JOURNAL OF Evolutionary Biology

doi: 10.1111/jeb.12264

SHORT COMMUNICATION

The effect of *Wolbachia* on the lifetime reproductive success of its insect host in the field

M. SEGOLI*, R. STOUTHAMER†, C. M. STOUTHAMER*, P. RUGMAN-JONES†
& J. A. ROSENHEIM*

*Department of Entomology, University of California, Davis, CA, USA
†Department of Entomology, University of California, Riverside, CA, USA

Keywords:

Anagrus sophiae;
body size;
eggload;
Prokelesia;
reproductive success;
Wolbachia.

Abstract

Wolbachia is a widespread endosymbiont that induces dramatic manipulations of its host's reproduction. Although there has been substantial progress in the developing theory for *Wolbachia*–host interactions and in measuring the effects of *Wolbachia* on host fitness in the laboratory, there is a widely recognized need to quantify the effects of *Wolbachia* on the host fitness in the field. The wasp *Anagrus sophiae*, an egg parasitoid of planthoppers, carries a *Wolbachia* strain that induces parthenogenesis, but its effects on the fitness of its *Anagrus* host are unknown. We developed a method to estimate the realized lifetime reproductive success of female wasps by collecting them soon after they die naturally in the field, counting the number of eggs remaining in their ovaries and quantifying *Wolbachia* density in their body. We sampled from a highly infected *A. sophiae* population and found no evidence for *Wolbachia* virulence and possible evidence for positive effects of *Wolbachia* on realized reproductive success.

Introduction

Of all microbial symbionts thus far discovered, perhaps the most widespread is *Wolbachia*, which is estimated to be hosted by ~40% of all arthropod species (Zug & Hammerstein, 2012). *Wolbachia* is primarily transmitted from females to their offspring through the eggs. To enhance its transmission, *Wolbachia* is known to induce a variety of reproductive manipulations, including the induction of parthenogenesis (asexual reproduction), feminization and male killing (O'Neill *et al.*, 1997). Laboratory-based empirical studies testing the effect of *Wolbachia* on host fitness are numerous and show a wide range of costs (Calvitti *et al.*, 2010; Sarakatsanou *et al.*, 2011; Vasquez *et al.*, 2011; White *et al.*, 2011) and benefits (Dong *et al.*, 2007; Brownlie *et al.*, 2009; Frentiu *et al.*, 2010; Guruprasad *et al.*, 2011; Unckless & Jaenike, 2012). The direction and magnitude of the observed fitness effects have been shown to depend on environmental conditions such as host density, nutrition and temperature (Olsen *et al.*, 2001; Brownlie *et al.*, 2009; Gavotte *et al.*, 2010; McMeniman & O'Neill, 2010). This highlights a need to test the effects of *Wolbachia* infection under natural conditions. However, the effects of *Wolbachia* on their host's fitness have rarely been tested directly in the field (Wenseleers *et al.*, 2002; Bellini *et al.*, 2010). Additionally, most studies have tested the effect of *Wolbachia* on host fitness by utilizing 'experimental' populations in which *Wolbachia* is simply classified as present or absent. However, *Wolbachia* density has been shown to vary tremendously among individuals in natural populations, and this is likely to play an important role in determining fitness effects (Unckless *et al.*, 2009). The absence of good field measurements of fitness effects is an obstacle for our basic understanding of the evolutionary ecology of *Wolbachia*–host interactions and also for our ability to manipulate these associations for our own benefit [e.g. for biological control of disease (Iturbe-Ormaetxe *et al.*, 2011) or agricultural pests (Stouthamer, 1993)]. The goal of this study was to characterize the relationship between *Wolbachia* density and host fitness in a natural ecosystem using a new approach for quantifying realized reproductive success for a minute insect in the field.

