Journal of Invertebrate Pathology 111 (2012) 197–204

Contents lists available at SciVerse ScienceDirect

Journal of Invertebrate Pathology

journal homepage: www.elsevier.com/locate/jip

Wolbachia gonadal density in female and male *Drosophila* vary with laboratory adaptation and respond differently to physiological and environmental challenges

Claudia C. Correa [a], J. William O. Ballard [a,b,*]

[a] School of Biotechnology and Biomolecular Sciences, University of New South Wales, Sydney 2052, Australia
[b] Evolution and Ecology Research Centre, School of Biological, Earth and Environmental Sciences, University of New South Wales, Sydney 2052, Australia

ARTICLE INFO

Article history:
Received 29 May 2012
Accepted 9 August 2012
Available online 16 August 2012

Keywords:
Wolbachia density
Ovaries
Testes
qPCR

ABSTRACT

In symbiotic associations such as those between *Wolbachia* and insects, the within-host symbiont density plays an important role in the maintenance of the infection in natural populations, as it relates to transmission fidelity and pathogenicity of the symbiont. Within-host density is speculated to be the result of complex interactions between the bacterial genotype, the host genotype and the environment, which may account for the substantial variation in *Wolbachia* titres among wild collected individuals compared to laboratory lines. Using quantitative PCR, we screened the *Wolbachia* gonadal density of individuals from 50 isofemale *Drosophila simulans* lines raised in standard conditions for at least two generations after collection from the wild. Although these newly collected lines displayed significant variation of ovarian *Wolbachia* titres, such variation was lost by F_{19}. Assaying these flies at different ages and under different environmental conditions indicated that symbiont titres in female gonads were not affected by the conditions tested. However, *Wolbachia* density in male gonads was consistently affected by these treatments in a line-specific way. We propose that the differences in *Wolbachia* densities among ovaries of F_4 flies are the consequence of large differences in the field-collected females caused by the variable environment, and carried over for at least four generations. In addition, we provide evidence of sex-specific dynamics of *Wolbachia* in gonads of females and males. In combination, our results support the view of sex-specific *Wolbachia* evolutionary interactions for males and females, which has been predicted by theory and observed experimentally.

1. Introduction

Symbiotic associations between vertically transmitted microbes and arthropods are common (Hilgenboecker et al., 2008; Moran et al., 2008). The intracellular, maternally transmitted *Wolbachia* is perhaps the best studied of these symbiotic microbes, as it has the capacity to induce reproductive modifications in their hosts, such as cytoplasmic incompatibility (CI), male killing or parthenogenesis. Frequently, *Wolbachia* infection ultimately increases the fitness of infected females and the number of infected females in a population (Poinsot and Merçot, 1997; Turelli, 1994). The density of *Wolbachia* within the arthropod host has been recognised as a critical factor in these associations, as it affects both transmission fidelity and pathogenicity of the infection (McGraw et al., 2002). Several studies suggest that, apart from its role in transmission efficiency and virulence, the within-host density of *Wolbachia* also correlates positively with the strength of the reproductive modifications. Therefore, the density dynamics within individuals may govern the prevalence of infection in host populations (reviewed in Jaenike, 2009).

The regulation of *Wolbachia* density is hypothesised to be the result of highly complex interactions involving the host and symbiont genotypes, as well as the environment (Mouton et al., 2007). Previous studies have shown clear evidence of the participation of both host and symbiont genotypes in the regulation of the within-individual *Wolbachia* titres. For example, in multiply *Wolbachia* infected wasps, the specific density of each strain remains constant independently of the presence of others; suggesting regulatory mechanisms in the host are specific to the microbe strain (Mouton et al., 2003). Similarly, Ikeda and colleagues (2003) artificially exchanged naturally occurring *Wolbachia* strains between the moths *Ephestia kuehniella* and *Cadra cautella* and found that the host genetic background strongly influenced the proliferation of each *Wolbachia* strain. Environmental and host physiological factors have also been shown to influence symbiont titres as well as the strength of the reproductive modifications induced by *Wolbachia*. The effects of a variety of factors including age (Clark et al., 2002; Tortosa et al., 2010; Unckless et al., 2009), larval crowding (Wiwatanaratanabutr and Kittayapong, 2009), developmental and

* Corresponding author at: School of Biotechnology and Biomolecular Sciences, University of New South Wales, Sydney 2052, Australia. Fax: +61 2 9385 1483.
E-mail addresses: cccorreao@gmail.com (C.C. Correa), w.ballard@unsw.edu.au (J. William O. Ballard).

0022-2011/$ - see front matter © 2012 Elsevier Inc. All rights reserved.
http://dx.doi.org/10.1016/j.jip.2012.08.003

adult temperature (Bordenstein and Bordenstein, 2011; Mouton et al., 2006, 2007), dietary antibiotics (Clancy and Hoffmann, 1998) and the presence of insecticide resistance genes (Berticat et al., 2002) have been shown to impact *Wolbachia* titres in several host species. Although the causative mechanisms of such effects are not known, it is speculated that they affect the balance of the symbiotic relationship, perhaps by altering the within host availability of nutrients, increasing the ability of the bacteria to replicate or altering the ability of the host to control the symbiont (Mouton et al., 2006).

