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SCIENTIFIC NOTE

FREQUENCY OF *WOLBACHIA* INFECTION IN LABORATORY AND FIELD SAND FLY (DIPTERA: PSYCHODIDAE) POPULATIONS

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ABSTRACT. Using a polymerase chain reaction (PCR) assay with primers designed for the *Wolbachia* 16S rRNA and outer surface protein (*wsp*) gene, we screened 11 laboratory colonies and 4 field samples of 6 sand fly species for *Wolbachia* infection. Infection was only detected in 3 laboratory colonies of *Phlebotomus papatasi* Scopoli, originally collected in Israel, Egypt, and Saudi Arabia.

KEY WORDS Sand fly, *Wolbachia*, laboratory colony, *Lutzomyia, Phlebotomus, Sergentomyia*

Wolbachia is a genus of alpha-proteobacteria living as intracellular symbionts in the reproductive tissues of a wide range of arthropods (insects, mites, and isopods). Recent surveys have found these bacteria in more than 16% of insect species (Werren 1997). In the hosts, *Wolbachia* infection causes a number of reproductive abnormalities such as cytoplasmic incompatibility between strains, parthenogenesis, and feminization (Werren 1997). Because *Wolbachia* infection confers a reproductive advantage in infected females, these bacterial endosymbionts are likely to spread rapidly in a population. As a result, *Wolbachia* could become a practical tool for introducing and spreading genes that limit the vectorial capacity of wild insect populations (Beard et al. 1993, Curtis and Sinkins 1998).

Here we report the result of screening for *Wolbachia* infection in 11 laboratory colonies and 4 field samples of sand flies, vectors of human and animal leishmaniasis. The 11 laboratory colonies, *Phlebotomus papatasi* Scopoli (Israel), *P. papatasi* (Saudi Arabia), *P. papatasi* (Jordan), *P. papatasi* (North Sinai, Egypt), *P. sergenti* Parrot (Jordan), *P. sergenti* (South Sinai, Egypt), *P. argentipes* Annandale and Brunetti (India), *P. dubosqi* Neveu-Lemaire (Kenya), *Lutzomyia longipalpis* Lutz and Neiva (Jacofena, Brazil), *L. longipalpis* (La Pena, Brazil), and *Sergentomyia schwetzi* Alder, Theodor and Parrot (Kenya) were established from field-collected adult sand flies and reared in the laboratory (Lawyer et al. 1991). Four samples of *L. longipalpis* (Honduras) were collected in 1996 from island, coast, plains, and mountains in Honduras. Ten female and 10 male sand fly adults from each colony or field sample were pooled for DNA extraction (Cui and Webb 1997). One microgram of the DNA was used for 35 cycles of polymerase chain reaction (PCR) amplifications (94°C for 30 sec, 55°C for 30 sec, and 72°C for 1 min) with 2 primers designed for *Wolbachia pipientis* Hertig 16S rRNA gene (5′-TTGTAGCCTGCTATGGTATAACT-3′ and 5′-GAATAGGTATGATTTTCATGT-3′; O'Neill et al. 1992) and 2 primers for the *wsp* gene (81F: 5′-TGGTCCAATAAGTGATGAAGAAAC-3′ and 691R: 5′-AAAAATTAAACGCTACTCCA-3′; Zhou et al. 1998). With the 16S rRNA primers, a product of ≈0.9 kilobase pairs (kbp) was amplified only from 3 laboratory colonies of *P. papatasi*, originally collected from Israel, Egypt, and Saudi Arabia (Table 1). The recently established colony of *P. papatasi*, collected from Jordan, did not harbor *Wolbachia*. Because the sequences of the *wsp* gene from different strains of *Wolbachia* have a high level of variability and are useful for *Wolbachia* strain typing (Braig et al. 1998), we further tested if these *Wolbachia* strains are different in the size of the PCR product with the *wsp* gene primers. Using the *wsp* gene primers, a DNA fragment of 590–632 bp was amplified, depending on the *Wolbachia* strains (Zhou et al. 1998). Our result with the *wsp* primers confirmed the detection of *Wolbachia* in

Table 1. Presence of *Wolbachia* infection in sand flies.

Species (location)	Approximate time in colony (years)	Presence of *Wolbachia* infection
Lutzomyia longipalpis (Jacofena Brazil)	16	–
L. longipalpis (Brazil, La Pena Cave)	10	–
L. longipalpis (Honduras)[1]	—	–
Phlebotomus argentipes (India)	5	–
P. dubosqi (Kenya)	?	–
P. sergenti (Jordan)	1	–
P. sergenti (South Sinai, Egypt)	2	–
P. papatasi (Israel)	>20	+
P. papatasi (Jordan)	1	–
P. papatasi (Saudi Arabia)	5	+
P. papatasi (North Sinai, Egypt)	>5	+
Sergentomyia schwetzi (Kenya)	>5	–

[1] *Lutzomyia longipalpis* (Honduras) was collected at 4 locations in Honduras.

the 3 *P. papatasi* colonies. A product of ≈0.6 kbp was amplified from the 3 *P. papatasi* colonies; no apparent size difference in PCR product was observed (data not shown). With the same *wsp* primers, Zhou et al. (1998) also detected the *Wolbachia* symbiont in a laboratory colony of *P. papatasi* (Israel). The absence of *Wolbachia* infection in the other 9 groups of sand flies suggests that this symbiont is probably uncommon in sand flies. On the one hand, the scarcity of *Wolbachia* infection in wild sand fly populations suggests that an artificial means of dissemination would be necessary to use *Wolbachia* as a carrier of genes that limit vectorial capacity. On the other hand, the absence of *Wolbachia* in sand fly populations would relieve such a management scheme from the need to replace current symbiotic infections with new ones.

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