Wolbachia strains for disease control: ecological and evolutionary considerations

Ary A. Hoffmann, Perran A. Ross and Gordana Rašić

Pest and Environmental Adaptation Research Group, School of BioSciences, Bio21 Institute, The University of Melbourne, Parkville, Vic., Australia

Keywords
Aedes, deployment issues, disease control, fitness, strain attributes, virus, Wolbachia.

Abstract
Wolbachia are endosymbionts found in many insects with the potential to suppress vectorborne diseases, particularly through interfering with pathogen transmission. Wolbachia strains are highly variable in their effects on hosts, raising the issue of which attributes should be selected to ensure that the best strains are developed for disease control. This depends on their ability to suppress viral transmission, invade host populations, persist without loss of viral suppression and not interfere with other control strategies. The potential to achieve these objectives is likely to involve evolutionary constraints; viral suppression may be limited by the ability of infections to spread due to deleterious host fitness effects. However, there are exceptions to these patterns in both natural infections and in novel associations generated following interspecific transfer, suggesting that pathogen blockage, deleterious fitness effects and changes to reproductive biology might be at least partly decoupled to achieve ideal infection attributes. The stability of introduced Wolbachia and its effects on viral transmission remain unclear, but rapid evolutionary changes seem unlikely. Although deliberate transfers of Wolbachia across species remain particularly challenging, the availability of strains with desirable attributes should be expanded, taking advantage of the diversity available across thousands of strains in natural populations.

Introduction
There is currently a high level of interest in using Wolbachia to indirectly suppress the incidence of vectorborne human diseases such as malaria, dengue fever or filariasis (McGraw and O’Neill 2013; Sinkins 2013; Bourtzis et al. 2014), or plant diseases caused by mosaic viruses and other disease agents (Box 1). Wolbachia are endosymbiotic bacteria living in all orders of insects and in other invertebrates. They are often promoted as a ‘natural’ way of manipulating insect hosts, in contrast to other approaches for manipulating vector biology, particularly through genetic modification, that are often regarded with suspicion because they involve novel constructs that are not present in the environment with the potential to spread to other nonvector species. While Wolbachia are already widespread in the environment, they are restricted to living exclusively within host cells and spread by changing the biology of their host species (O’Neill et al. 1997). Wolbachia can be used in a variety of ways for disease suppression, by decreasing the size of a vector population through (i) the release of Wolbachia-infected males that are incompatible with females (O’Connor et al. 2012) or (ii) the invasion of a Wolbachia strain that produces deleterious fitness effects particularly under seasonally variable environments (Rašić et al. 2014a), and particularly by (iii) decreasing the ability of the vector population to transmit diseases through the invasion of a Wolbachia strain that interferes directly with transmission (Teixeira et al. 2008; Kambris et al. 2009; Moreira et al. 2009; Walker et al. 2011). The third option is considered particularly promising because it may not require ongoing management by health authorities; once a Wolbachia strain blocking disease transmission has invaded a target vector population by altering host reproduction, the Wolbachia strain should stay at a high frequency in that population without further releases being required (Hoffmann et al. 2011). It is also important to note that the three strategies are not mutually exclusive but rather complementary.
Despite the promise held by Wolbachia-based disease suppression programmes particularly for viral diseases spread by mosquito vectors (Box 1), there are still substantial challenges in their widespread deployment. In particular, strains of Wolbachia for release need to be carefully selected and evaluated to ensure long-term viability of the strategy in the face of ongoing evolutionary changes, and to meet any regulatory and community concerns. In this study, we focus on these issues, using information that has been collected on insects naturally infected with Wolbachia and on artificial introductions of Wolbachia into new hosts. We consider the development of strains and host backgrounds that combine desirable attributes for disease suppression with those required for rapid invasion into target vector populations, as well as the likely long-term evolutionary stability of effects generated by Wolbachia in these populations. Much of the information we discuss comes from research in Drosophila species where Wolbachia infections have been investigated within an evolutionary and ecological context since the early 80s, and where a large number of infections have been transferred across species to investigate the interaction and evolution of host and Wolbachia genomes.

**Diversity of Wolbachia and their effects**

There is an enormous diversity of Wolbachia strains in nature. DNA sequence data have been used to demonstrate the presence of multiple Wolbachia variants within the same individual host, the presence of variation among Wolbachia sequences collected from different conspecific individuals, and molecular changes in the same Wolbachia infection when it is transferred to different host species. Most molecular comparisons have focussed on describing variation in Wolbachia infections across related species to characterize patterns of horizontal and vertical transmission of infections across time (e.g. Bing et al. 2014; Morrow et al. 2014), using sets of conserved primers for a series of genes such as the MLST set (Baldo et al. 2006). Many studies have used primer sets to demonstrate variation in Wolbachia strains within the same host. An example of a species carrying a complex of infections is the tsetse fly, where 37 different Wolbachia variants have been identified (Symula et al. 2013). Numerous Wolbachia strains have also been identified in mosquitoes of the Culex pipiens species complex (Atyame et al. 2011; Morningstar et al. 2012) where they (rather than the nuclear background) largely control patterns of cytoplasmic incompatibility (Duron et al. 2006). Variability among Wolbachia within the same host could arise through recombination and mutation (Klason et al. 2009; Atyame et al. 2011), and a new Wolbachia strain could spread if it has a selective advantage and/or generates patterns of cytoplasmic incompatibility or other changes to host reproduction that favour its spread. Horizontal transmission of Wolbachia across hosts such as mediated through a parasitoid could also result in the introduction of a new Wolbachia strain into a host population. Once new Wolbachia strains arise, they can displace existing strains at a rapid rate, as indicated by the replacement of wAu by wRi in Australian populations of Drosophila simulans (Kriesner et al. 2013), but these types of replacements are thought to occur very rarely (Richardson et al. 2012).
The majority of Wolbachia strains have undescribed effects, having been detected in organisms via molecular tools (Hilgenboecker et al. 2008; Ahmed et al. 2013) and not further studied experimentally. Many of these strains may well have little impact on their host, but nevertheless persist because of a high fidelity of vertical transmission from mothers to offspring. Such infections with no apparent phenotypic effects on hosts have been described in Drosophila species (e.g. Hoffmann et al. 1996). Other Wolbachia strains are likely to have dramatic effects on their host; the most widespread of these effects is cytoplasmic incompatibility, where Wolbachia presence leads to the death of embryos and sometimes immature offspring when infected fathers mate with uninfected mothers (or mothers carrying a different Wolbachia strain). There are also Wolbachia infections that cause the death of male offspring only (male killers) and others that lead to parthenogenetic reproduction in haplodiploid organisms or feminization of male offspring (reviewed in O’Neill et al. 1997). Even when Wolbachia appear to have no phenotypic effects on their host’s reproduction, they might nevertheless have other effects that only become evident once appropriate host challenges are provided; for instance, the viral blocking activity of Wolbachia strains only became apparent once infected Drosophila strains were challenged with RNA viruses (Teixeira et al. 2008; Hedges et al. 2008, Osborne et al. 2009).

Wolbachia effects exerted on hosts typically fall along a continuum; for instance, cytoplasmic incompatibility can range from complete (all offspring die) as in the case of many Wolbachia infections from mosquitoes (e.g. Rason and Scott 2003), to relatively weak (a small proportion of offspring die) as in the case of particular Drosophila infections (e.g. Reynolds et al. 2003). Moreover, the effects of Wolbachia on hosts can change markedly depending on environmental conditions and the age of the insect. Factors, such as the presence of natural antibiotics (Clancy and Hoffmann 1998; Lu et al. 2012), temperature extremes (Mouton et al. 2007; Bordenstein and Bordenstein 2011), the age of the male and female (Unckless et al. 2009; Tortosa et al. 2010) and interactions among these factors (Mouton et al. 2007; Bordenstein and Bordenstein 2011), can all influence the density of Wolbachia in host tissues and host effects such as cytoplasmic incompatibility.

Wolbachia density often varies substantially among individuals under field conditions (e.g. Ahantarig et al. 2008). This variation could potentially influence the transmission, fitness effects and expression of cytoplasmic incompatibility, which has been characterized in detail in Drosophila populations where variability in cytoplasmic incompatibility is high (e.g. Turelli and Hoffmann 1995) and in Culex populations where the variability is low (Rason and Scott 2003). However, it is not clear whether the variability reflects Wolbachia/host genomic variation or environmentally induced variation that might only have a temporary effect on density and host phenotypes. For instance, when the wHa infection in D. simulans was tested in multiple host lines derived from the field, variation in the ovarian density of the Wolbachia infection among host lines was maintained for several generations, but was eventually lost (Correa and Ballard 2012). Therefore, while experimental studies might indicate a clear correlation between Wolbachia density and cytoplasmic incompatibility/deleterious effects (e.g. Clancy and Hoffmann 1998), it is not clear whether density variation is necessarily linked to variation in the Wolbachia genome. Recently, a group of Wolbachia genes associated with density variation (the Octomom region) has been identified in the wMelPop strain of D. melanogaster (Chrostek and Teixeira 2015) and might provide candidates for affecting density in field samples.

Unpredictable phenotypic effects in new hosts

A substantial number of Wolbachia strains have now been transferred through microinjection across species boundaries, particularly in the genus Drosophila, but also across genera within and among insect orders (Table 1, Appendix S1). Successful Wolbachia transfers can be challenging, although those involving Drosophila species have been undertaken for some time and have become fairly routine (e.g. Poinset et al. 1998; Charlat et al. 2002). Cross-infection experiments where Wolbachia are artificially transferred from one species to another have demonstrated (particularly in Drosophila) that host effects associated with a particular Wolbachia strain can persist or be modified after transfer to a new host (e.g. Ikeda et al. 2003; Osborne et al. 2012; Veneti et al. 2012).