In addition to understanding the host, *Wolbachia* and environmental contributions to the regulation of within-individual symbiont titres, the study of sex-specific *Wolbachia* dynamics may shed further light on the evolutionary processes that regulate these interactions. Given its maternal transmission, *Wolbachia* have been predicted to evolve toward mutualism with the maternal host lineage, as their reproductive success is directly related to that of their female hosts (Herre et al., 1999). Infected females with CI-inducing *Wolbachia* have a reproductive advantage associated with the infection, as they produce viable offspring regardless of the infection status of the male (Friberg et al., 2011). Therefore, selection on both female host and symbiont is expected to converge towards improved transmission and low pathogenicity in females. Contrary to females, the CI-inducing *Wolbachia* infection in males represents a fitness compromise, as successful matings would occur only with infected females. In response, males genotypes may evolve modifiers that ultimately reduce the strength of the reproductive manipulation and overcome the fitness cost associated with the infection (Koehncke et al., 2009). Additionally, like mitochondria in most metazoans, males represent an evolutionary dead end for the symbiont. As a consequence, symbiont-host adaptations that are advantageous for females might not result in adaptive advantages for males; this being more evident for sexually dimorphic traits (Innocenti et al., 2011). Evidence of such sexually-antagonistic evolution has been observed in that natural populations of *Aedes albopictus*, in which males lose their *Wolbachia* *w*AlbA infection very early in their adult life, while in females it is maintained throughout their lifespan (Dutton and Sinkins, 2004; Tortosa et al., 2010).

Two approaches are usually used to investigate the regulation of within-host *Wolbachia* density: the exchange of *Wolbachia* strains between genetically distinct hosts (usually by microinjection) and the comparison of distinct *Wolbachia* strains within the same host species. An alternative strategy that has the potential to shed light on infection prevalence and plausibly, the strength of reproductive alterations in natural populations (Jaenike, 2009) is to screen a population for differences in *Wolbachia* titres among individuals or families. Despite this potential, a limited number of studies have been dedicated to explore this latter strategy. Unckless and colleagues (2009) screened more than 2000 wild collected *Drosophila inubila* females for *Wolbachia* density in ovaries and found around 20,000-fold difference between the least and the most heavily infected individuals. Similar results were obtained for the mosquito *Ae. albopictus*, with the levels of *Wolbachia* infection in F_1 offspring of wild caught animals varying by approximately 180,000-fold (Ahantarig et al., 2008). Whether these remarkable differences in *Wolbachia* titres are heritable has yet to be explored.

In this study, we investigated the possible heritability of intra-population *Wolbachia* titres by measuring the ovarian *Wolbachia* density in individuals from 50 isofemale lines of *Drosophila simulans* four generations after field collection and repeated the measurement for a subset of those lines after 15 generations of laboratory rearing. We concomitantly studied *Wolbachia* densities in male gonads to investigate if symbiont titre dynamics differ between females and males in sexually dimorphic tissues. We selected the *D. simulans* population from Hawaii because this fly population is exclusively infected by the strong-CI inducing *w*Ha strain of *Wolbachia* (James and Ballard, 2000). This strain displays very high transmission fidelity, infection frequencies near fixation (Ballard, 2004; James and Ballard, 2000; Rousset et al., 1992; Turelli and Hoffmann, 1995) as well as no apparent fitness costs or benefits to the female host compared to uninfected individuals (Poinsot and Merçot, 1997). We subsequently examined the effects of age, temperature, immune challenge and diet on gonadal titres of females and males in two different isofemale lines in order to explore the environmental effects of infection levels among different fly lines.

Overall, our results show that gonadal *Wolbachia* density (i) is highly variable in newly collected females, but such variation is lost during the process of laboratory adaptation (ii) does not correlate between females and males from the same isofemale line, and (iii) is very unresponsive to environmental treatments in ovaries while densities in testes are affected in a line specific manner. Our study highlights the importance of considering the effects of laboratory rearing on the study of *Wolbachia* population dynamics and that sex-specific dynamics of *Wolbachia* gonadal titres may provide clues to understanding the evolutionary processes of this symbiont in nature.

2. Materials and methods

2.1. Drosophila collection and rearing

A total of 91 female *Drosophila simulans* were collected in July 2009 from Honolulu, Hawaii. Flies were placed in individual vials and the male offspring confirmed the species status of *D. simulans*. Lines were transferred first through quarantine in Canberra, Australia and then to the laboratory in Sydney. In the laboratory flies were maintained in discrete generations at low densities on standard treacle-semolina-yeast-agar media at 23 °C at 50% humidity and under a 12-h light–dark cycle. Flies were assayed after being raised for at least two generations in standard laboratory conditions.

2.2. Wolbachia infection frequency and genetic variation

Founder females and four to five F_1 individuals of each line were stored in Gentra Puregene® cell lysis solution (Gentra Systems Inc., Minneapolis, USA) for *Wolbachia* infection determination by polymerase chain reaction (PCR). Genomic DNA (gDNA) was extracted from whole flies using the Gentra Puregene® Cell Kit (Gentra Systems Inc., Minneapolis, USA) following the Isolation from Solid Tissue protocol. PCR was performed using primers amplifying the *wsp* gene following Zhou et al. (1998). Infection was corroborated by amplification of a second, independently extracted DNA sample. Amplification of the COI barcoding region using universal mitochondrial primers (Folmer et al., 1994) was used to validate DNA quality. Numbers of infected and uninfected lines in this study were compared against previously reported data for this host/symbiont population (James and Ballard, 2000; Rousset et al., 1992; Turelli and Hoffmann, 1995).

We determined the *Wolbachia* strain and genetic variation in the population by sequencing a 549 bp region of the *wsp* gene following Zhou et al. (1998) and an additional 439 bp of the cell cycle-gene *ftsZ* following Baldo et al. (2006). These two loci are commonly used to determine *Wolbachia* strain infecting arthropod hosts as they have shown to have highly variable regions (Baldo et al., 2006; Werren et al., 1995; Zhou et al., 1998). Sequencing was carried out for a subset of 20 lines, which were determined to be *Wolbachia* infected. Chromatograms were visualised and edited using Sequencer 5.1 (Gene Codes, Ann Arbor, USA).