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Wolbachia–host interactions: connecting phenotype to genotype

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The long-established view of *Wolbachia* as reproductive parasites of insects is becoming complicated as an increasing number of papers describe a richer picture of *Wolbachia*-mediated phenotypes in insects. The search for the molecular basis for this phenotypic variability has been greatly aided by the recent sequencing of several *Wolbachia* genomes. These studies have revealed putative genes and pathways that are likely to be involved in the host–symbiont interaction. Whereas significant progress is being made from comparative genomic studies together with the use of model host systems like *Drosophila*, the ultimate linking of phenotype to genotype will require the development of genetic manipulation technology for both host and symbiont.

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Current Opinion in Microbiology 2007, **10**:221–224

This review comes from a themed issue on
Ecology and industrial microbiology
Edited by Victor de Lorenzo and Margaret J McFall-Ngai

Available online 29th May 2007

1369-5274/$ – see front matter
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DOI 10.1016/j.mib.2007.05.002

Introduction

The endosymbiotic α-proteobacterium *Wolbachia pipientis* was discovered in 1924 in the ovaries of *Culex pipiens* mosquitoes [1] and is thought to infect more than 20% of all insect species [2,3], as well as spiders, mites, terrestrial crustaceans and most filarial nematode species [4–9], making it one of the most successful intracellular symbionts yet described. This success has been attributed to its ability to modify host reproductive biology in order to favour its own transovarial transmission. The most common reproductive phenotype induced by *Wolbachia* in insects is cytoplasmic incompatibility (CI), a type of embryonic lethality that occurs when *Wolbachia*-infected males mate with females that do not harbour the same *Wolbachia* strain [10]. Other common phenotypes include the selective killing of male offspring [11], the conversion of genetic males into functional phenotypic females and the induction of parthenogenesis [10].

The ability of *Wolbachia* to manipulate host reproductive biology to its own benefit represents a very successful evolutionary strategy, contrary to conventional wisdom that tightly linked associations should evolve towards mutualism [12]. Not surprisingly, *Wolbachia* research has largely focused on reproductive parasitism traits, and to some extent this has channelled thinking within the field so that other phenotypic outcomes of infection have received less attention. The discovery of obligate *Wolbachia* infections in filarial nematodes demonstrated that some *Wolbachia* strains also possessed the capability to act as conventional mutualists, because their removal disrupts host development, moulting, fertility, viability and lifespan [13,14]. Indeed it is now clear that *Wolbachia* is able to influence host biology in a number of different ways beyond reproductive parasitism.

Phenotypic variability

Examples of this complexity have recently been demonstrated in the parasitoid wasp *Asobara tabida* where the production of oocytes and their development into viable offspring is dependent on the presence of *Wolbachia* [15]. In this case *Wolbachia* seems to act by influencing programmed cell death processes, preventing apoptosis of nurse cells and allowing oocyte maturation [16••]. A similar observation was recently made in the date stone beetle *Coccotrypes dactyliperda* [17•], where virgin females fed on antibiotics showed arrested oogenesis. In this study a *Rickettsia*-like symbiont was found, as well as *Wolbachia*, and the relative roles of each symbiont in the observed phenotype has yet to be determined. The interaction of *Wolbachia* with host oogenesis processes has also been observed in *Drosophila*, where *Wolbachia* infection has been shown to rescue mutations in the sex-lethal (*Sxl*) gene [18], a splicing and translational regulator involved in somatic sex determination, oogenesis and meiotic recombination. Other studies in *Drosophila* suggest that *Wolbachia* might interact with *chico*, a gene encoding an insulin receptor substrate involved in growth regulation [19•]. In this case some *chico* alleles are lethal in the absence of the *Wolbachia* infection. Whether this effect is directly associated with *chico* or another gene that interacts with *chico* is not clear at present.