The marked changes in cytoplasmic incompatibility and other reproductive effects, as well as host fitness effects, are typified by the wAu infection and lack of fitness effects in its native host but life shortening following transfer to D. melanogaster (Chrostek et al. 2014), and the absence of male killing when Wolbachia from Drosophila innubila are transferred to D. melanogaster and D. simulans (Veneti et al. 2012). As another example, wCauA causes cytoplasmic incompatibility in its native host, Cadra cautella (Sasaki and Ishikawa 1999), but when transferred to Ephesia kuehniella, it causes male killing (Sasaki et al. 2002) (see Appendix S1). There are also several other instances where shifts in cytoplasmic incompatibility occur when Wolbachia from one host are transferred to a different species within the same genus (e.g. Boyle et al. 1993), and clearly, viral interference will also depend on host effects as reflected by the limited blockage provided by wAlbB in its native Ae. albopictus host compared to strong blockage when this
Table 1. Stable Wolbachia infections in native and transinfected hosts, their reproductive effects (CI—cytoplasmic incompatibility, MT—maternal transmission), fitness effects, and viral blocking effectiveness where demonstrated (?—information unavailable). Effect size is denoted as: high (>90%), moderate/partial (20–90%), low (<20%) and none (no detectible effects). More details are found in Appendix S1.

| Strain | NATIVE HOST | CI | MT | Fitness cost | Viral blockage | TRANSFECTED HOST | CI | MT | Fitness cost | Viral blockage |
|--------|-------------|----|----|-------------|--------------|-----------------|----|----|-------------|--------------|
| wMel   | D. melanogaster | Partial/1,2 | Partial/4 | None/6,6 | some benefits/3,7 | Aedes aegypti/12 | High/12 | High/12 | Low/12,13 | High/12 |
| wMelPop| D. melanogaster | Partial/22 | none/6,12,23 | High/24 | High/21,22,24 | High/10,25 | Aedes albopictus/16 | High/16 | High/18 | None/17 | High/16,17 |
| wAu    | D. simulans/33 | None/32 | Low/5-7 | High/33 | ? | Aedes albopictus/31 | High/23,24,32 | High/23,24,32 | ? | High/23,24,32 |
| wMelCS | D. melanogaster | Low/5-7 | None/37 | Moderate/10 | ? | D. simulans/34 | Low/none/34 | High/19,20 | Moderate/10,25 | ? |
| wInn   | D. innubila/39 | Male killing/39 | High/39 | None/35,38 | Some benefits/40 | D. simulans/41 | High/19,20 | High/19,20 | Low/19 | ? |
| wRi    | D. simulans/42 | Partial/42 | High/43 | partial/14 | Low/43 | D. simulans/41 | None/41 | High/41 | Some benefits/41 | None/41 |
| wAlbB  | Ae. albopictus/52 | High/53,54,55 | High/56 | None/57 | some benefits/54,55 | Ae. aegypti/60,61 | Low/17,58,59 | High/60 | partial/61 | Moderate/60 |
| wPip   | Culex pipiens complex/60,66 | High/67 | High/67 | Low/68, none/67 | Ae. albopictus/69 | High/19,20 | High/19,20 | Low/19 | ? |

1Hoffmann (1988); 2Reynolds and Hoffmann (2002); 3Fry et al. (2004); 4Hoffmann et al. (1998); 5Harcombe and Hoffmann (2004); 6Montenegro et al. (2006); 7Fry and Rand (2002); 8Glaser and Meola (2010); 9Teixeira et al. (2008); 10Chrostek et al. (2011); 11Rances et al. (2012); 12Walker et al. (2011); 13Hoffmann et al. (2014a); 14van den Hurk et al. (2012); 15Hussain et al. (2013); 16Hoffmann et al. (2014b); 17Blagrove et al. (2013); 18Veneti et al. (2012); 19Hoffmann et al. (1986); 20Hoffmann et al. (1990); 21Hoffmann et al. (1995); 22Boyle et al. (1993); 23Clancy and Hoffmann (1997); 24Zabalou et al. (2004); 25Kang et al. (2003); 26Sinkins et al. (1995); 27Dobson et al. (2001); 28Dobson et al. (2004); 29Dobson et al. (2002); 30Kittayapong et al. (2002); 31Calvitti et al. (2009); 32Hedges et al. (2008); 33Hedges et al. (2008); 34McMeniman et al. (2009); 35Yeap et al. (2011); 36McMeniman and O’Neill (2010); 37Turley et al. (2009); 38Moore et al. (2009); 39Suh et al. (2009); 40Carrington et al. (2010); 41Carrington et al. (2010); 42Carrington et al. (2010); 43Hofmann et al. (1996); 44Yamada et al. (2011); 45Chrostek et al. (2014); 46Seligman et al. (1994); 47Holden et al. (1993); 48Seiga et al. (2014); 49Dyer and Jaenike (2004); 50Uncless and Jaenike (2011); 51Veneti et al. (2012); 52Hoffmann et al. (1986); 53Hoffmann et al. (1990); 54Hoffmann et al. (1995); 55Weeks et al. (2007); 56Xu et al. (2006); 57Fu et al. (2010); 58Boyle et al. (1993); 59Clancy and Hoffmann (1997); 60Zabalou et al. (2004); 61Kang et al. (2003); 62Sinkins et al. (1995); 63Dobson et al. (2001); 64Dobson et al. (2004); 65Dobson et al. (2002); 66Kittayapong et al. (2002); 67Calvitti et al. (2009); 68Mousson et al. (2012); 69Mousson et al. (2010); 70Xi et al. (2005); 71Ruang-Areerate and Kittayapong (2006); 72Bian et al. (2010); 73Andrews et al. (2012); 74Bian et al. (2013a); 75Hertig and Wolbach (1924); 76Yen and Barr (1973); 77Ragson and Scott (2003); 78de Almeida et al. (2011); 79Calvitti et al. (2010); 80Moretti and Calvitti (2013). *High for one day old males, but decreases rapidly with increasing male age; †increases with higher Octomom copy numbers (Chrostek and Teixeira 2015); ‡inferred based on routine propagation of transinfected lines without loss of infection over time; §unpublished work by J Axford and AA Hoffmann.
infection is transferred to Ae. aegypti (Bian et al. 2010) and other examples (Table 1, Appendix S1).

Desirable attributes of Wolbachia strains for disease suppression

With many thousands of Wolbachia strains existing in nature and interacting with host genomes and local environments in different ways, Wolbachia could be used in a variety of ways for disease control strategies aimed at suppressing vector populations and directly interfering with disease transmission. Some important transfers of Wolbachia to disease vectors have now been achieved, including transfers of Wolbachia from Drosophila to Aedes mosquitoes for the production of vectors that exhibit shortened lifespan (McMeniman et al. 2009) and suppression of RNA viruses and other disease agents (Kambris et al. 2009; Moreira et al. 2009; Walker et al. 2011). In addition, there have been successful transfers of Wolbachia from Aedes albopictus to Aedes aegypti to achieve virus suppression (Xi et al. 2005; Bian et al. 2010). These transfers capture a tiny fraction of the vast diversity of Wolbachia strains available in natural populations of insects related to mosquitoes. Yet, they are already raising questions about how Wolbachia strains and host backgrounds might be developed for disease suppression.

Different strain attributes are required by the three strategies that use Wolbachia to reduce disease transmission. The simplest requirement is for population suppression via male release where the main attribute is for released males to exhibit strong cytoplasmic incompatibility when they mate with field females. Released males also need to be competitive with males from natural populations. Competitive ability could be reduced if Wolbachia in the release strain directly reduces male competitive fitness and/or if the host nuclear background of the release strain has a detrimental effect on male field competitiveness. At least for Ae.aegypti carrying the wMel or wMelPop infection, there is no evidence that Wolbachia directly reduces male competitive fitness (Segoli et al. 2014), while Ae. polynesiensis carrying Wolbachia are also competitive in field releases (O’Connor et al. 2012). Detrimental host nuclear effects might develop if the release strain evolves and becomes adapted to conditions used for artificial rearing. This can be circumvented through backcrossing the release strain to field-sourced material prior to releases taking place, although it may then be more difficult to rear the strain under the artificial conditions if adaptation has taken place. Male competitiveness also needs to be high for successful Wolbachia strategies involving invasion (that utilize deleterious fitness effects and viral interference) because strong cytoplasmic incompatibility is required to drive the infection into a target population. In addition, several other attributes will be required for invasion-based strategies.

Ease of invasion into field populations

To produce disease suppression by interfering with pathogen transmission or expressing deleterious fitness effects, Wolbachia strains need to invade and reach high frequencies in focal populations. In Wolbachia strains that have so far been introduced into Ae. aegypti populations, cytoplasmic incompatibility has been complete or nearly complete with uninfected target populations (Xi et al. 2005; McMeniman et al. 2009; Walker et al. 2011; Yeap et al. 2011), facilitating invasions. As long as there are no substantial deleterious effects of the Wolbachia on the hosts and as long as the infection is transmitted with a relatively high fidelity, invasion should be possible under strong cytoplasmic incompatibility. However, if a focal population is already infected with a Wolbachia strain that shows bidirectional incompatibility with the release strain, invasion becomes more difficult. Under bidirectional incompatibility between two Wolbachia strains with equivalent deleterious effects on a host, the infection frequency of an introduced strain has to exceed 50% to achieve invasion (Hoffmann and Turelli 1997). This situation applies to the wMel infection introduced into Ae. albopictus (Table 1) which is bidirectionally incompatible with the naturally occurring Wolbachia of this species (Blagrove et al. 2012). Invasion will also depend on other fitness attributes such as the ability of females carrying the Wolbachia strain to feed and locate breeding sites and the ability of larvae with the Wolbachia strain to compete against other conspecific larvae and other species.

Reduced pathogen transmission

For effective suppression of vectorborne diseases (strategy (iii) from above), Wolbachia strains will need to directly interfere with pathogen transmission in vector species. In Aedes mosquitoes, this has often been assessed in laboratory-based assays where blood is mixed with virus cultures to mimic titres that might be found in infected humans (Moreira et al. 2009). However, it is ideally assessed by feeding mosquitoes directly on blood from infected humans and assessing pathogens in tissue through which transmission occurs, such as the salivary glands and saliva of mosquitoes (Ferguson et al. 2015).

The ability of Wolbachia to block viruses and other microbes will depend on the nature of the viruses and the Wolbachia strains. In Drosophila, it appears that some types of viruses (DNA viruses in particular) are not affected by the presence of Wolbachia in host cells,
whereas RNA viruses appear to be inhibited (Teixeira et al. 2008). The extent of inhibition varies dramatically between *Wolbachia* strains, such that some strains cause a dramatic reduction of the viral load in the host, whereas others have little impact (Table 1). In *Aedes* mosquitoes where stable *Wolbachia* infections have been established, the potential for *Wolbachia* to block different dengue virus serotypes and other RNA viruses seems to be high (Table 1, Appendix S1). The wMelPop infection appears to be highly efficacious in blocking different dengue serotypes as well as other arboviruses, at least in laboratory-based assays (Moreira et al. 2009; van den Hurk et al. 2012). For other *Wolbachia* infections, particularly wMel and wAlbB, blockage against dengue serotypes also appears robust (Bian et al. 2010; Frentiu et al. 2014), but somewhat weaker than provided by wMelPop (Walker et al. 2011). Recent data for wMel feeding on blood from infected human patients also point to strong blockage of dengue in saliva but show some differences among serotypes (Ferguson et al. 2015).

**Stable effects on hosts**

Once a high frequency of infection is reached through releases and subsequent invasion driven by cytoplasmic incompatibility and other effects, *Wolbachia* effects on hosts and on viral transmission need to be stable, even if there are evolutionary changes in the virus and/or changes in the host’s nuclear genome and *Wolbachia* genome. Data on the stability of *Wolbachia* effects following deliberate introductions are only just starting to emerge (Frentiu et al. 2014a; Hoffmann et al. 2014a), but there is some relevant information from natural *Wolbachia* infections in other systems and particularly in *Drosophila* (Chrostek et al. 2013). Strategies that utilize the deleterious host effects associated with *Wolbachia* infections (strategy ii from above) also require that such effects remain stable even when there might be strong selection in the host genome to counter them.