Correspondence: Michal Segoli, Department of Entomology, University of California, 1 Shields Ave., Davis, CA 95616, USA.
Tel.: +1 530 7524481; fax: +1 530 7521537; e-mail: msegoli@ucdavis.edu

Materials and methods

Study organisms

We studied the parasitoid wasp *Anagrus sophiae* (body length < 1 mm) that parasitizes the eggs of the planthoppers *Prokelesia marginata* and *P. dolus*, major herbivores of *Spartina* spp. cordgrasses in salt marshes in North America (Denno *et al.*, 1987). *Anagrus sophiae* populations in Florida and along the Gulf Coast are sexual (i.e. arrhenotokous) and appear to be largely or entirely free of *Wolbachia*, whereas populations along the northern Atlantic and Pacific coasts are almost entirely female and are nearly fixed for *Wolbachia* infection. The inference that *Wolbachia* induce parthenogenesis in nearly all-female populations is reinforced by the observation of rare males that test negative for *Wolbachia*. Preliminary data collected in 2009 revealed substantial variation in the density of *Wolbachia* among individual females (M. Segoli, unpublished data). We exploited this variation to test the effect of *Wolbachia* on female reproductive success. We sampled parasitoids from the salt marshes fringing the Petaluma River in northern San Francisco Bay [GPS: 38.116315,-122.504581] during August–September 2010.

Reproductive success estimates

We estimated *potential reproductive success* by collecting *Spartina* leaves harbouring planthopper eggs from the field and placing them in emergence cages. Emerging females were dissected in a drop of water under a dissecting microscope to count the number of eggs in their ovaries. *Anagrus sophiae* females are pro-ovigenic, emerging with their full lifetime complement of eggs. Thus, initial eggload represents the maximal number of eggs a female can lay during its lifetime. The head and thorax of each female were retained for DNA extraction. In addition, we measured the length of the hind tibia of each female [a good estimate of body size in *Anagrus* spp. (Cronin & Strong, 1996)]. This was performed to characterize the relationship between parasitoid size and initial eggload, which was later used to estimate realized reproductive success (see below).

In a separate collection, we estimated *realized reproductive success* of females as their oviposition success. *Anagrus sophiae* forage on *Spartina* foliage for planthopper eggs and upon death fall out of the plant canopy. We captured these dead wasps on collecting trays, where they were retained using a system of baffles that prevented them from being blown out by the wind. Trays were left in the field for 24 h with the aid of a flotation system that prevented them from being inundated during high tides (Fig. 1). All parasitoids were returned to the laboratory, and females were dissected, as above, allowing us to estimate their realized lifetime reproductive success as their estimated initial eggload (based on the relationship between initial eggload and body size) minus their actual eggload at death. Similar methods have previously been used to quantify the egg loads of dead females from the field in three additional minute insects: a gall midge (Rosenheim *et al.*, 2008), a fig wasp (Dunn *et al.*, 2011) and a parasitoid wasp (Segoli & Rosenheim, in press).

Estimates of *Wolbachia* density

Wolbachia density within individual females was estimated using a TaqMan®-based multiplex quantitative PCR (qPCR) (Sigma-Genosys, The Woodlands, TX, USA) and the –dd*C*t method (Schmittgen & Livak, 2008), where –dd*C*t was calculated for each sample as the differ-