Wolbachia infections have also been implicated in influencing a number of fitness traits, sometimes in apparently contradictory ways. In some cases, such as in the parasitoid wasp *Leptopilina heterotoma*, *Wolbachia* can negatively affect fecundity, locomotor performance and longevity [20]. In *Drosophila simulans*, *Wolbachia* has been reported to reduce sperm production [21]. On the contrary, single and double *Wolbachia* infections have been reported to improve fecundity in *Aedes albopictus* [22] and in both *Drosophila melanogaster* and *D. simulans*, infections

have been reported to induce variable fecundity and longevity effects depending on the genetic strain of fly used [23,24•].

A further complication in *Wolbachia* biology is the observation that some strains have not been shown to induce any phenotype that can help explain their presence in host populations, for example the *w*Au strain that infects *D. simulans* [25] or the global selective sweep of *w*Mel in *D. melanogaster* [26•]. In the absence of substantial horizontal transmission these *Wolbachia* must affect hosts in ways that are not apparent at the present time, presumably through mechanisms unrelated to reproductive parasitism.

Wolbachia genomics

Whereas our understanding of the phenotypic outcomes of *Wolbachia* infection is rapidly expanding, our knowledge of the molecular mechanisms that mediate these outcomes is very rudimentary. A key step forward has been the recent sequencing of two complete *Wolbachia* genomes, that of the *w*Mel strain that induces CI in *D. melanogaster* [27••] and that of the *w*Bm strain, an obligate mutualist of the filarial nematode *Brugia malayi* [28••]. Various other genomes representative of the phenotypic diversity of *Wolbachia* are currently the focus of different sequencing projects and will soon provide a wealth of additional data [29]. In addition, useful *Wolbachia* genomic information has been obtained recently by data mining the sequencing projects of host insects that are infected with *Wolbachia* [30•].

The comparative value of these genomic data is being enhanced as whole genome sequences of closely related pathogens that don't cause any of the same phenotypes as *Wolbachia*, such as several species of *Rickettsia* [31–33], *Anaplasma marginale* [34] and *Ehrlichia rumiantium* [35], are becoming available. The comparison of *Wolbachia* with these genomes will assist with the identification of the molecular basis underlying the various phenotypes. In addition, ongoing projects to sequence unrelated symbiont genomes that induce similar phenotypic effects in hosts will be of great interest. For example, the future sequencing of *Cardinium hertigii*, an arthropod symbiont not related to *Wolbachia* but that induces most phenotypes traditionally associated with *Wolbachia*, such as CI [36,37], parthenogenesis [38] and feminization [39], will provide valuable comparative insights.

To date the analysis of *Wolbachia* genomes has revealed the loss of multiple metabolic pathways, the abundance of repetitive DNA and the presence of a series of genes with potential roles in host interaction [27••,28••]. For example, the *w*Mel genome contains a large number of genes that have variable numbers of ankyrin domains that appear to be candidates for involvement in the cytoplasmic incompatibility phenotype [40••]. These genes are quite common in *Wolbachia* but very rare in most other known bacterial genomes [41]. Comparative analysis of orthologues of these genes in different *Wolbachia* strains infecting both *Drosophila* and *Culex pipiens* has shown them to be extremely variable [40••,42••]. This variation was reflected in the following: first, the presence/absence of transmembrane domains, probably affecting their subcellular localization; second, the number of ANK repeats, probably affecting the strength and/or specificity of their interaction with other proteins; and third, the absence of particular orthologues, or their disruption by insertion elements, in *Wolbachia* strains that are known to be incapable of inducing CI. The prevalence of these ANK genes in *Wolbachia*, their potential role in protein–protein interactions, and the results of comparative analyses suggests that they are probable candidates to be involved in host communication and potential reproductive phenotypes.

Analysis of genome data has also revealed that in *Wolbachia* numerous prophage genes are present and that phages are likely to play a significant role in the ecology of *Wolbachia* through the regulation of infection densities within hosts [27••,43••]. A correlation between sequence variability in phage structural genes and the expression of reproductive phenotypes has yet to show a relationship between particular phage infections and reproductive phenotypes [44,45]; however, a role for phage-associated genes, such as some ANK genes or virulence determinants cannot be excluded.