**Evolutionary changes in the host genome**

Evolution of host genomes in response to *Wolbachia* is certainly possible and is dramatically illustrated by the changes that nullify male killing by a natural *Wolbachia* infection in the butterfly *Hypolimnas bolina* (Hornett et al. 2006). Other relevant sources of evidence for such changes include experimental populations and longitudinal studies of natural populations.

Phenotypic changes in the expression of *Wolbachia* effects due to changes in the host nuclear genome have been documented in experimental host populations maintained both with and without deliberate selection pressures. These include evidence for nuclear-based attenuation of wMelPop effects on longevity in *D. melanogaster* hosts (Carrington et al. 2009) and in the novel host *D. simulans* (Carrington et al. 2010). When the wMelPop infection was transferred from *D. melanogaster* to *D. simulans*, it initially caused large fitness effects such as reducing fecundity and decreasing longevity as in its native host (McGraw et al. 2002). However, these effects attenuated quickly (Reynolds et al. 2003), such that wMelPop-infected *D. simulans* eventually exhibited an increase in longevity in some genetic backgrounds (Carrington et al. 2010). In *Ae. aegypti* mosquitoes, host genome changes can cause a decrease in deleterious effects of the introduced wMelPop on egg viability (A. Callahan and A. A. Hoffmann, unpublished data). The impact of host nuclear genomic backgrounds on virus blocking by *Wolbachia* has not yet been systematically investigated within either *Drosophila* or mosquito species. However, because the upregulation of immune response genes seems to be restricted to recently transferred infections in mosquitoes rather than native infections, an eventual decrease in blockage might be expected, given the likely high cost of constitutive immune gene expression.

The deliberate release of *Wolbachia* infections into natural mosquito populations provides an opportunity to test for host nuclear responses in natural populations across a period of a few years. In particular, the release of wMel into uninfected *Ae. aegypti* populations in 2011 in two areas around Cairns, Australia (Hoffmann et al. 2011), provided an opportunity to monitor changes in both the viral interference effect and deleterious host effect across a three-year time span. These comparisons have indicated that dengue interference was not altered within this period (Frentiu et al. 2014) and neither were fitness effects of *Wolbachia* on its host (Hoffmann et al. 2014a). Because there is ongoing gene flow into these populations as inferred from infection frequencies and a lack of maternal leakage (Hoffmann 2014b), changes in the nuclear genome due to *Wolbachia* are only expected if selection is relatively strong.

Although the host genome can have a substantial effect on the expression of cytoplasmic incompatibility, deleterious effects and viral interference, it is not yet clear whether there will be rapid changes in the host genome that might affect the success of *Wolbachia* releases aimed at disease suppression. The most rapid host changes are expected in response to any deleterious effects induced by *Wolbachia*, whereas selection for altered effects of *Wolbachia* on viral interference should be weak unless the virus has a particularly large impact on host fitness (in which case selection would favour ongoing interference by *Wolbachia*). The host genome is therefore most likely to influence the success of a suppression strategy based
on the expression of deleterious effects following invasion.

**Evolutionary changes in the Wolbachia genome**

Evidence for possible changes in the *Wolbachia* genome comes from analysis of changes in laboratory and natural populations. In addition, the phenotypic effects associated with particular *Wolbachia* strains that are maintained following interspecific transfers (as in the case of the *wMelPop* infection following transfer from *D. melanogaster* to *D. simulans* and *Ae. aegypti* – Table 1) also point to effects on hosts mediated by the *Wolbachia* genome rather than the host genome.

It is still difficult to predict whether genomic changes in *Wolbachia* will be rapid enough to be detectable in experimental populations. For the virulent *wMelPop* infection, there have only been minor genomic changes since its introduction from *D. melanogaster* into the new host *Ae. aegypti* (Woolfit et al. 2013). On the other hand, in laboratory *D. melanogaster* populations, Octomom copy number seems to be able to evolve rapidly to alter the density of *wMelPop* (Chrostek and Teixeira 2015). There is also evidence from comparisons of conspecific populations of *D. melanogaster* that interactions between *wMel Wolbachia* and host genomes can evolve fairly rapidly (Olsen et al. 2001; Fry et al. 2004). The *wRi* infection of *D. simulans* is another such example (Weeks et al. 2007). The deleterious effects of this infection on female reproduction were first characterized in the late 1980s (Hoffmann et al. 1990). Twenty years on, such effects were no longer evident, and some infected females even showed a fecundity advantage over uninfected hosts, largely attributable to changes in *wRi* or another maternally inherited factor (Weeks et al. 2007).

These findings suggest that while there is ample evidence for variation in the *Wolbachia* genome resulting in multiple strains of *Wolbachia* occurring in the same host and/or conspecific individuals carrying different *Wolbachia* strains, it is not clear whether there will be rapid changes in *Wolbachia* strains being released for disease suppression. As in the case of host genome changes, any changes will most likely lead to *Wolbachia* strains that exert a reduced deleterious effect on their host, which might only indirectly influence viral interference.

**Evolutionary changes in the viral genome**

While viruses evolve rapidly, changes in the virus genome in response to *Wolbachia* are largely unpredictable due to a lack of relevant background information and clarity around selective factors involved (Bull and Turelli 2013). Selection on viral resistance to the blocking effects of *Wolbachia* might be expected, particularly given that there are differences in the extent to which dengue serotypes are blocked by *Wolbachia* (Frentiu et al. 2014; Ferguson et al. 2015). However, only some types of interactions between *Wolbachia* and viruses (such as direct competition between viruses and *Wolbachia*) are expected to lead to evolutionary changes (Bull and Turelli 2013). Moreover, viral evolutionary dynamics are affected by a number of factors unconnected to *Wolbachia* that drive viral strain replacements (Vu et al. 2010; Lambrechts et al. 2012). *Wolbachia* and/or host genomes could also evolve in response to any changes in the virus, particularly if these affect the fitness of the vector host, although (at least in the case of dengue) viral effects on hosts remain unclear (Maciel-de-Freitas et al. 2011).

**Other effects of Wolbachia**

Even though *Wolbachia* can decrease transmission of many viral infections, its effects on others remain uncertain. A comparison of *Wolbachia*-infected and cured *D. melanogaster* strains and *Culex quinquefasciatus* strains suggested that *Wolbachia* might block West Nile virus (Glaser and Meola 2010). However, most *Culex quinquefasciatus* populations appear naturally infected with *Wolbachia* but are still capable of transmitting West Nile (Micieli and Glaser 2014). This may reflect the fact that *Wolbachia* densities in natural infections are too low to have much impact on transmission of this virus. On the other hand, in a recent study where *Wolbachia* from another mosquito were injected into *Culex dorsalis* females, the titre of West Nile virus increased (Dodson et al. 2014), although this may have been an effect of the infection process; the effect of *Wolbachia* on West Nile needs to be investigated in a host mosquito species carrying a stably introduced *Wolbachia* infection. In *Spodoptera* moths, *Wolbachia* may also increase susceptibility to a virus (Graham et al. 2012); infection by nucleopolyhedrovirus was associated with moths carrying different strains of *Wolbachia*, and laboratory tests with one of the *Wolbachia* strains (likely a male killer) indicated much higher mortality levels following the viral infection. Because nucleopolyhedrovirus is being explored as a potential biopesticide, this result might point to a potentially novel application of *Wolbachia* releases for pest control.

It is not yet clear whether *Wolbachia*-based strategies will be effective against microbes other than viruses. *Wolbachia* introduced into the major malaria vector *Anopheles stephensi* protects against *Plasmodium* to some extent (Bian et al. 2013b), although perhaps insufficiently to provide much impact on disease transmission (Killeen et al. 2013). Moreover, it has been suggested that the presence of
Wolbachia may even enhance the incidence of malaria pathogens to some extent (Zélé et al. 2014) although this requires further validation. In Drosophila, Wolbachia infections appear to have few consistent effects on bacterial infections (Wong et al. 2011), while in mosquitoes, it has been suggested that any effects on bacteria will depend on whether the immune system is upregulated following Wolbachia transfer (Ye et al. 2013).

Another issue relevant to disease transmission is the potential interaction between Wolbachia and pesticide susceptibility. For Ae. aegypti mosquitoes that are artificially infected with Wolbachia, the infection does not affect susceptibility to commonly used insecticides (Endersby and Hoffmann 2013). However, in Culex pipiens naturally infected with Wolbachia, there was rapid evolutionary increase of Wolbachia density in an insecticide-resistant line (Echaubard et al. 2010), suggesting a dynamic interaction between the Wolbachia and/or host genomes evolving under insecticide exposure.

Because most Wolbachia-transfected lines originate from few or just one female (Xi et al. 2005; McMeniman et al. 2009), Wolbachia invasions can cause a dramatic reduction of mitochondrial haplotype diversity within and among populations (H. L. Yeap and A. A. Hoffmann, unpublished data; Armbruster et al. 2003). There is a growing body of evidence linking the mitochondrial polymorphisms with differences in metabolic rate and some fitness components in Drosophila (e.g. Ballard et al. 2007; Kurbanlija Novičić et al. 2015), suggesting that mitochondrial diversity in natural populations is maintained by natural selection. Mitochondrial variation might play an important role in the epistatic interaction between the mitochondrial and nuclear genomes in determining insect metabolic rate under varying environmental conditions (Arnvist et al. 2010). It is therefore possible that the loss of mitochondrial diversity following Wolbachia invasion could affect the performance of infected populations.

Finally, the various Wolbachia effects on host fitness could change the size and age distribution of the mosquito larval community in containers (Mains et al. 2013). These effects in turn might influence interspecific interactions, particularly under high-density larval conditions when fitness differences between Wolbachia-infected strains and uninfected strains can become accentuated (Ross et al. 2014). These ecological effects of Wolbachia need to be evaluated following invasions into natural communities and could have a substantial effect on disease transmission if vector populations become suppressed due to the detrimental effects of Wolbachia infection. The most dramatic example involves the wMelPop infection of Ae. aegypti, which reduces the viability of eggs when held in a dried state (Yeap et al. 2011). During a dry season, this effect could result in the complete collapse of an isolated population until there is a reinvansion from another source (Rašič et al. 2014a). Population cage experiments indicate that collapse is likely in populations that are completely Wolbachia-infected (S. Ritchie unpublished data).

