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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Introduction
The endosymbiotic α-proteobacterium Wolbachia pipiens 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 parthenogenogenesis [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, moultling, 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 Cocotrypes dactylipera [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 \( \omega \)Au strain that infects \( D. \) simulans [25] or the global selective sweep of \( \omega \)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 \( \omega \)Mel strain that induces CI in \( D. \) melanogaster [27**] and that of the \( \omega \)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 ruminantium* [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 \( \omega \)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 \( \omega \)Mel [27**] and \( \omega \)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.

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References and recommended reading
Papers of particular interest, published within the period of review, have been highlighted as:

* of special interest
** of outstanding interest


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.


This work reveals an interesting example of the mutualistic nature of some Wolbachia infections in beetles.


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.


In this paper the author shows that fitness benefits associated with a Wolbachia strain can depend on mitochondrial haplotype.


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.


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.


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.


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.