

Copulatory courtship song in *Lutzomyia migonei* (Diptera: Psychodidae)

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Lutzomyia migonei is a vector of leishmaniasis with a wide distribution in South America, which could favour population differentiation and speciation. Cryptic species of the *Lutzomyia longipalpis* complex, the widely distributed sand fly vector of visceral leishmaniasis in Latin America, have previously been shown to display distinct copulation songs. We found that *Lu. migonei* males also produce a song during copulation. This “lovesong” presents short trains (6–8 pulses) with an inter-pulse interval around 26 ms and is potentially involved in cryptic female choice and insemination success.

Key words: sand flies - lovesong - leishmaniasis

Lutzomyia migonei is a vector of American cutaneous leishmaniasis (Rangel & Lainson 2009) and it was recently suggested that *Lu. migonei* may also be a vector of American visceral leishmaniasis (de Carvalho et al. 2010, Salomon et al. 2010). This sand fly has a wide distribution in South America, being found from Argentina to Colombia across a variety of habitats (Rangel & Lainson 2003, 2009). The extensive distribution of this species may contribute to population differentiation and speciation due to geographical isolation and local adaptation, especially when in association with sexual selection (Ritchie 2007).

Lutzomyia longipalpis, a sand fly species that is also distributed over a large area in South and Central America, is a complex of sibling species (Ward et al. 1988, Lanzaro et al. 1993, Bauzer et al. 2002, 2007, Arrivillaga et al. 2003, Maingon et al. 2003, 2008). One important marker that has been used to differentiate the cryptic sibling species of this complex occurring in Brazil is the song produced by males during copulation (Souza et al. 2004, Araki et al. 2009). Differences in this copulatory courtship song probably contribute to the insemination failure observed in crosses between some Brazilian populations of this vector (Ward et al. 1983, 1988, Souza et al. 2008). Indeed, acoustic signals in insects are often involved in sexual selection and reproductive isolation of closely related species (Ewing 1989).

Copulation songs were also found in males of *Lutzomyia cruzi* (Vigoder et al. 2010) however, this species is very closely related to *Lu. longipalpis s.l.* and should

be considered a member of this complex (Vigoder et al. 2010). To the best of our knowledge, copulation songs have not been previously reported in other sand fly species. In the present paper we report that *Lu. migonei* males also produce a song during copulation.

Recordings were carried out using a Sony Hi8C-CD-TRV65 video camera and Sony SLV-77HFBR VCR, according to the procedure outlined by Souza et al. (2004). Briefly, a male and female virgin were placed inside the INSECTVOX (Gorczyca & Hall 1987) at 25°C ± 1°C for about 5 min. If no copulation occurred during this time, the couple was replaced for another one. Insects used in the present work were the F1 of females collected in Mesquita, state Rio de Janeiro, Brazil (22°46'S 43°25'W), an area of cutaneous leishmaniasis transmission (Meneses et al. 2002) and identified according to Young and Duncan (1994). A CED1401 A/D converter was used to digitalize the acoustic signals that were analyzed using Spike2 software (v4.08), both from Cambridge Electronic Design (UK). Five parameters were analyzed from the song of six different males: inter-pulse interval (IPI) and train length (TL), measured in milliseconds (ms), number of pulses per train (NP) and carrier frequency (FREQ), in hertz (Hz), and number of cycles per pulse (CPP).

We observed that *Lu. migonei* males also sing during copulation, similar to *Lu. longipalpis s.l.* (Souza et al. 2004, Araki et al. 2009). Figure 1A shows a segment of 2.5 s of the song of a *Lu. migonei* male with four trains and Figure 1B shows a single train in more detail. *Lu. migonei* males produce several short TL (153.44 ± 7.92 ms) composed of a small number of NP (6.95 ± 0.18) with a short IPI (25.87 ± 0.83 ms). These three song parameters make the copulation song pattern of *Lu. migonei* quite different from those observed in *Lu. longipalpis s.l.* (Souza et al. 2004, Araki et al. 2009). The song of *Lu. migonei* has also a low FREQ (280.87 ± 15.62 Hz) and has pulses about three CPPs (3.28 ± 0.12).

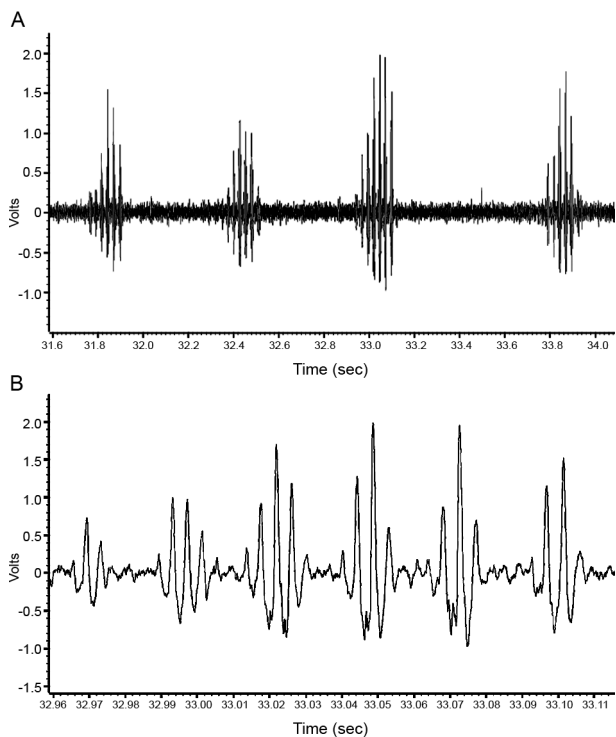
The *Lu. migonei* male song produced during copulation is probably part of courtship and potentially in-

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Lutzomyia migonei copulatory courtship song. A: segment of 2.5 sec of song with four trains; B: single train in more detail.

involved in cryptic female choice and reproductive isolation (Eberhard 1996). This has been shown in other insect species whose males also sing during copulation, such as *Drosophila birchii* and *Drosophila serrata* (Hoikkala & Crossley 2000, Hoikkala et al. 2000).

Lu. migonei and *Lu. longipalpis* are not very closely related (Mazzoni et al. 2002, Beati et al. 2004) and in fact some authors place the former species in a new genus, *Migonemyia*, proposed by Galati (1995). This suggests that copulatory courtship songs might be widespread in sand flies and could prove to be a powerful tool in studies aiming at disclosing cryptic species. In this respect, it would be interesting to record the song of males from populations of *Lu. migonei* from different geographical locations, including the areas where this species might be transmitting visceral leishmaniasis (Salomon et al. 2010), to see if there are differences that might suggest that this important vector is in fact a complex of cryptic species.

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