**Figure 1** The first two principal components explaining 80.6% of total variation among Wolbachia strains in natural and transfected hosts for the levels of: cytoplasmic incompatibility/male killing, maternal transmission, fitness costs and blockage of RNA viruses. Empty symbols denote natural Wolbachia infections, and filled symbols denote transfections. Each infection attribute is ordered as: 0 (no effect), 1 (low), 2 (medium/partial) or 3 (high/full effect). Fitness cost has an additional value of −1 for infection effects that are somewhat beneficial. Twenty-one data points summarize values extracted from Table 1. Overall effects in natural hosts seem different from those in transfected hosts, and the effects are also virus-dependent. Colinearity between fitness cost and viral blockage suggest that there is a possible trade-off between these effects, such that strains with strong viral protection might be difficult to spread due to higher deleterious effects on the host. Exceptions to these patterns, however, indicate that it may be possible to achieve a desirable combination of infection attributes, but more strains need to be tested.

**A pathogen interference/spread trade-off?**

It is possible that Wolbachia infections that provide the strongest blockage of pathogen transmission might not spread easily into populations (Fig. 1). This possibility arises because a high density of Wolbachia in hosts may increase viral blockage but decrease host fitness (Chrostek et al. 2013; Sinkins 2013; Martinez et al. 2014), and such a trade-off could have driven past cycles of Wolbachia strain replacements in natural populations. For instance, the wMel-CS strain in D. melanogaster which causes strong virus blockage (Table 1) may have been replaced with the wMel strain which causes weaker blockage but does not decrease longevity to the same extent in this host (Chrostek et al. 2013). Relevant information to explore the notion of such a trade-off comes from (i) comparisons of viral
suppression, host fitness and \textit{Wolbachia} density between infected hosts, (ii) inferences from natural populations and (iii) mechanistic understanding of the common basis of viral interference.

\textbf{Viral suppression vs host fitness and \textit{Wolbachia} density}

Several authors have contrasted viral blockage (measured as survival/longevity following pathogen infection) in \textit{Wolbachia} strains from \textit{Drosophila} with effects on host fitness (mostly measured as longevity in the absence of the infection) and on cytoplasmic incompatibility (Table 1). These comparisons involve a relatively limited number of \textit{Wolbachia} infections and a comparison of natural and introduced \textit{Wolbachia} strains which may have different dynamics (Table 1, Fig. 1). In \textit{D. simulans}, where the largest number of comparisons have been made involving 19 strains, survival following RNA viral infection is positively correlated with \textit{Wolbachia} tissue density, although there are strains with relatively high \textit{Wolbachia} densities that have a limited impact on survival (Martinez et al. 2014). Any association between deleterious \textit{Wolbachia} effects and viral blockage may also not be particularly strong. In a comparison of five \textit{Wolbachia} strains including one from a non-native host (\textit{D. melanogaster}), the wAu infection caused the strongest blockage and had the highest density across tissues (Osborne et al. 2009, 2012), yet this strain does not cause detectable cytoplasmic incompatibility or have deleterious fitness effects, and is also transmitted at a high fidelity under field conditions (Hoffmann et al. 1996).

The wMelPop infection was transferred from \textit{D. melanogaster} to \textit{Ae. aegypti} to generate a strain that has a reduced longevity and thereby a reduced propensity to transmit diseases requiring a long incubation period through older females (McMeniman et al. 2009). In subsequent experiments, this strain was shown to have very strong blockage of viral replication and disease transmission in laboratory assays (Moreira et al. 2009). However, the wMelPop infection also causes substantial fitness costs, not just to longevity but also for egg viability, particularly when eggs are in a quiescent stage (McMeniman and O’Neill 2010; Yeap et al. 2011). The wMelPop infection also has deleterious effects on larval development under crowded conditions (Ross et al. 2014) and on some adult traits (e.g. Turley et al. 2009). In contrast, the wMel infection causes somewhat weaker blockage of dengue and other viruses than wMelPop, but has fewer deleterious effects as well as having a lower titre in adults (Walker et al. 2011; Hoffmann et al. 2014a).

The wMel infection was also transferred to \textit{Ae. albopictus} where it causes strong blockage of chikungunya virus and dengue in laboratory assays, but has no apparent effects on longevity, hatch rates or other laboratory fitness parameters, despite generating strong cytoplasmic incompatibility (Blagrove et al. 2012, 2013). The wAlbB infection that blocks the transmission of dengue viruses in \textit{Ae.aegypti} (Xi et al. 2005; Bian et al. 2010) has deleterious fitness effects on its host including a decrease in the viability of quiescent eggs and a reduction in longevity, although these deleterious effects are weaker compared to those exerted by wMelPop (J. Axford, unpublished data). When the native wPolA infection in \textit{Ae. polynesiensis} was replaced with wAlbB from \textit{Ae. albopictus}, there was an increase in \textit{Wolbachia} density and evidence of dengue blocking in this species (Bian et al. 2013a), although it is not yet clear whether this transferred strain also produced deleterious fitness effects (Table 1).

\textit{Wolbachia} density represents a complex phenotype, typically measured in three contexts: (i) whole body density, usually measured in newly eclosed adults; (ii) tissue specific density, focussing on heads, abdomens, ovaries, testes, salivary glands and so on; and (iii) age-specific (and life stage-specific) density, which can indicate whether \textit{Wolbachia} continue to replicate when hosts have reached maturity or enter a quiescent phase. Changes in whole body density through exposure to low levels of antibiotics (usually tetracycline) typically reduce cytoplasmic incompatibility induced by \textit{Wolbachia}, as demonstrated in the case of \textit{D. simulans} (Clancy and Hoffmann 1998) and \textit{Nasonia} wasps (Breeuwer and Werren 1993), and also reduce viral interference as shown for wAu in \textit{D. simulans} (Osborne et al. 2012). These experimental data support the notion that differences in \textit{Wolbachia} density can be linked to the expression of host effects and support the notion of a blocking/spread trade-off, particularly given that strain variation in \textit{Wolbachia} density has a positive relationship to blockage in \textit{D. simulans} as noted above (Martinez et al. 2014). However, the expression of strong cytoplasmic incompatibility in the \textit{Drosophila paulistorum} species complex involves very low \textit{Wolbachia} titres that can only be detected although nonconventional molecular methods (Miller et al. 2010), whereas high-density infections of other \textit{Drosophila} species such as wAu (Osborne et al. 2012) have no detectable effects on cytoplasmic incompatibility or host fitness (Hoffmann et al. 1996). The effects of some infections can therefore be unconnected to their overall densities in hosts.

The tissue distribution of strains may influence pathogen blocking and host effects. For instance, the wRi and wHa infections in \textit{D. simulans} are restricted mostly to gonadal tissues (Binnington and Hoffmann 1989; Correa and Ballard 2014), have mild deleterious effects (Hoffmann et al. 1990; Turelli and Hoffmann 1995) and cause mid- to low-level viral blockage (Osborne et al. 2009). On the other
hand, the wAu and wMelPop infections may block pathogens effectively because they are found in a variety of tissues (Min and Benzer 1997; Osborne et al. 2012). In mosquitoes, Wolbachia presence in a variety of tissues through which a virus needs to pass to be transmitted may be crucial for generating strong transmission blockage; for instance, wMelPop which causes strong blockage is found in many tissues including the salivary glands of Ae. aegypti (Moreira et al. 2009). This feature seems particularly important for dengue viruses, where a density-dependent cellular relationship between Wolbachia and viral load has been reported (Lu et al. 2012).

Some Wolbachia infections attain higher densities at eclosion and replicate at a higher rate than others when hosts reach adulthood (Chrostek and Teixeira 2013), resulting in very high densities throughout the body as hosts age. While this high density might result in strong pathogen blockage, it could also eventually kill the host and limit the potential of such infections to spread. The reduced longevity of D. melanogaster infected by the wMelPop strain is thought to be due to ongoing replication and increasing density of this virus (Min and Benzer 1997), as is the reduction in longevity and increased mortality of quiescent eggs in Ae. aegypti artificially infected by wMelPop (McMeniman and O’Neill 2010; Yeap et al. 2011). Continued Wolbachia replication may also contribute to hybrid sterility in crosses between D. paulistorum semi-species (Miller et al. 2010).

The distribution of Wolbachia within hosts is expected to be altered due to evolutionary changes in the host and Wolbachia. The distribution of Wolbachia densities across tissues in long-standing infections is expected to become more variable if there is no evolution towards obligate relationship with the host (Correa and Ballard 2014). Strong cytoplasmic incompatibility with infected sperm should favour accurate transmission of an infection across generations, resulting in strong tissue tropism. However, for old infections where cytoplasmic incompatibility is weak (e.g. wMa in D. simulans), Wolbachia density in tissues is expected to be variable because selection pressures for accurate transmission are weak (Correa and Ballard 2014). Such evolutionary changes are expected to weaken any blocking/spread trade-off.

These examples provide some support for a possible relationship between viral blockage, deleterious host effects and Wolbachia density, but too few strains have so far been examined. Moreover, the Drosophila data suggest that it is possible to identify infected lines demonstrating strong blockage, strong cytoplasmic incompatibility and no apparent fitness effects on the host. However, it is not yet clear whether such lines can be developed from novel combinations of hosts and infections generated through artificial transfers of Wolbachia.

Inferences from changes in natural populations

Although the potential benefits that hosts gain from pathogen blocking have so far only been demonstrated in laboratory tests (Chrostek et al. 2013), it seems likely that similar benefits will occur under field conditions. Recently, the wAu infection in D. simulans which causes strong viral blockage but no detectable cytoplasmic incompatibility (Hoffmann et al. 1996) has nevertheless been shown to induce a rapid increase in viral load in infectious vectors (Kriesner et al. 2013), suggesting that the infection provides a fitness advantage to its host which may include viral blocking. Another example is the wMel infection of D. melanogaster, which exhibits a stable cline in eastern Australia suggestive of selection (Hoffmann et al. 1994), but causes only partial cytoplasmic incompatibility in matings with young males (Reynolds et al. 2003). Given that this infection shows incomplete maternal transmission, it is hard to explain its persistence in D. melanogaster populations without assuming some sort of fitness benefit (Hoffmann et al. 1994). However, we still lack field data testing for a direct association between Wolbachia infection and natural viral load. If field strains exist that provide a fitness advantage under a high viral load but have few other effects on hosts, these would indicate that a blocking/spread trade-off can be avoided.