Genome sequencing has also revealed the presence of complete operons encoding Type IV secretion systems in both *w*Mel [27••] and *w*Bm [28••] genomes. A better understanding of these secretion systems and the effector molecules they translocate will be fundamental to a future understanding of host–symbiont interactions.

Conclusions

The ability to use the genetic tools of *Drosophila* in the analysis of host–symbiont interactions has the potential to greatly accelerate our progress in understanding how *Wolbachia* generates host phenotypes. For example, a subtractive hybridisation approach has been used recently to identify host genes whose transcription is altered by the presence of *Wolbachia* [46••]. One gene found with this approach was the non-muscle myosin II gene *zipper*, which was found to be upregulated in *Wolbachia*-infected *D. simulans*. Subsequent overexpression of this gene in *D. melanogaster* was shown to mimic the CI phenotype in the absence of *Wolbachia* indicating a potential functional role. However, *Wolbachia* infection was unable to rescue the effect in examined lines. A derivation on this experimental approach is the potential expression of *Wolbachia* genes directly in *Drosophila* to examine possible phenotypes.

However the ultimate confirmation of the functional role of *Wolbachia* genes in host interactions requires the ability

to directly manipulate the *Wolbachia* genome. Currently, *Wolbachia* gene function can only be inferred from comparative genomics or assessed using model-host genetic tools or heterologous expression systems. Recent advances in the development of *Wolbachia* genetic transformation methodologies using targeted homologous recombination (Iturbe-Ormaetxe, unpublished) creates optimism that these tools will soon be available.

Acknowledgements

We'd like to thank Jeremy Brownlie, Markus Riegler, Conor McMeniman and Begoña Heras for helpful and constructive comments on the manuscript. This work was supported by the Bill & Melinda Gates Grand Challenges in Global Health Initiative and by grants from the Australian Research Council. A full list of *Wolbachia* literature and resources can be found at the *Wolbachia* website (http://www.wolbachia.sols.uq.edu.au).

References and recommended reading

Papers of particular interest, published within the period of review, have been highlighted as:

- • of special interest
- •• of outstanding interest

1. Hertig M, Wolbach SB: **Studies of *Rickettsia*-like microorganisms in insects**. *Journal of Medical Research* 1924, **44**:329-374.

2. Jeyaprakash A, Hoy MA: **Long PCR improves *Wolbachia* DNA amplification: wsp sequences found in 76% of sixty-three arthropod species**. *Insect Mol Biol* 2000, **9**:393-405.

3. Werren JH, Windsor D, Guo LR: **Distribution of *Wolbachia* among Neotropical Arthropods**. *Proc Biol Sci* 1995, **262**:197-204.

4. Bouchon D, Rigaud T, Juchault P: **Evidence for widespread *Wolbachia* infection in isopod crustaceans: molecular identification and host feminization**. *Proc Biol Sci* 1998, **265**:1081-1090.

5. Breeuwer JAJ, Jacobs G: ***Wolbachia*: intracellular manipulators of mite reproduction**. *Exp Appl Acarol* 1996, **20**:421-434.

6. Oh HW, Kim MG, Shin SW, Bae KS, Ahn YJ, Park HY: **Ultrastructural and molecular identification of a *Wolbachia* endosymbiont in a spider, *Nephila clavata***. *Insect Mol Biol* 2000, **9**:539-543.

7. Rowley SM, Raven RJ, McGraw EA: ***Wolbachia* pipientis in Australian Spiders**. *Curr Microbiol* 2004, **49**:208-214.

8. Taylor MJ, Bilo K, Cross HF, Archer JP, Underwood AP: **16S rDNA phylogeny and ultrastructural characterization of *Wolbachia* intracellular bacteria of the filarial nematodes *Brugia malayi*, *B. pahangi*, and *Wuchereria bancrofti***. *Exp Parasitol* 1999, **91**:356-361.

9. Bandi C, Dunn AM, Hurst GD, Rigaud T: **Inherited microorganisms, sex-specific virulence and reproductive parasitism**. *Trends Parasitol* 2001, **17**:88-94.