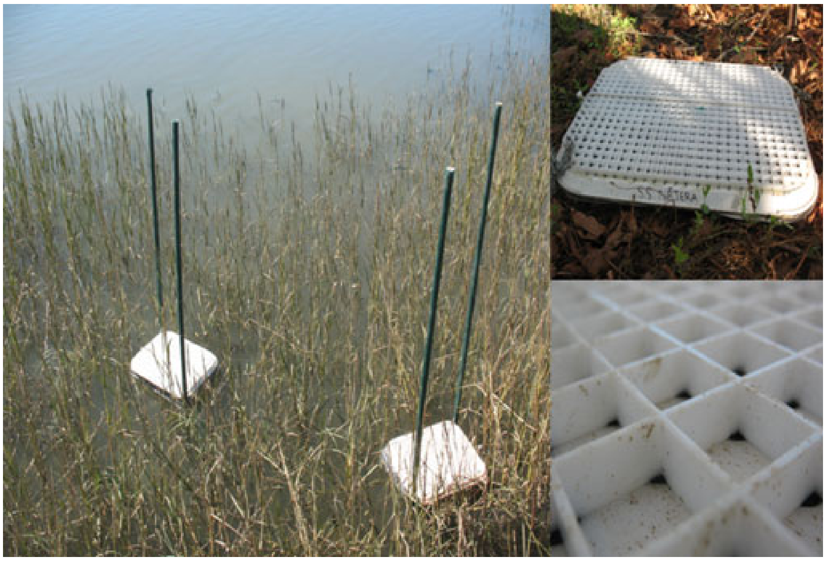

Fig. 1 Modified capture trays to collect dead *Anagrus* wasps upon their death. The trays have a floatation device to prevent them from being inundated during the high tides in the salt marshes.

ence between the *C*t (number of cycles required to reach a threshold) for the 16S gene of *Wolbachia* and the *C*t for the EF1α gene of the host, minus the same difference for a calibrating sample (extraction from 10 females). *Wolbachia* was detected in 94% of the females tested ($N = 77$). See appendix S1 for additional details.

Results

Potential reproductive success

In a multiple regression with *Wolbachia* relative density (–dd*C*t) and body size (tibia length) as independent factors and potential reproductive success (initial eggload) as a dependent variable, we found no effect of *Wolbachia* density (Fig. 2, N = 27, $R^2 = 0.28$, $F_{1,26} = 0.27$, $P = 0.11$) and a significant positive effect of body size on potential reproductive success ($F_{1,26} = 5.65$, $P = 0.03$).

Realized reproductive success

Eggloads of dead females collected by the capture trays ranged from 1 to 42. In a multiple regression with *Wolbachia* relative density (–dd*C*t) and *Anagrus* body size (tibia length) as independent factors and realized reproductive success (the estimated number of eggs laid by a female during its lifetime) as a dependent variable, we found a positive effect of *Wolbachia* density (Fig. 3, N = 37, $R^2 = 0.31$, $F_{1,36} = 11.71$, $P = 0.002$), but no effect of tibia length on female realized reproductive success ($F_{1,36} = 2.86$, $P = 0.10$).

Discussion

Using novel techniques, we were able to estimate potential and realized reproductive success for a minute parasitoid in the most direct way applied so far and to relate it to *Wolbachia* density within individual females. None of our fitness measures provides any support for the possibility that *Wolbachia* is virulent in this system. Instead, our observations provide possible evidence that *Wolbachia* is beneficial for its host in nature.

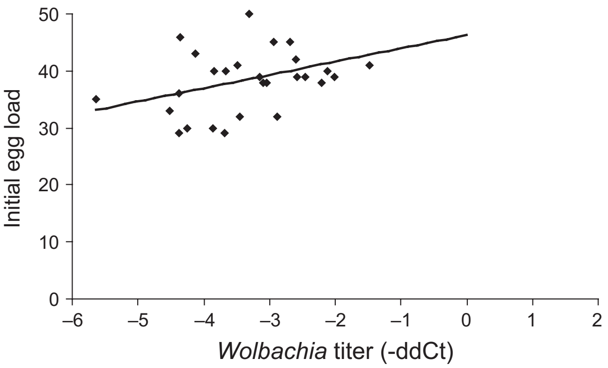

Fig. 2 Relationship between parasitoid potential fitness (initial eggload) and *Wolbachia* relative density (–dd*C*t).

We found no significant association between *Wolbachia* density and the total number of eggs matured by females during development. Increasing *Wolbachia* density was, however, significantly correlated with realized fitness, measured as the number of eggs laid over a female's full lifetime. The underlying cause for this association is currently unknown; *Wolbachia* might have increased the lifespan of females or their oviposition rate, as has been observed for other species in the laboratory (Dobson *et al.*, 2001; Fry & Rand, 2002; Dong *et al.*, 2007). Alternatively, *Wolbachia* could be associated with some other unidentified factor that increases reproductive success. Additional work is required to distinguish among these possibilities. This could be achieved by recording the longevity, mobility and oviposition rate of infected versus uninfected females in traditional laboratory experimentation, but will also require revalidation under natural or semi-natural conditions.

