Wolbachia is an obligate intracellular α-proteobacterium associated with arthropods and nematodes (O’Neill et al. 1997; Bazzochi et al. 2000). Wolbachia is transovarially transmitted by females to their progeny, and infections often are associated with reproductive anomalies in their host (O’Neill et al. 1997). In parasitoids, Wolbachia can cause cytoplasmic incompatibility (Stouthamer et al. 1999), thelytoky (parthenogenesis) (Stouthamer et al. 1990), and alter aspects of fecundity (Grenier et al. 2002).

In Florida, colonies of the thelytokous endoparasitoid Diaphorencyrtus aligarhensis (Hymenoptera: Encyrtidae) (Shafee, Alam and Agarwal) and the arrenhotokous ectoparasitoid Tamarixia radiata Waterston (Hymenoptera: Eulophidae) were imported from Taiwan and Vietnam, respectively, and released in a classical biological control program against the Asian citrus psyllid, Diaphorina citri Kuwayama (Hemiptera: Psyllidae) (Hoy & Nguyen 2000). Morphological differences were observed between female and male D. aligarhensis (Fig. 1). The male abdomen was small and all black, but the female abdomen was larger and was yellowish and black (Fig. 1A, D). Both the geniculate antennae (Fig. 1B, E) and genitalia (Fig. 1C, F) of female and male D. aligarhensis were structurally distinguishable. The antennae of male D. aligarhensis in an arrhenotokous population from Asia (Shafee et al. 1975) were similar to those observed in male D. aligarhensis produced here.

Molecular analyses were used to determine if Wolbachia was eliminated from male D. aligarhensis. DNA was isolated from each of 3 individual females and males D. aligarhensis with PUREGENE reagents (Gentra Systems, Minneapolis, MN) according to the manufacturer’s protocol. A 25-μL high-fidelity polymerase chain reaction (PCR) was conducted according to Hoy et al. (2001) to detect the wsp gene of Wolbachia with the primers wsp 81F (5’-TGGTCCTAAATGTAAGAG AAAC-3’) and wsp 691R (5’-AAAATTAACCGACTTCCA-3’) (Braig et al. 1998). For a DNA template control, the mitochondrial cytochrome c oxidase I gene (COI) was amplified with the primers CI-J1632.
(5'-TGATCAAATTTATAAT-3') and Cl-N-2191 (5'-GGTAAAATTAAAATATAAACTTC-3') (Kambhampati & Smith 1995). PCR amplification products were analyzed, purified, cloned, and sequenced according to Hoy & Jeyaprakash (2005).

The \textit{wsp} gene was PCR-amplified from all female \textit{D. aligarhensis} \((n = 3)\) but not from any males \((n = 3)\) (Fig. 2). The mitochondrial COI gene was amplified from all samples indicating that the DNA was adequate for the PCR (Fig. 2). No amplification products were detected in the negative control for both the \textit{wsp} and COI genes.

PCR products from the COI gene of both female and male \textit{D. aligarhensis} were cloned and sequenced, and the resulting 552-bp sequences were 100% identical (GenBank accession EF431956). This indicated that the males were the same species as the female \textit{D. aligarhensis} treated with tetracycline and that another parasitoid had not unexpectedly invaded the laboratory colony.

Fig. 1. Adult female compared to a male \textit{D. aligarhensis} produced by tetracycline treatment. (A) Adult female; (B) Antenna: adult female; (C) Terminal abdominal segment: adult female (ventral view); (D) Adult male; (E) Antenna: adult male; (F) Terminal abdominal segment: adult male (dorsal view). Scale: (A, D) 0.25 mm; (B, C, E, F) 0.1 mm.

The morphological and molecular data support our hypothesis that \textit{Wolbachia} causes thelytoky in our laboratory colony of \textit{D. aligarhensis}, the first report of this phenomenon in this genus. Male production also has been documented following elimination of \textit{Wolbachia} from other thelytokous parasitoids in the families Encyrtidae (Pijs et al. 1996), Trichogrammitidae (Stouthamer et al. 1990), Scelionidae (Arakaki et al. 2000), Eulophidae (Argov et al. 2000), and Aphelinidae (De Barro & Hart 2001). It is possible that the titer of \textit{Wolbachia} in male \textit{D. aligarhensis} was below the sensitivity of the high-fidelity PCR assay, which detects as few as 100 copies of the target template 100% of the time and as few as 10 copies 50% of the time (Hoy et al. 2001), but, if so, this titer reduction could still result in male production. No bacterial symbionts other than \textit{Wolbachia} were detected in a molecular survey of the imported population of \textit{D. aligarhensis} (Meyer 2007). Although unlikely, it cannot be excluded that uni-
dentified microbial species in D. aligarhensis also influence thelytokous reproduction. Male and female D. aligarhensis exhibited mating behavior when they were held together in a 50-mL centrifuge tube (Meyer 2007). First the male faced the female, then moved behind and climbed on the female, and finally attempted to copulate by bending its abdomen to contact the female abdomen. Males attempted to mate with multiple females. Further studies are needed to determine if males produce viable sperm that are transferred to the female during mating, and if a Wolbachia-free bisexual line of D. aligarhensis can be produced.

The authors thank Jennifer Zaspel for assistance with photography. This research was funded by the Davies, Fischer and Eckes Endowment in biological control to M. A. Hoy.

SUMMARY

Wolbachia is associated with thelytokous reproduction in D. aligarhensis. Male D. aligarhensis were produced following antibiotic treatment of their mothers, indicating that Wolbachia is associated with thelytoky in this population. Wolbachia appears to be missing in males of D. aligarhensis produced by tetracycline treatment of their mothers, indicating that Wolbachia is associated with thelytoky in this population. Wolbachia appears to be missing in males of D. aligarhensis produced by tetracycline treatment of their mothers, indicating that Wolbachia is associated with thelytoky in this population. Wolbachia appears to be missing in males of D. aligarhensis produced by tetracycline treatment of their mothers, indicating that Wolbachia is associated with thelytoky in this population. Wolbachia appears to be missing in males of D. aligarhensis produced by tetracycline treatment of their mothers, indicating that Wolbachia is associated with thelytoky in this population. Wolbachia appears to be missing in males of D. aligarhensis produced by tetracycline treatment of their mothers, indicating that Wolbachia is associated with thelytoky in this population. Wolbachia appears to be missing in males of D. aligarhensis produced by tetracycline treatment of their mothers, indicating that Wolbachia is associated with thelytoky in this population. Wolbachia appears to be missing in males of D. aligarhensis produced by tetracycline treatment of their mothers, indicating that Wolbachia is associated with thelytoky in this population.

REFERENCES CITED

found in 76% of sixty-three arthropod species. Insect Mol. Biol. 9: 393-405.