10. Stouthamer R, Breeuwer JA, Hurst GD: ***Wolbachia* pipientis: microbial manipulator of arthropod reproduction**. *Annu Rev Microbiol* 1999, **53**:71-102.

11. Hurst GDD, Jiggins FM, von der Schulenburg JHG, Bertrand D, West SA, Goriacheva II, Zakharov IA, Werren JH, Stouthamer R, Majerus MEN: **Male-killing *Wolbachia* in two species of insect**. *Proc R Soc Lond B Biol Sci* 1999, **266**:735-740.

12. Werren JH, O'Neill SL: **The evolution of heritable symbionts**. In *Influential Passengers. Inherited Microorganisms and Arthropod Reproduction.* Edited by O'Neill SL, Hoffmann AA, Werren JH: Oxford University Press; 1997:1-41.

13. Bandi C, McCall JW, Genchi C, Corona S, Venco L, Sacchi L: **Effects of tetracycline on the filarial worms *Brugia pahangi* and *Dirofilaria immitis* and their bacterial endosymbionts *Wolbachia***. *Int J Parasitol* 1999, **29**:357-364.

14. Hoerauf A, Nissen-Pahle K, Schmetz C, Henkle-Duhrsen K, Blaxter ML, Buttner DW, Gallin MY, Al-Qaoud KM, Lucius R, Fleischer B: **Tetracycline therapy targets intracellular bacteria in the filarial nematode *Litomosoides sigmodontis* and results in filarial infertility**. *J Clin Invest* 1999, **103**:11-18.

15. Dedeine F, Vavre F, Fleury F, Loppin B, Hochberg ME, Bouletreau M: **Removing symbiotic *Wolbachia* bacteria specifically inhibits oogenesis in a parasitic wasp**. *Proc Natl Acad Sci USA* 2001, **98**:6247-6252.

16. •• Pannebakker BA, Loppin B, Elemans CP, Humblot L, Vavre F: **Parasitic inhibition of cell death facilitates symbiosis**. *Proc Natl Acad Sci USA* 2007, **104**:213-215.
This paper shows that *Wolbachia* influences programmed cell death by inhibiting apoptosis of nurse cells in the wasp *Asobara tabida*. As a consequence, the host requires *Wolbachia* for oogenesis. Authors propose that the targeting of apoptotic pathways could be a common trend in bacteria–host interactions.

17. • Zchori-Fein E, Borad C, Harari AR: **Oogenesis in the date stone beetle, *Coccotrypes dactyliperda*, depends on symbiotic bacteria**. *Physiol Entomol* 2006, **31**:164-169.
This work reveals an interesting example of the mutualistic nature of some *Wolbachia* infections in beetles.

18. Starr DJ, Cline TW: **A host parasite interaction rescues *Drosophila* oogenesis defects**. *Nature* 2002, **418**:76-79.

19. • Clark ME, Anderson C, Cande J, Karr TL: **Widespread prevalence of *Wolbachia* in laboratory stocks and the implications for *Drosophila* research**. *Genetics* 2005, **170**:1667-1675.
This works presents a survey of *Wolbachia* infections in *Drosophila* stock centers and highlights the possible interaction between Wolbachia and different *Drosophila* traits. It describes the possible interaction between *Wolbachia* and the *chico* locus.

20. Fleury F, Vavre F, Ris N, Fouillet P, Bouletreau M: **Physiological cost induced by the maternally-transmitted endosymbiont *Wolbachia* in the *Drosophila* parasitoid *Leptopilina heterotoma***. *Parasitology* 2000, **121**:493-500.

21. Snook RR, Cleland SY, Wolfner MF, Karr TL: **Offsetting effects of *Wolbachia* infection and heat shock on sperm production in *Drosophila simulans*: analyses of fecundity, fertility and accessory gland proteins**. *Genetics* 2000, **155**:167-178.

22. Dobson SL, Rattanadechakul W, Marsland EJ: **Fitness advantage and cytoplasmic incompatibility in *Wolbachia* single- and superinfected *Aedes albopictus***. *Heredity* 2004, **93**:135-142.