Mechanistic understanding of viral interference/host effects – immune priming and other effects

If the mechanisms involved in viral blockage, cytoplasmic incompatibility, and host fitness effects were understood, it might help in predicting likely interactions among Wolbachia effects. Viral blocking by Wolbachia seems to involve a number of subcomponents (Rances et al. 2013; Sinkins 2013). Part of the blockage may come from the upregulation of the immune system, as suggested by the increased expression of some immune response genes following recent Wolbachia transfers in mosquitoes (Kambris et al. 2009; Lu et al. 2012). However, cross-species transfers of Wolbachia do not necessarily lead to immune priming, as in the case of the experimental wAu infection of D. melanogaster (Chrostek et al. 2014). Other mechanisms have also been implicated, such as competition for resources such as cholesterol, interactions involving various metabolites, and the expression of microRNAs (Caragata et al. 2013; Zhang et al. 2013). Blockage mechanisms may be partly related to changes in the tissue distribution and density of Wolbachia particularly following transfer to a new host. For instance, native Wolbachia infections of Ae. albopictus have a relatively low density; the natural wAlbB infection of Ae. albopictus seems to cause some suppression of dengue and chikungunya viruses in its native host.
(Mousson et al. 2012). However, following transfer from *Ae. albopictus* into *Ae. aegypti*, the same infection develops a much higher density and blocking effect (Lu et al. 2012).

Overall, these different lines of evidence point to a complicated pattern of interaction between pathogen blockage, deleterious fitness effects and cytoplasmic incompatibility. Host effects are not necessarily tightly linked mechanistically or through density, and a trade-off between blockage and spread might exist when host effects are predominantly related to density, but might in other cases be circumvented (Fig. 1). The *Drosophila* data indicate that strains such as *wAu* with strong blockage, no deleterious effects, high densities and no cytoplasmic incompatibility exist in populations alongside strains such as *wHa* that cause strong cytoplasmic incompatibility, but no blockage or large deleterious effects. A range of infections with different combinations of attributes occur in natural populations, including strains that might exhibit relatively strong blockage while also being able to easily spread in the absence of over replication after eclosion, and a high density in reproductive tissues to ensure strong cytoplasmic incompatibility and high maternal transmission. Unfortunately, the same combination of attributes might not be maintained after such a strain is transferred to a target vector host. For example, the *wMel* infection causes weak cytoplasmic incompatibility in its native *Drosophila* host but complete cytoplasmic incompatibility once transferred to *Ae. aegypti*, which has been essential for its successful spread (Hoffmann et al. 2011). Similarly, the *wAu* infection has no detectable fitness effect in its native host *D. simulans*, but causes a sharp reduction in lifespan and exhibits exponential growth when transferred to *D. melanogaster* (Chrostek et al. 2014). Therefore, intra- and intergeneric transfers across host species have unexpected consequences that may affect the suitability of strains for disease suppression.

**Other deployment issues**

**Host population ecology**

The successful invasion of *Wolbachia* infections will depend on the ecology of the host population. For example, if wMelPop is released into a host mosquito population where breeding sites lead to rapid egg hatch and where larvae develop under low densities, *Wolbachia* is more likely to invade. This is because the wMelPop infection does not strongly affect host viability and development time under low-density conditions and in the absence of dry conditions (McMeniman and O’Neill 2010; Yeap et al. 2011). On the other hand, there are development time and viability costs when wMelPop-infected mosquitoes are reared at a high density in competition with uninfected larvae (Ross et al. 2014). High-density conditions coupled with an extended period of dry season will raise costs and the threshold *Wolbachia* frequency required for a wMelPop invasion (Hancock et al. 2011; Yeap et al. 2014).

Areas of high mosquito density could be identified through factors such as housing characteristics, distribution of breeding containers and so on if this information is available from past surveys. Such information can be used to inform local invasion rates (Hoffmann et al. 2014b) and potential pockets where uninfected mosquitoes might persist and require additional treatment. Local knowledge of the ecology of mosquito populations should be used to inform release strategies; for instance, breeding containers that fill only occasionally after rain may need to be treated to remove sources of uninfected mosquitoes.

Release programmes also need to take into account expected movement patterns of mosquitoes and variation in host density across the region. Information on natural movement patterns from mark-release experiments or genetic analyses of local populations (e.g. Harrington et al. 2005; Olanratmanee et al. 2013) can provide a picture of likely movement patterns. By characterizing thousands of SNP markers, a much higher level of resolution of population structure can be obtained, and the movement of related individuals across a region can also be followed (Rašić et al. 2014b).

*Wolbachia* invasion into an isolated uninfected population of a target host only occurs if *Wolbachia* frequencies consistently exceed a particular frequency set by the size of the deleterious effects of *Wolbachia* on its host, levels of cytoplasmic incompatibility and to a lesser extent by the fidelity of maternal transmission (Hoffmann and Turelli 1997; Turelli 2010). If deleterious host effects associated with *Wolbachia* infections are too large, *Wolbachia* invasion into target host populations becomes difficult and high infection frequencies might not be sustained even if invasion succeeds. Invasion and persistence become increasingly unlikely if there is ongoing immigration of uninfected individuals into a relatively small release area (Barton and Turelli 2011) and if there are fitness effects of *Wolbachia* that decrease the size of the target population, making reinvasion by uninfected mosquitoes more likely (Rašić et al. 2014a).

A benefit of releasing infections with at least some deleterious fitness effects is that infections are expected to remain contained within an area rather than spreading rampantly (Barton and Turelli 2011; Hancock and Godfray 2012). This prediction is consistent with field experience from wMel releases around Cairns, Australia, where wMel did not spread outside areas where they were released even though *Wolbachia* were occasionally detected in other areas (Hoffmann et al. 2011, 2014b). Spread only occurs relatively slowly through a continuous residential area and is likely to be stopped by barriers to movement and high-density areas occupied by uninfected mosquitoes (Barton and...
Wolbachia strains for disease control

Turelli 2011; Hancock and Godfray 2012; Hoffmann et al. 2014b). Spread is much easier to achieve when a large area with a high host density has been invaded and the surrounding area has a low density; an increase in host density outside the invaded zone can stop Wolbachia spread, particularly if the invasion point is high (Barton and Turelli 2011), as in the case of wMelPop (Yeap et al. 2011). Moreover, invasions might then fail to persist with a moderate influx of migrants into a population (Hancock et al. 2011).

Although the host fitness costs associated with Wolbachia infections could be used to suppress and even eradicate mosquito hosts in some isolated areas (Rašić et al. 2014a), they provide challenges for the infection spreading in large and continuously distributed mosquito populations. So far, attempts to spread the high cost wMelPop infection into relatively isolated natural populations in Vietnam and northern Australia have failed, despite high release rates and some success in getting the infection to a high frequency (T. H. Nguyen, unpublished data). The wMelPop infection did successfully invade semi-field population cages, but only when release rates were high and sustained for many weeks (Walker et al. 2011). Several strategies could assist in spreading infections with high deleterious effects, such as through the suppression of host populations across all life stages just prior to release (Hoffmann 2014), through the release of male-biased sex ratios (Hancock et al. 2011) or through the use of pesticide resistance genes and application of pesticides during the release process (Hoffmann and Turelli 2013). These strategies should assist in introducing such infections into relatively isolated populations, but the infection is unlikely to spread further outside these areas (Barton and Turelli 2011).

Community acceptance

Although the likely benefits and costs of Wolbachia-based strategies for disease suppression can be identified to some extent, the final strategy and strain adopted will also depend on community acceptance and regulatory approval. A challenge for Wolbachia releases aimed at invasion and replacement is that there will be a period of time when mosquito numbers are increased above background levels to ensure that the Wolbachia infection exceeds an invasion threshold. As long as there are no fitness costs associated with the infection, Wolbachia is expected to spread from a very low starting frequency (close to 0%) depending on stochastic factors, with a slow rate of spread initially (Jansen et al. 2008). This type of spread has been observed in natural infections of D. simulans where resident populations number in the millions (Kriesner et al. 2013). However, with a threshold frequency of around 20–30%, the wMel invasion into uninfected Ae. aegypti required releases across 10 weeks, at which time adult numbers increased by a factor of 1.5–2 (Hoffmann et al. 2011; Ritchie et al. 2013). The period of time and relative increase in mosquito numbers required will be greater if infections are costly, and/or if the release material has a relatively low fitness.

While a 1.5–2 fold increase in mosquito numbers might seem trivial, particularly when only one mosquito species is being targeted in release areas where several species are likely to co-occur, implementation of such a strategy can be challenging. In most countries where dengue is endemic and attributable to Aedes aegypti mosquitoes which breed around houses, communities are encouraged to decrease the availability of breeding sites, removing containers that might hold standing water, treating containers with chemicals, and perhaps fogging an entire area with pesticides. Such combined programmes can be effective in reducing mosquito densities (Erlanger et al. 2008), but often there is little impact on mosquito populations due to factors such as cryptic breeding sites that cannot be easily targeted (Heintze et al. 2007; Eisen et al. 2009). These strategies can also generate additional problems such as the evolution of pesticide resistance in hosts (Maciel-de-Freitas et al. 2014). Furthermore, there is often a poor correlation between measures of mosquito numbers and disease incidence (Bowman et al. 2014), making it difficult to justify such campaigns. Nevertheless, while education and engagement campaigns can help increase acceptance of Wolbachia releases (McNaughton and Huong 2014), communities may be reluctant to participate in Wolbachia release programmes and regulatory authorities may be reluctant to approve strategies where there is a deliberate increase in mosquito numbers over a period of time.

This issue becomes particularly important where the Wolbachia strains being introduced have high invasion thresholds and therefore require high release numbers across an extended period of time. For instance, wMelPop failed to invade isolated field populations despite releases across several months where frequencies exceeded 70% (T. H. Nguyen, unpublished data). Even when this infection invaded semi-field cages, it required more than 80 days before the infection reached fixation in one cage, despite a starting infection frequency of 65% (Walker et al. 2011). In contrast, infections such as wMel seem to invade quite readily, at least based on experience in Australia.

One of the advantages of Wolbachia releases is that they are not necessarily incompatible with other control programmes. For instance, during the 2011 release of wMel around Cairns, Australia, pesticides were applied by the health authorities to a residential block within the release site where a dengue case had been reported, and this did not inadvertently affect the local rate of increase of Wolbachia (Hoffmann et al. 2011). In this case, both the resident uninfected population of Aedes aegypti and the released mosquitoes did not contain appreciable levels of insecticide
resistance. In contrast, in many communities where there has been widespread application of pyrethroids and other insecticides over some time, resistance levels in uninfected *Ae. aegypti* are expected to be high (Ranson et al. 2009). In such cases, insecticide application during the release could lead to a preferential removal of the infected released mosquitoes. However, it should be possible to minimize this issue by backcrossing infected release stock to the local genetic background of a target population with high insecticide resistance.

Finally, when there are community concerns about release numbers increasing above background levels, suppression of mosquitoes prior to starting releases could help to alleviate community concerns, as well as speeding up *Wolbachia* invasions by increasing the frequency of *Wolbachia*, and by producing vacant breeding sites for infected released females. In addition, it may be possible to release large numbers of nonbiting infected male mosquitoes to facilitate invasions when these males generate cytoplasmic incompatibility with uninfected mosquitoes (Hancock et al. 2011). Pesticide applications could also assist invasions if the release material carries a higher level of resistance than the resident population (Hoffmann and Turelli 2013). Although there is little risk that resistance alleles will spread to the uninfected resident populations as long as cytoplasmic incompatibility is complete and maternal transmission is high, this strategy is unlikely to be approved by regulators except in limited circumstances, for instance, where relevant genes are already present in a target population.

