8* (4), 375-380.

Jensen K.M.V., Jespersen J.B., Birkett M.A., Pickett J.A., Thomas G., Wadhams L.J. & Woodcock C.M. Variation in the load of the horn fly, *Haematobia irritans*, in cattle herds is determined by the presence or absence of individual heifers. *Medical and Veterinary Entomology*, 2004, *18*, 275-280

Krishnakumari B., Sarita Raj K. & Hamilton J.G.C. Synthesis of 9-methylgermacrene from germacrone, an active analogue of (S)-9-methylgermacrene-B, sex pheromone of Phlebotomine sandfly, *Lutzomyia longipalpis*, from Lapinha Brazil. P-183, IUPAC International conference on Biodiversity and Natural Products: Chemistry and Medical Applications, 26-31 January 2004, New Delhi (India).

Lainson R. & Rangel E.F. *Lutzomyia longipalpis* and the eco-epidemiology of American visceral leishmaniasis, with particular reference to Brazil: a review. *Memorias do Instituto Oswaldo Cruz*, 2005, *100*, 811-827.

Lainson R., Shaw J.J., Silveira F.T. & Fraiha H. Leishmaniasis in Brazil. XIX: visceral leishmaniasis in the Amazon region, and the presence of *Lutzomyia longipalpis* on the island of Marajó, Pará State. *Transaction of the Royal Society of Tropical Medicine and Hygiene*, 1983, *7*, 323-330.

Lane R.P. Sandflies (Phlebotominae), in: Medical insects and arachnids. Lane R.P. & Crosskey R.W. (eds). Chapman and Hall, London, 1993, 78-119.

Lane R.P. & Ward R.D. The morphology and possible function of abdominal patches in males of two forms of the leishmaniasis vector *Lutzomyia longipalpis* (Diptera: Phlebotominae). *Cahiers de l'ORSTOM, Serie Entomologie Medicale et Parasitologie*, 1984, *22*, 245-249.

Lane R., Phillips A., Molyneux D.H., Procter G. & Ward R.D. Chemical analysis of the abdominal glands of two forms of *Lutzomyia longipalpis*: site of a possible sex pheromone? *Annals of Tropical Medicine and Parasitology*, 1985, *79*, 225-229.

Maingon R.D.C., Ward R.D., Hamilton J.G.C., Bauzer L.G. & Peixoto A.A. The *Lutzomyia longipalpis* species complex: does population sub-structure matter to *Leishmania* transmission? *Trends in Parasitology*, 2008, *24* (1), 12-17.

Mangabeira O.F. Sóbre a sistemática e biologia dos *Phlebotomus* do Ceaió. *Revista Brasileiros Malariologica Doencias Tropical*, 1969, *12*, 3-26.

McCall P.J. & Cameron M.M. Oviposition pheromones in insect vectors. *Parasitology Today*, 1995, *11*, 315-356.

Morrison A.C., Ferro C., Pardo R., Torres M., Wilson M.L. & Tesh R.B. Nocturnal activity patterns of *Lutzomyia longipalpis* (Diptera, Psychodidae) at an endemic focus of visceral leishmaniasis in Colombia. *Journal of Medical Entomology*, 1995, *32*, 605-617.

Morton I.E. & Ward R.D. Laboratory response of female *Lutzomyia longipalpis* sandflies to a host and male pheromone source over distance. *Medical and Veterinary Entomology*, 1989, *3*, 219-223.

Nigam Y. & Ward R.D. The effects of male sandfly pheromone and host factors as attractants for female *Lutzomyia longipalpis*. *Physiological Entomology*, 1991, *16*, 305-312.

Oshaghi M., McCall P. & Ward R. Response of adult sandflies, *L. longipalpis* (Diptera:Psychodidae), to sticky traps baited with host odour and tested in the laboratory. *Annals of Tropical Medicine and Parasitology*, 1994, *88*, 439-444.

Pinto M. C., Campbell-Lendrum D.H., Lozovei A.L., Teodoro U. & Davies C.R. Phlebotomine sand fly responses to carbon dioxide and human odor in the field. *Medical and Veterinary Entomology*, 2001, *15*, 132-139.

Qiu Y., Smallegange R., Van Loon J., Ter Braak C. & Takken W. Interindividual variation in the attractiveness of human odours to the malaria mosquito *Anopheles gambiae* s. s. *Medical and Veterinary Entomology*, 2006, *20* (3), 280-287.

Quinnell R. J. & Dye C. An experimental study of the peridomestic distribution of *Lutzomyia longipalpis* (Diptera: Psychodidae). *Bulletin of Entomological Research*, 1994, *84*, 379-382.

Rebollar-Tellez E.A., Hamilton J.G.C. & Ward R.D. Response of female *Lutzomyia longipalpis* to host odour kairomones from human skin. *Physiological Entomology*, 1999, *24*, 220-226.

TAKKEN W. The role of olfaction in host-seeking of mosquitos – a review. *Insect Science and its Application*, 1991, *12*, 287-295.

WARD R.D., RIBEIRO A.L., READY P.R. & MURTAGH A. Reproductive isolation between different forms of *Lutzomyia longipalpis* (Lutz & Neiva) (Diptera: Psychodidae), the vector of *Leishmania donovani chagasi* Cunha & Chagas and its significance to kala-azar distribution in South America. *Memorias do Instituto Oswaldo Cruz*, 1983, *78*, 269-280.

WATTS P.C., HAMILTON J.G.C., WARD R.D., NOYES H.A., SOUZA N.A., KEMP S.J., FELICIANGELI D.M., BRAZIL R. & MAINGON R.D.C. Male sex pheromones and the phylogeographic structure of the L*utzomyia longipalpis* species complex (Diptera: Psychodidae) From Brazil and Venezuela. *American Journal of Tropical Medicine and Hygiene*, 2005, *73* (4), 734-743.

WORLD HEALTH ORGANISATION, 2008. http://www.who.int/leishmaniasis/burden/magnitude/burden_magnitude/en/index.html

YOUNG D.M. & DUNCAN M.A. Guide to the identification and geographic distribution of *Lutzomyia* sandflies in Mexico, Central and South America (Diptera: Psychodidae). Memoirs of the American Entomological Institute, 1994, No. 54, Assoc. Pub, Gainesville, Florida.