23. Fry AJ, Palmer MR, Rand DM: **Variable fitness effects of *Wolbachia* infection in *Drosophila melanogaster***. *Heredity* 2004, **93**:379-389.

24. • Dean MD: **A *Wolbachia*-associated fitness benefit depends on genetic background in *Drosophila simulans***. *Proc Biol Sci* 2006, **273**:1415-1420.
In this paper the author shows that fitness benefits associated with a *Wolbachia* strain can depend on mitochondrial haplotype.

25. Hoffmann AA, Clancy D, Duncan J: **Naturally-occurring *Wolbachia* infection in *Drosophila simulans* that does not cause cytoplasmic incompatibility**. *Heredity* 1996, **76**:1-8.

26. • Riegler M, Sidhu M, Miller WJ, O'Neill SL: **Evidence for a global *Wolbachia* replacement in *Drosophila melanogaster***. *Curr Biol* 2005, **15**:1428-1433.
This paper reports genetic variability in *Wolbachia* associated with *D. melanogaster* in field and lab populations. It presents the first evidence for a worldwide replacement of a *Wolbachia* strain in an insect host species.

27. •• Wu M, Sun LV, Vamathevan J, Riegler M, Deboy R, Brownlie JC, McGraw EA, Martin W, Esser C, Ahmadinejad N *et al.*: **Phylogenomics of the reproductive parasite *Wolbachia pipientis* wMel: A streamlined genome overrun by mobile genetic elements**. *Plos Biol* 2004, **2**:327-341.
The authors describe the genome sequence of the *Wolbachia* strains that infect *Drosophila melanogaster*. They highlight the importance of repetitive DNA, mobile elements and phages in *Wolbachia* biology. This work reports the abundance of ANK domain genes in *Wolbachia*, potentially implicated in *Wolbachia*–host interactions and phenotypic manipulation.

28. Foster J, Ganatra M, Kamal I, Ware J, Makarova K, Ivanova N,
•• Bhattacharyya A, Kapatral V, Kumar S, Posfai J *et al.*: **The *Wolbachia* genome of *Brugia malayi*: endosymbiont evolution within a human pathogenic nematode**. *PLos biol* 2005, **3**:e121.
The authors describe the first genome sequence from a mutualistic *Wolbachia* that infects filarial nematodes. Analysis provides hypothesis on the molecular basis underpinning the nematode's dependence on *Wolbachia*.

29. Liolios K, Tavernarakis N, Hugenholtz P, Kyrpides NC: **The Genomes on line database (GOLD) v.2: a monitor of genome projects worldwide**. *Nucleic Acids Res* 2006, **1**:D332-D334.

30. Salzberg SL, Hotopp JC, Delcher AL, Pop M, Smith DR, Eisen MB,
• Nelson WC: **Serendipitous discovery of *Wolbachia* genomes in multiple *Drosophila* species**. *Genome Biol* 2005, **6**:R23.
Salzberg *et al*. show that data mining of *Drosophila* trace archives can yield useful *Wolbachia* genome sequence data. The authors reveal partial genome information from two *Wolbachia* infections from *D. ananassae, and D. simulan* (see subsequent erratum).

31. Andersson SG, Zomorodipour A, Andersson JO, Sicheritz-Ponten T, Alsmark UC, Podowski RM, Naslund AK, Eriksson AS, Winkler HH, Kurland CG: **The genome sequence of *Rickettsia prowazekii* and the origin of mitochondria**. *Nature* 1998, **396**:133-140.

32. McLeod MP, Qin X, Karpathy SE, Gioia J, Highlander SK, Fox GE, McNeill TZ, Jiang H, Muzny D, Jacob LS *et al.*: **Complete genome sequence of *Rickettsia typhi* and comparison with sequences of other *Rickettsiae***. *J Bacteriol* 2004, **186**:5842-5855.

33. Ogata H, La Scola B, Audic S, Renesto P, Blanc G, Robert C, Fournier PE, Claverie JM, Raoult D: **Genome sequence of *Rickettsia bellii* illuminates the role of amoebae in gene exchanges between intracellular pathogens**. *PLoS Genet* 2006, **2**:e76.