**Conclusions**

Selecting a suitable strain of *Wolbachia* for release is not a straightforward process, and involves a balance between minimizing fitness costs while maximizing cytoplasmic incompatibility and blockage of disease agents, as well as considering community and regulatory issues. It is not yet clear to what extent desirable strain qualities can be combined or whether there are trade-offs that limit the options available. It seems essential to create and test a number of *Wolbachia* infections for releases, despite the challenges associated with this exercise that require thousands of microinjections to achieve success (McMeniman et al. 2009; Bian et al. 2013b). Nevertheless, there are many natural *Wolbachia* strains available within Diptera for potential introduction into disease vectors. Once a suitable strain has been identified, it will be necessary to monitor the long-term stability of the desirable effects because there may be further evolutionary changes in the host, *Wolbachia* and pathogen genomes that could modify *Wolbachia* effects, even though current data suggest they are relatively stable.

**Literature cited**

Ahantarg, A., W. Trinachartvanit, and P. Kittayapong 2008. Relative *Wolbachia* density of field-collected *Aedes albopictus* mosquitoes in Thailand. Journal of Vector Ecology 33:173–177.

Ahmed, M. Z., O. F. C. Greyvenstein, C. Erasmus, J. J. Welch, and J. M. Greeff 2013. Consistently high incidence of *Wolbachia* in global *Aedes* wasp communities. Ecological Entomology 38:147–154.

de Almeida, F., A. S. Moura, A. F. Cardoso, C. E. Winter, A. T. Bijuovsky, and L. Suebek 2011. Effects of *Wolbachia* on fitness of *Culex quinquefasciatus* (Diptera: Culicidae). Infection, Genetics and Evolution 11:2138–2143.

Andrews, E. S., P. R. Crain, Y. Fu, D. K. Howe, and S. L. Dobson 2012. Reactive oxygen species production and *Brugia pahangi* surviviorship in *Aedes polynesiensis* with artificial *Wolbachia* infection types. PloS Pathogens 8:e1003075.

Armbruster, P., W. E. Damsky, R. Giordano, J. Birungi, L. E. Munstermann, and J. E. Conn 2003. Infection of new- and old-world *Aedes albopictus* (Diptera: Culicidae) by the intracellular parasite *Wolbachia*: implications for host mitochondrial DNA evolution. Journal of Medical Entomology 40:356–360.

Armynist, G., D. K. Dowling, P. Eady, L. Gay, T. Tregenza, M. Tuda, and D. J. Hosken 2010. Genetic architecture of metabolic rate: environment specific epistasis between mitochondrial and nuclear genes in an insect. Evolution 64:3354–3363.

Attyame, C. M., F. Delsuc, N. Pasteur, M. Weill, and O. Duron 2011. Diversification of *Wolbachia* endosymbiont in the *Culex pipiens* mosquito. Molecular Biology and Evolution 28:2761–2772.

Baldo, L., J. C. D. Hotopp, K. A. Jolley, S. R. Bordenstein, S. A. Biber, R. R. Choudhury, C. Hayashi et al. 2006. Multilocus sequence typing system for the endosymbiont *Wolbachia pipientis*. Applied and Environmental Microbiology 72:7098–7110.

Ballard, J. W. O., R. G. Melvin, S. D. Katewa, and K. Maas 2007. Mitochondrial DNA variation is associated with measurable differences in life-history traits and mitochondrial metabolism in *Drosophila simulans*. Evolution 61:1735–1747.

Barton, N. H., and M. Turelli 2011. Spatial waves of advance with bistable dynamics: cytoplasmic and genetic analogues of Allee effects. The American Naturalist 178:E48–E75.

Bian, G. W., Y. Xu, P. Lu, Y. Xie, and Z. Y. Xi 2010. The endosymbiotic bacterium *Wolbachia* induces resistance to dengue virus in *Aedes aegypti*. PloS Pathogens 6:e1000833.

Bian, G. W., G. L. Zhou, P. Lu, and Z. Y. Xi 2013a. Replacing a native *Wolbachia* with a novel strain results in an increase in endosymbiont load and resistance to dengue virus in a mosquito vector. PloS Neglected Tropical Diseases 7:e22350.

Bian, G. W., D. Joshi, Y. M. Dong, P. Lu, G. L. Zhou, X. L. Pan, Y. Xu et al. 2013b. *Wolbachia* invades *Anopheles stephensi* populations and induces refractoriness to *Plasmodium* infection. Science 340:748–751.

Bing, X. L., W. Q. Xia, J. D. Gui, G. H. Yan, X. W. Wang, and S. S. Liu 2014. Diversity and evolution of the *Wolbachia* endosymbionts of *Bemisia* (Hemiptera: Aleyrodidae) whiteflies. Ecology and Evolution 4:2714–2737.

Binnington, K. C., and A. A. Hoffmann 1989. *Wolbachia* like organisms and cytoplasmic incompatibility in *Drosophila simulans*. Journal of Invertebrate Pathology 58:344–352.

Blagrove, M. S. C., A. Arias-Goeta, A. B. Failloux, and S. P. Sinkins 2012. *Wolbachia* strain wMel induces cytoplasmic incompatibility and blocks dengue transmission in *Aedes albopictus*. Proceedings of the National Academy of Sciences of the USA 109:255–260.
Blagrove, M. S. C., C. Arias-Goeta, C. Di Genua, A. B. Failloux, and S. P. Sinkins 2013. A Wolbachia wMel transfection in Aedes albopictus is not detrimental to host fitness and inhibits Chikungunya virus. PloS Neglected Tropical Diseases 7:e2152.

Bordenstein, S. R., and S. R. Bordenstein 2011. Temperature affects the tripartite interactions between bacteriophage WO, Wolbachia, and cytoplasmic incompatibility. PLoS ONE 6:e29106.

Bourtzis, K., S. L, Dobson, Z. Xi, J. L, Rasgon, M. Calvitti, L. A. Moreira, H. C. Bossin et al. 2014. Harnessing mosquito-Wolbachia symbiosis for vector and disease control. Acta Tropica 132:S150–S163.

Bowman, L. R., S. Runge-Ranzinger, and P. J. McCall 2014. Assessing the relationship between vector indices and dengue transmission: a systematic review of the evidence. PloS Neglected Tropical Diseases 8:e2848.

Boyle, L., S. L. O’Neill, H. M. Robertson, and T. L. Karr 1993. Interspecific and intraspecific horizontal transfer of Wolbachia in Drosophila. Science 260:1796–1799.

Breuner, J. A. J., and J. H. Werren 1993. Cytoplasmic incompatibility and bacterial density in Nasonia vitripennis. Genetics 135:565–574.

Bull, J. J., and M. Turelli 2013. Wolbachia versus dengue: evolutionary forecasts. Evolution, Medicine, and Public Health 1:197–207.

Calvitti, M., R. Moretti, D. Forretta, R. Bellini, and S. Urbanelli 2009. Effects on male fitness of removing Wolbachia infections from the mosquito Aedes albopictus. Medical and Veterinary Entomology 23:132–140.

Calvitti, M., R. Moretti, E. Lamparzi, R. Bellini, and S. L. Dobson 2010. Characterization of a new Aedes albopictus (Diptera: Culicidae) Wolbachia pipiens (Rickettsiiales: Rickettsiaceae) symbiotic association generated by artificial transfer of the wPip Strain from Culex pipiens (Diptera: Culicidae). Journal of Medical Entomology 47:179–187.

Caragata, E. P., E. Rances, L. M. Hedges, A. W. Gofton, K. N. Johnson, S. L. O’Neill, and E. A. McGraw 2013. Dietary cholesterol modulates pathogen blocking by Wolbachia. PloS Pathogens 9:e1003459.

Carrington, L. B., J. Leslie, A. R. Weeks, and A. A. Hoffmann 2009. The popcorn Wolbachia infection of Drosophila melanogaster: can selection alter Wolbachia longevity effects? Evolution 63:2648–2657.

Carrington, L. B., A. A. Hoffmann, and A. R. Weeks 2010. Monitoring long-term evolutionary changes following Wolbachia introduction into a novel host: the Wolbachia popcorn infection in Drosophila simulans. Proceedings of the Royal Society B-Biological Sciences 277:2059–2068.

Charlat, S., A. Nirgianaki, K. Bourtzis, and H. Mercot 2002. Evolution of Wolbachia-induced cytoplasmic incompatibility in Drosophila simulans and D. sechellia. Evolution 56:1735–1742.

Chrostek, E., and L. Teixeira 2015. Mutualism breakdown by amplification of Wolbachia genes. PloS Biology 13:e1002065.

Chrostek, E., M. S. P. Marialva, S. S. Esteves, L. A. Weinert, J. Martinez, F. M. Jiggins, and L. Teixeira 2013. Wolbachia variants induce differential protection to viruses in Drosophila melanogaster: a phenotypic and phylogenomic analysis. PloS Genetics 9:e1003896.

Chrostek, E., M. S. P. Marialva, R. Yamada, S. L. O’Neill, and L. Teixeira 2014. High anti-viral protection without immune upregulation after interspecies Wolbachia transfer. PLoS ONE 9:e99025.

Clancy, D. J., and A. A. Hoffmann 1997. Behavior of Wolbachia endosymbionts from Drosophila simulans in Drosophila serrata, a novel host. The American Naturalist 149:975–988.

Clancy, D. J., and A. A. Hoffmann 1998. Environmental effects on cytoplasmic incompatibility and bacterial load in Wolbachia-infected Drosophila simulans. Entomologia Experimentalis Et Applicata 86:13–24.

Correa, C. C., and J. W. O. Ballard 2012. Wolbachia gonadal density in female and male Drosophila vary with laboratory adaptation and respond differently to physiological and environmental challenges. Journal of Invertebrate Pathology 111:197–204.

Correa, C. C., and J. W. O. Ballard 2014. What can symbiont titres tell us about co-evolution of Wolbachia and their host? Journal of Invertebrate Pathology 118:20–27.

Dobson, S. L., E. J. Marsland, and W. Rattanadechakul 2001. Wolbachia-induced cytoplasmic incompatibility in single- and superinfected Aedes albopictus (Diptera: Culicidae). Journal of Medical Entomology 38:382–387.

Dobson, S. L., E. J. Marsland, and W. Rattanadechakul 2002. Mutualistic Wolbachia infection in Aedes albopictus: accelerating cytoplasmic drive. Genetics 160:1087–1094.

Dobson, S. L., W. Rattanadechakul, and E. J. Marsland 2004. Fitness advantage and cytoplasmic incompatibility in Wolbachia single- and superinfected Aedes albopictus. Heredity 93:135–142.