Although our regression analyses revealed significant positive relationships between realized reproductive success and *Wolbachia* density, the *R*-squared value was relatively low. This may be, in part, due to the weaknesses of our estimation methods. However, the high variation in our data set could also reflect the importance of additional factors in determining the oviposi-

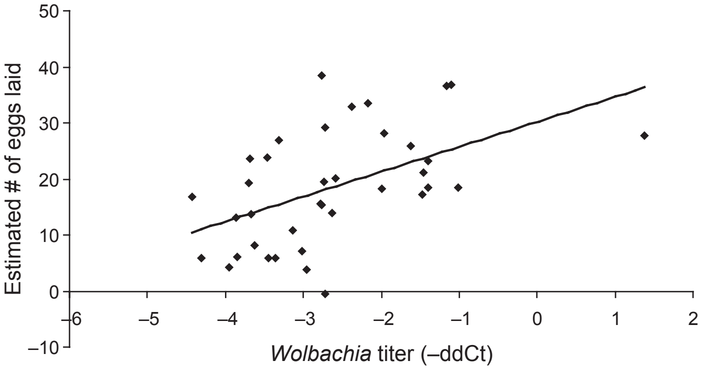

Fig. 3 Relationship between realized fitness (lifetime number of eggs laid) of *Anagrus sophiae* females and *Wolbachia* relative density (–dd*C*t).

tion success of parasitoids in the field. These may include spatial and temporal variation in environmental factors such as food availability, host availability and temperature (Ellers *et al.*, 2001; Lee & Heimpel, 2008). The salt marsh environment is particularly unpredictable, and its inhabitants may experience extreme temperatures, strong winds, high tides and patchy resources (Packham & Willis, 1997), all of which may have strong effects on minute, mobile and short-lived insects such as *A. sophiae*.

Our results are consistent with the theoretical prediction of reduced virulence in populations where *Wolbachia* infection has approached fixation (Stouthamer, 1997). Additional indirect support for this prediction comes from parasitoids in the genus *Trichogramma*, in which *Wolbachia* has been found to have negative effects in some species that have low infection frequencies, and where sexual reproduction therefore still occurs, and positive effects in species where the infection has progressed to fixation (Pintureau *et al.*, 2002). However, a crucial test of this hypothesis requires comparisons among different populations of the same species. The occurrence of both sexual and asexual *A. sophiae* populations along the salt marshes of the Atlantic and Gulf coasts suggests that such comparisons may be feasible in this species.

In conclusion, *Wolbachia* effects on its host are known to be highly dependent on environmental conditions, underscoring the need to quantify fitness effects under realistic natural conditions. Our results, and the general applicability of our approach, provide one step towards building our ability to test general predictions for the evolutionary ecology of *Wolbachia*–host interactions in nature.

Acknowledgments

We thank Cory Fernandez, Timothy Fong, Tobias Glik, Tierra Groff, Orly Oren, Michael Park, Moran Segoli, Ian Taff, Scott Richie and Lucinda Ye for technical support, fruitful discussions and comments on the manuscript. We thank Giselle Block, Chris Chamberlain, Brian Collins, Jim Cronin, Hanna Garland, David Kimbro, Leonard Liu, Jen McBroom and Brian Swedberg for aid in obtaining samples, permits and access to study sites. This study was partially funded by grants from the Israel Science Foundation (ISF), the Bi-national Agricultural Research and Development (BARD) and the United States National Science Foundation (NSF: DMS-1022639).

References

Bellini, R., Albieri, A., Balestrino, F., Carrieri, M., Porretta, D., Urbanelli, S. *et al.* 2010. Dispersal and survival of *Aedes albopictus* (Diptera: Culicidae) males in Italian urban areas and significance for sterile insect technique application. *J. Med. Entomol.* **47**: 1082–1091.