34. Brayton KA, Kappmeyer LS, Herndon DR, Dark MJ, Tibbals DL, Palmer GH, McGuire TC, Knowles DP Jr: **Complete genome sequencing of Anaplasma marginale reveals that the surface is skewed to two superfamilies of outer membrane proteins**. *Proc Natl Acad Sci USA* 2005, **102**:844-849.

35. Collins NE, Liebenberg J, de Villiers EP, Brayton KA, Louw E, Pretorius A, Faber FE, van Heerden H, Josemans A, van Kleef M *et al.*: **The genome of the heartwater agent *Ehrlichia ruminantium* contains multiple tandem repeats of actively variable copy number**. *Proc Natl Acad Sci USA* 2005, **102**:838-843.

36. Hunter MS, Perlman SJ, Kelly SE: **A bacterial symbiont in the Bacteroidetes induces cytoplasmic incompatibility in the parasitoid wasp *Encarsia pergandiella***. *Proc Biol Sci* 2003, **270**:2185-2190.

37. Gotoh T, Noda H, Ito S: ***Cardinium* symbionts cause cytoplasmic incompatibility in spider mites**. *Heredity* 2007, **98**:13-20.

38. Zchori-Fein E, Gottlieb Y, Kelly SE, Brown JK, Wilson JM, Karr TL, Hunter MS: **A newly discovered bacterium associated with parthenogenesis and a change in host selection behavior in parasitoid wasps**. *Proc Natl Acad Sci USA* 2001, **98**:12555-12560.

39. Weeks AR, Marec F, Breeuwer JA: **A mite species that consists entirely of haploid females**. *Science* 2001, **292**:2441-2443.

40. Iturbe-Ormaetxe I, Burke GR, Riegler M, O'Neill SL: **Distribution,
•• expression, and motif variability of ankyrin domain genes in *Wolbachia* pipientis**. *J Bacteriol* 2005, **187**:5136-5145.
A comprehensive comparative analysis of the ANK domain genes from different *Wolbachia* strains infecting *Drosophila*.

41. Li J, Mahajan A, Tsai MD: **Ankyrin repeat: a unique motif mediating protein-protein interactions**. *Biochemistry* 2006, **45**:15168-15178.

42. Sinkins SP, Walker T, Lynd AR, Steven AR, Makepeace BL,
•• Godfray HC, Parkhill J: ***Wolbachia* variability and host effects on crossing type in Culex mosquitoes**. *Nature* 2005, **436**:257-260.
This paper describes two ANK domain genes that are associated with different crossing types in *Culex* mosquitoes. One of these genes, *pk2,* has host sex-specific expression.

43. Bordenstein SR, Marshall ML, Fry AJ, Kim U, Wernegreen JJ: **The
•• tripartite associations between bacteriophage, *Wolbachia*, and arthropods**. *PLoS Pathog* 2006, **2**:e43.
This work shows that *Wolbachia* phage might influence symbiont densities within hosts, which in turn can modulate expression of reproductive phenotypes.

44. Sanogo YO, Eitam A, Dobson SL: **No evidence for bacteriophage WO orf7 correlation with *Wolbachia*-induced cytoplasmic incompatibility in the *Culex pipiens* complex (Culicidae: Diptera)**. *J Med Entomol* 2005, **42**:789-794.

45. Gavotte L, Henri H, Stouthamer R, Charif D, Charlat S, Bouletreau M, Vavre F: **A survey of the bacteriophage WO in the endosymbiotic bacteria *Wolbachia***. *Mol Biol Evol* 2007, **24**:427-435.

46. Clark ME, Heath BD, Anderson CL, Karr TL: **Induced paternal
•• effects mimic cytoplasmic incompatibility in *Drosophila***. *Genetics* 2006, **173**:727-734.
By using host genetic tools the authors report that the overexpression of the *Drosophila* gene zipper, found to be induced in infected fly testes, can mimic the CI modification phenotype, although it cannot rescue it.