Dodson, B. L., G. L. Hughes, O. Paul, A. C. Matacchiero, L. D. Kramer, and J. L. Rasgon 2014. Wolbachia enhances West Nile virus (WNV) infection in the mosquito Culex tarsalis. PloS Neglected Tropical Diseases 8:e2965.

Duron, O., C. Bernard, S. Unal, A. Berthomieu, C. Berticat, and M. Weill 2006. Tracking factors modulating cytoplasmic incompatibilities in the mosquito Culex pipiens. Molecular Ecology 15:3061–3071.

Dyer, K. A., and J. Jaenike 2004. Evolutionarily stable infection by a male-killing endosymbiont in Drosophila innubila: molecular evidence from the host and parasite genomes. Genetics 168:1443–1455.

Echaubard, P., O. Duron, P. Agniew, C. Sidobre, V. Noel, M. Weill, and Y. Michalakis 2010. Rapid evolution of Wolbachia density in insecticide resistant Culex pipiens. Heredity 104:15–19.

Eisen, L. B., J. Beaty, A. C. Morrison, and T. W. Scott 2009. Proactive vector control strategies and improved monitoring and evaluation practices for dengue prevention. Journal of Medical Entomology 46:1245–1255.

Endersby, N. M., and A. A. Hoffmann 2013. Effect of Wolbachia on insecticide susceptibility in lines of Aedes aegypti. Bulletin of Entomological Research 103:269–277.

Erlanger, T. E., J. Keiser, and J. Utzinger 2008. Effect of dengue vector control interventions on entomological parameters in developing countries: a systematic review and meta-analysis. Medical and Veterinary Entomology 22:203–221.

Ferguson, N. M., D. T. H. Kien, H. Clapham, R. Aguas, V. T. Tuan Trung, T. N. B. Chau, J. Popovic et al. 2015. Modeling the impact on virus transmission of Wolbachia-mediated blocking of dengue virus infection of Aedes aegypti. Science Translational Medicine 7:279ra37.

Frentiu, F. D., T. Zakir, T. Walker, J. Popovic, A. T. Van Pyke, A. den Hurk, E. A. McGraw et al. 2014. Limited dengue virus replication in field-collected Aedes aegypti mosquitoes infected with Wolbachia. PloS Neglected Tropical Diseases 8:e2688.

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

Fry, A. J., M. R. Palmer, and D. M. Rand 2004. Variable fitness effects of Wolbachia infection in Drosophila melanogaster. Heredity 93:379–389.

Fu, Y., L. Gavotte, D. R. Mercer, and S. L. Dobson 2010. Wolbachia-induced cytoplasmic incompatibility in Drosophila simulans yields a new pattern of unidirectional cytoplasmic incompatibility. Applied Environmental Microbiology 76:5887–5891.

Glaser, R. L., and M. A. Meola 2010. The native Wolbachia endosymbionts of Drosophila melanogaster and Culex quinquefasciatus
increase host resistance to West Nile virus infection. PLoS ONE 5: e11977.

Graham, R. I., D. Grzywacz, W. L. Mushobozi, and K. Wilson 2012. Wolbachia in a major African crop pest increases susceptibility to viral disease rather than protects. Ecology Letters 15:993–1000.

Hancock, P. A., and S. P. Sinkins 2011. Strategies for introducing Wolbachia to reduce transmission of mosquito-borne diseases. PLoS Neglected Tropical Diseases 5:e1024.

Harcombe, W., and A. A. Hoffmann 2004. Wolbachia effects in Drosophila melanogaster: in search of fitness benefits. Journal of Invertebrate Pathology 87:45–50.

Harrington, L. C., T. W. Scott, K. Lordhusnee, R. C. Coleman, A. Costero, G. G. Clark, J. J. Jones et al. 2005. Dispersal of the dengue vector Aedes aegypti within and between rural communities. American Journal of Tropical Medicine and Hygiene 72:209–220.

Hedges, L. M., J. C. Brownlie, S. L. O’Neill, and K. N. Johnson 2008. Wolbachia and virus protection in insects. Science 322:702.

Heintze, C., M. V. Garrido, and A. Kroeber 2007. What do community-based dengue control programmes achieve? A systematic review of published evaluations. Transactions of the Royal Society of Tropical Medicine and Hygiene 101:317–325.

Hertig, M., and S. B. Wolbach 1924. Studies on rickettsia-like micro-organisms in insects. The Journal of Medical Research 44:329–378.

Hilgenboecker, K., P. Hammerstein, P. Schlattmann, A. Telschow, and J. Ritchie 2014b. Invasion of Wolbachia at the residential block level is associated with local abundance of Stegomyia aegypti populations and property attributes. Medical and Veterinary Entomology 28(Suppl. 1):90–97.

Holden, P. R., P. Jones, and J. F. Y. Brookfield 1993. Evidence for a Wolbachia symbiont in Drosophila melanogaster. Genetical Research 62:23–29.

Hornett, E. A., S. Charlat, A. M. R. Duplouy, N. Davies, G. K. Roderick, N. Wedell, and G. D. D. Hurst 2006. Evolution of male-killer suppression in a natural population. PLoS Biology 4:1643–1648.

van den Hurk, A. F., S. Hall-Mendelin, A. T. Pyke, F. D. Frentiu, K. McElroy, A. Day, S. Higgs et al. 2012. Impact of Wolbachia on infection with chikungunya and yellow fever viruses in the mosquito vector Aedes aegypti. PLoS Neglected Tropical Diseases 6:e1892.

Hussain, M., G. Lu, S. Torres, J. H. Edmonds, B. H. Kay, A. Khromykh, and S. Asgari 2013. Effect of Wolbachia on replication of West Nile virus in a mosquito cell line and adult mosquitoes. Journal of Virology 87:851–858.

Ikeda, T., H. Ishikawa, and T. Sasaki 2003. Regulation of Wolbachia density in the mediterranean flour moth, Ephesia kuehniella, and the almond moth, Cadra cautella. Zoological Science 20:153–157.

Jansen, V. A. A., M. Turelli, and H. C. J. Godfray 2008. Stochastic spread of Wolbachia. Proceedings of the Royal Society B-Biological Sciences 275:2769–2776.

Kambiris, Z., P. E. Cook, H. K. Phuc, and S. P. Sinkins 2009. Immune activation by life-shortening Wolbachia and reduced filarial competence in mosquitoes. Science 326:134–136.

Kang, L., X. Ma, L. Cai, S. Liao, L. Sun, H. Zhu, X. Chen et al. 2003. Superinfection of Lusodelphax striatellus with Wolbachia from Drosophila simulans. Heredity 90:71–76.

Killeen, G. F., C. Barillas-Mury, M. B. Thomas, and B. Greenwood 2013. Modulating malaria with Wolbachia. Nature Medicine 19:974–975.

Kittayapong, P., K. J. Baisley, R. G. Sharpe, V. Baimai, and S. L. O’Neill 2002. Maternal transmission efficiency of Wolbachia superinfections in Aedes albopictus populations in Thailand. The American Journal of Tropical Medicine and Hygiene 66:103–107.

Klasson, L., J. Westberg, P. Sapountzis, K. Naslund, Y. Lutnaes, A. C. Darby, Z. Veneti et al. 2009. The mosaic genome structure of the Wolbachia wRi strain infecting Drosophila simulans. Proceedings of the National Academy of Sciences of the USA 106:5725–5730.

Kriesner, P., A. A. Hoffmann, S. F. Lee, M. Turelli, and A. R. Weeks 2013. Rapid sequential spread of two Wolbachia variants in Drosophila simulans. PLoS Pathogens 9:e1003607.

Kurralja Novićić, Z., E. Immonen, M. Jelić, M. AnĐelković, M. Stamenković-Radak, and G. Armqvist. 2015. Within-population genetic effects of mtDNA on metabolic rate in Drosophila subobscura. Journal of Evolutionary Biology 28:338–346.

Lambrechts, L., T. Fansiri, A. Pongsiri, B. Thaisomboonsuk, C. Klungthong, J. H. Richardson, A. Ponlavit et al. 2012. Dengue-1 virus clade replacement in Thailand associated with enhanced mosquito transmission. Journal of Virology 86:1853–1861.
Lu, P., G. W. Bian, X. L. Pan, and Z. Y. Xi 2012. Wolbachia induces density-dependent inhibition to dengue virus in mosquito cells. PloS Neglected Tropical Diseases 6:e1754.

Maciel-de-Freitas, R., J. C. Koella, and R. Lourenco-de-Oliveira 2011. Lower survival rate, longevity and fecundity of Aedes aegypti (Diptera: Culicidae) females orally challenged with dengue virus serotype 2. Transactions of the Royal Society of Tropical Medicine and Hygiene 105:452–458.

Maciel-de-Freitas, R., F. C. Avendanho, R. Santos, G. Sylvestre, S. C. Araujo, J. B. P. Lima, A. J. Martins et al. 2014. Undesirable consequences of insecticide resistance following Aedes aegypti control activities due to a dengue outbreak. PloS ONE 9:e92424.

Mains, J. W., C. L. Brelsford, P. R. Crain, Y. X. Huang, and S. L. Dobson 2013. Population impacts of Wolbachia on Aedes albopictus. Ecological Applications 23:493–501.

Martinez, J., B. Longdon, S. Bauer, Y. S. Chan, W. J. Miller, K. Bourtzis, L. Teixeira et al. 2014. Symbionts commonly provide broad spectrum resistance to viruses in insects: a comparative analysis of Wolbachia strains. PloS Pathogens 10:e1004369.

McGraw, E. A., and S. L. O’Neill 2013. Beyond insecticides: new thinking on an ancient problem. Nature Reviews Microbiology 11:181–193.

McGraw, E. A., D. J. Merritt, J. N. Droller, and S. L. O’Neill 2001. Wolbachia-mediated spermat modification is dependent on the host genotype in Drosophila. Proceedings of the Royal Society B-Biological Sciences 268:2565–2570.

McGraw, E. A., D. J. Merritt, J. N. Droller, and S. L. O’Neill 2002. Wolbachia density and virulence attenuation after transfer into a novel host. Proceedings of the National Academy of the Sciences of the USA 99:2918–2923.

McMeniman, C. J., and S. L. O’Neill 2010. A virulent Wolbachia infection decreases the viability of the dengue vector Aedes aegypti during periods of embryonic quiescence. PloS Neglected Tropical Diseases 4:e748.

McMeniman, C. J., R. V. Lane, B. N. Cass, A. W. C. Fong, M. Sidhu, Y. F. Wang, and S. L. O’Neill 2009. Stable introduction of a life-shortening Wolbachia infection into the mosquito Aedes aegypti. Science 323:141–144.

McNaughton, D., and D. T. T. Huong 2014. Designing a community engagement framework for a new dengue control method: a case study from central Vietnam. PloS Neglected Tropical Diseases 8:e2794.