Brownlie, J.C., Cass, B.N., Riegler, M., Witsenburg, J.J., Iturbe-Ormaetxe, I., McGraw, E.A. *et al.* 2009. Evidence for metabolic provisioning by a common invertebrate endosymbiont, *Wolbachia pipientis*, during periods of nutritional stress. *PLoS Pathog.* **5**: e1000368.

Calvitti, M., Moretti, R., Lampazzi, E., Bellini, R. & Dobson, S.L. 2010. Characterization of a new *Aedes albopictus* (Diptera: Culicidae)-*Wolbachia pipientis* (Rickettsiales: Rickettsiaceae) symbiotic association generated by artificial transfer of the wPip Strain from *Culex pipiens* (Diptera: Culicidae). *J. Med. Entomol.* **47**: 179–187.

Cronin, J.T. & Strong, D.R. 1996. Genetics of oviposition success of a thelytokous fairyfly parasitoid, *Anagrus delicatus*. *Heredity* **76**: 43–54.

Denno, R.F., Schauff, M.E., Wilson, S.W. & Olmstead, K.L. 1987. Practical diagnosis and natural-history of 2 sibling salt marsh-inhabiting planthoppers in the genus *Prokelisia* (Homoptera, Delphacidae). *Proc. Entomol. Soc. Wash.* **89**: 687–700.

Dobson, S.L., Marsland, E.J. & Rattanadechakul, W. 2001. *Wolbachia*-induced cytoplasmic incompatibility in single-and superinfected *Aedes albopictus* (Diptera: Culicidae). *J. Med. Entomol.* **38**: 382–387.

Dong, P., Wang, J.J., Hu, F. & Jia, F.X. 2007. Influence of *Wolbachia* infection on the fitness of the stored-product pest *Liposcelis tricolor* (Psocoptera: Liposeelididae). *J. Econ. Entomol.* **100**: 1476–1481.

Dunn, D.W., Jansen-Gonzalez, S., Cook, J.M., Yu, D.W. & Pereira, R.A.S. 2011. Measuring the discrepancy between fecundity and lifetime reproductive success in a pollinating fig wasp. *Entomol. Exp. Appl.* **140**: 218–225.

Ellers, J., Bax, M. & van Alphen, J.J.M. 2001. Seasonal changes in female size and its relation to reproduction in the parasitoid *Asobara tabida*. *Oikos* **92**: 309–314.

Frentiu, F.D., Robinson, J., Young, P.R., McGraw, E.A. & O'Neill, S.L. 2010. *Wolbachia*-mediated resistance to Dengue virus infection and death at the cellular level. *PLoS ONE* **5**: e13398.

Fry, A.J. & Rand, D.M. 2002. *Wolbachia* interactions that determine *Drosophila melanogaster* survival. *Evolution* **56**: 1976–1981.

Gavotte, L., Mercer, D.R., Stoeckle, J.J. & Dobson, S.L. 2010. Costs and benefits of *Wolbachia* infection in immature *Aedes albopictus* depend upon sex and competition level. *J. Invertebr. Pathol.* **105**: 341–346.

Guruprasad, N.M., Mouton, L. & Puttaraju, H.P. 2011. Effect of *Wolbachia* infection and temperature variations on the fecundity of the Uzi fly *Exorista sorbillans* (Diptera: Tachinidae). *Symbiosis* **54**: 151–158.

Iturbe-Ormaetxe, I., Walker, T. & O' Neill, S.L. 2011. *Wolbachia* and the biological control of mosquito-borne disease. *EMBO Rep.* **12**: 508–518.

Lee, J.C. & Heimpel, G.E. 2008. Floral resources impact longevity and oviposition rate of a parasitoid in the field. *J. Anim. Ecol.* **77**: 565–572.

McMeniman, C.J. & O'Neill, S.L. 2010. A virulent *Wolbachia* infection decreases the viability of the Dengue vector *Aedes aegypti* during periods of embryonic quiescence. *PLoS Negl. Trop. Dis.* **4**: e748.

Olsen, K., Reynolds, K.T. & Hoffmann, A.A. 2001. A field cage test of the effects of the endosymbiont *Wolbachia* on *Drosophila melanogaster*. *Heredity* **86**: 731–737.

O'Neill, S.L., Hoffmann, A.A. & Werren, J.H. 1997. *Influential Passengers: Inherited Microorganisms and Arthropod Reproduction*. Oxford University Press, New York.

Packham, J.R. & Willis, A. 1997. *Ecology of Dunes, Salt Marsh, and Shingle*. Chapman & Hall, London.

Pintureau, B., Grenier, S., Heddi, A. & Charles, H. 2002. Biodiversity of *Wolbachia* and of their effects in *Trichogramma* (Hymenoptera: Trichogrammatidae). *Ann. Soc. Entomol. Fr.* **38**: 333–338.

Rosenheim, J.A., Jepsen, S.J., Matthews, C.E., Smith, D.S. & Rosenheim, M.R. 2008. Time limitation, egg limitation, the cost of oviposition, and lifetime reproduction by an insect in nature. *Am. Nat.* **172**: 486–496.

Sarakatsanou, A., Diamantidis, A.D., Papanastasiou, S.A., Bourtzis, K. & Papadopoulos, N.T. 2011. Effects of *Wolbachia* on fitness of the Mediterranean fruit fly (Diptera: Tephritidae). *J. Appl. Entomol.* **135**: 554–563.

Schmittgen, T.D. & Livak, K.J. 2008. Analyzing real-time PCR data by the comparative CT method. *Nat. Protoc.* **3**: 1101–1108.

Segoli, M. & Rosenheim, J.A. in press. Limits to the reproductive success of two insect parasitoid species in the field. *Ecology* doi: org/10.1890/13-0262.1.

Stouthamer, R. 1993. The use of sexual versus asexual wasps in biological-control. *Entomophaga* **38**: 3–6.

Stouthamer, R. 1997. *Wolbachia*-induced parthenogenesis. In: *Influential Passengers: Inherited Microorganisms and Arthropod Reproduction* (S.L. O'Neill, A.A. Hoffmann & J.H. Werren, eds), pp. 102–124. Oxford University Press, New York, NY, USA.

Unckless, R.L. & Jaenike, J. 2012. Maintenance of a Male-Killing *Wolbachia* in *Drosophila innubila* by Male-Killing dependent and Male-Killing independent mechanisms. *Evolution* **66**: 678–689.

Unckless, R.L., Boelio, L.M., Herren, J.K. & Jaenike, J. 2009. *Wolbachia* as populations within individual insects: causes and consequences of density variation in natural populations. *Proc. Biol. Sci.* **276**: 2805–2811.

Vasquez, C.J., Stouthamer, R., Jeong, G. & Morse, J.G. 2011. Discovery of a CI-inducing *Wolbachia* and its associated fitness costs in the biological control agent *Aphytis melinus* DeBach (Hymenoptera: Aphelinidae). *Biol. Control* **58**: 192–198.

Wenseleers, T., Sundstrom, L. & Billen, J. 2002. Deleterious *Wolbachia* in the ant *Formica truncorum*. *Proc. Biol. Sci.* **269**: 623–629.

White, J.A., Kelly, S.E., Cockburn, S.N., Perlman, S.J. & Hunter, M.S. 2011. Endosymbiont costs and benefits in a parasitoid infected with both *Wolbachia* and *Cardinium*. *Heredity* **106**: 585–591.

Zug, R. & Hammerstein, P. 2012. Still a host of hosts for *Wolbachia*: analysis of recent data suggests that 40% of terrestrial arthropod species are infected. *PLoS ONE* **7**: e38544.

Supporting information

Additional Supporting Information may be found in the online version of this article:
Appendix S1 Molecular methods.

Data deposited at Dryad: doi:10.5061/dryad.3t30n

Received 24 July 2013; revised 2 September 2013; accepted 7 September 2013