Micieli, M. V., and R. L. Glaser 2014. Somatic Wolbachia strains for disease control. Molecular Ecology 23:361–369.

McMeniman, C. J., and S. L. O’Neill 2010. A virulent Wolbachia infection decreases the viability of the dengue vector Aedes aegypti during periods of embryonic quiescence. PloS Neglected Tropical Diseases 4:e748.

Mains, J. W., C. L. Brelsford, P. R. Crain, Y. X. Huang, and S. L. Dobson 2013. Population impacts of Wolbachia on Aedes albopictus. Ecological Applications 23:493–501.

Martinez, J., B. Longdon, S. Bauer, Y. S. Chan, W. J. Miller, K. Bourtzis, L. Teixeira et al. 2014. Symbionts commonly provide broad spectrum resistance to viruses in insects: a comparative analysis of Wolbachia strains. PloS Pathogens 10:e1004369.

Mao, C., Y. C. Chen, and C. L. Hsu 2004. The relative importance of innate immune priming in Wolbachia-mediated dengue virus interference. PloS Pathogens 6:e1000656.

Maciel-de-Freitas, R., J. C. Koella, and R. Lourenco-de-Oliveira 2011. Lower survival rate, longevity and fecundity of Aedes aegypti (Diptera: Culicidae) females orally challenged with dengue virus serotype 2. Transactions of the Royal Society of Tropical Medicine and Hygiene 105:452–458.

Maciel-de-Freitas, R., F. C. Avendanho, R. Santos, G. Sylvestre, S. C. Araujo, J. B. P. Lima, A. J. Martins et al. 2014. Undesirable consequences of insecticide resistance following Aedes aegypti control activities due to a dengue outbreak. PloS ONE 9:e92424.

Mains, J. W., C. L. Brelsford, P. R. Crain, Y. X. Huang, and S. L. Dobson 2013. Population impacts of Wolbachia on Aedes albopictus. Ecological Applications 23:493–501.

Martinez, J., B. Longdon, S. Bauer, Y. S. Chan, W. J. Miller, K. Bourtzis, L. Teixeira et al. 2014. Symbionts commonly provide broad spectrum resistance to viruses in insects: a comparative analysis of Wolbachia strains. PloS Pathogens 10:e1004369.
Ritchie, S. A., B. L. Montgomery, and A. A. Hoffmann 2013. Novel estimates and infection dynamics in natural populations. Genetics 165:2029–2038.

Raii, G., N. M. Endersby, C. Williams, and A. A. Hoffmann 2014a. Using Wolbachia-based releases for suppression of Aedes mosquitoes: insights from genetic data and population simulations. Ecological Applications 24:1226–1234.

Raii, G., I. Filipovic, A. R. Weeks, and A. A. Hoffmann 2014b. Genome-wide SNPs lead to strong signals of geographic structure and relatedness patterns in the major arbovirus vector, Aedes aegypti. Bmc Genomics 15:275.

Reynolds, K. T., and A. A. Hoffmann 2002. Male age, host effects and the weak expression or nonexpression of cytoplasmic incompatibility in Drosophila strains infected by maternally transmitted Wolbachia. Genetical Research 80:79–87.

Reynolds, K. T., L. J. Thomson, and A. A. Hoffmann 2003. The effects of host age, host nuclear background and temperature on phenotypic effects of the virulent Wolbachia strain popcorn in Drosophila melanogaster. Genetics 164:1027–1034.

Richardson, M. F., L. A. Weinert, J. J. Welch, R. S. Linheiro, M. M. Magwire, F. M. Jiggins, and C. M. Bergman 2012. Population genomics of the Wolbachia endosymbiont in Drosophila melanogaster. PloS Genetics 8:e1003129.

Ritchie, S. A., B. L. Montgomery, and A. A. Hoffmann 2013. Novel estimates of Aedes aegypti (Diptera: Culicidae) population size and adult survival based on Wolbachia releases. Journal of Medical Entomology 50:624–631.

Ross, P. A., N. M. Endersby, H. L. Yeap, and A. A. Hoffmann 2014. Larval competition extends developmental time and decreases adult size of wMelPop Wolbachia-infected Aedes aegypti. American Journal of Tropical Medicine and Hygiene 91:198–205.

Ruangs-Areerate, T., and P. Kittayapong 2006. Wolbachia transfection in Aedes aegypti: a potential gene driver of dengue vectors. Proceedings of the National Academy of Sciences of the USA 103:12534–12539.

Sasaki, T., and H. Ishikawa 1999. Wolbachia infections and cytoplasmic incompatibility in the almond moth and the mediterranean flour moth. Zoological Science 16:739–744.

Sasaki, T., T. Kubo, and H. Ishikawa 2002. Interspecific transfer of Wolbachia between two lepidopteran insects expressing cytoplasmic incompatibility: a Wolbachia variant naturally infecting Cadra cautella causes male killing in Euphestia kuehniella. Genetics 162:1313–1319.

Segoli, M., A. A. Hoffmann, J. Lloyd, G. J. Omodei, and S. A. Ritchie 2014. The effect of virus-blocking Wolbachia on male competitiveness of the dengue vector mosquito, Aedes aegypti. PloS Neglected Tropical Diseases 8:e3294.

Serga, S., O. Maistrenko, A. Rozhok, T. Mousseau, and I. Kozeretska 2012. Functional test of the influence of Wolbachia strains for disease control. Drosophila melanogaster. Applied Environmental Microbiology 75:7783–7788.

Simula, R. E., U. Alam, C. Breilsfoard, Y. N. Wu, R. Echoud, L. M. Okedi, S. Aksoy et al. 2013. Wolbachia association with the tsetse fly, Glossina fuscipes fuscipes, reveals high levels of genetic diversity and complex evolutionary dynamics. Bmc Evolutionary Biology 13:12.

Teixeira, L., A. Ferreira, and M. Ashburner 2008. The bacterial symbiont Wolbachia induces resistance to RNA viral infections in Drosophila melanogaster. PloS Biology 6:2753–2763.

Tortosa, P., S. Charlat, P. Labbe, J.-S. Dehecq, H. Barre, and M. Weill 2010. Wolbachia age-sex-specific density in Aedes albopictus: a host evolutionary response to cytoplasmic incompatibility? PloS ONE 5: e9700.

Turelli, M. 2010. Cytoplasmic incompatibility in populations with overlapping generations. Evolution 64:232–241.

Turelli, M., and A. A. Hoffmann 1995. Cytoplasmic incompatibility in Drosophila simulans – dynamics and parameter estimates from natural populations. Genetics 140:1319–1338.

Turley, A. P., L. A. Moreira, S. L. O’Neill, and E. A. McGraw 2009. Wolbachia infection reduces blood-feeding success in the dengue fever mosquito, Aedes aegypti. PloS Neglected Tropical Diseases 3:e3516.

Unckless, R. L., and J. Jaenike 2011. Maintenance of a male-killing Wolbachia in Drosophila immigrata by male-killing dependent and male-killing independent mechanisms. Evolution 66:878–899.

Unckless, R. L., L. M. Boelio, J. K. Herren, and J. Jaenike 2009. Wolbachia a as populations within individual insects: causes and consequences of density variation in natural populations. Proceedings of the Royal Society B-Biological Sciences 276:2805–2811.

Veneti, Z., S. Zabulou, G. Papafotiou, C. Paraskevopoulos, S. Pattas, I. Livadas, G. Markakis et al. 2012. Loss of reproductive parasitism following transfer of male-killing Wolbachia to Drosophila melanogaster and Drosophila simulans. Heredity 109:306–312.

Vu, T. T. H., E. C. Holmes, D. Veesna, T. Q. Nguyen, T. H. Tran, M. Quail, C. Churcher et al. 2010. Emergence of the Asian 1 genotype of dengue virus serotype 2 in Viet Nam: in vivo fitness advantage and lineage replacement in South-East Asia. PloS Neglected Tropical Diseases 4:e757.

Walker, T., P. H. Johnson, L. A. Moreira, I. Iturbe-Ormaetxe, F. D. Frentiu, C. J. McMeniman, Y. S. Leong et al. 2011. The wMel Wolbachia strain blocks dengue and invades caged Aedes aegypti populations. Nature 476:450–453.

Weeks, A. R., M. Turelli, W. R. Harcombe, K. T. Reynolds, and A. A. Hoffmann 2007. From parasite to mutualist: rapid evolution of Wolbachia in natural populations of Drosophila. PloS Biology 5:997–1005.

Wong, Z. S., L. M. Hedges, J. C. Brownlie, and K. N. Johnson 2011. Wolbachia-mediated antibacterial protection and immune gene regulation in Drosophila. PloS ONE 6:e25430.

Wooffitt, M., I. Iturbe-Ormaetxe, J. C. Brownlie, T. Walker, M. Riegler, A. Sleznev, J. Popovic et al. 2013. Genomic evolution of the pathogenic Wolbachia strain, wMelPop. Genome Biology and Evolution 5:2189–2204.

Xi, Z. Y., C. C. H. Khoo, and S. L. Dobson 2005. Wolbachia establishment and invasion in an Aedes aegypti laboratory population. Science 310:326–328.

Xi, Z. Y., C. C. H. Khoo, and S. L. Dobson 2006. Interspecific transfer of Wolbachia into the mosquito disease vector Aedes albopictus. Proceedings of the Royal Society B-Biological Sciences 273:1317–1322.
Wolbachia strains for disease control

Hoffmann et al.

incompatibility expression in Drosophila melanogaster. Insect Molecular Biology 20:75–85.
Ye, Y. X. H., M. Woolfit, E. Rances, S. L. O’Neill, and E. A. McGraw 2013. Wolbachia-associated bacterial protection in the mosquito Aedes aegypti. PLoS Neglected Tropical Diseases 7:e2362.
Yeap, H. L., P. Mee, T. Walker, A. R. Weeks, S. L. O’Neill, P. Johnson, S. A. Ritchie et al. 2011. Dynamics of the “popcorn” Wolbachia infection in outbred Aedes aegypti informs prospects for mosquito vector control. Genetics 187:583–595.
Yeap, H. L., J. K. Axford, J. Popovici, N. M. Endersby, I. Iturbe-Ormaetxe, S. A. Ritchie, and A. A. Hoffmann 2014. Assessing quality of life-shortening Wolbachia-infected Aedes aegypti mosquitoes in the field based on capture rates and morphometric assessments. Parasites & Vectors 7:13.
Yen, J. H., and A. R. Barr 1973. The etiological agent of cytoplasmic incompatibility in Culex pipiens. Journal of Invertebrate Pathology 22:242–250.
Zabalou, S., S. Charlat, A. Nirgianaki, D. Lachaise, H. Mercot, and K. Bourtzis 2004. Natural Wolbachia infections in the Drosophila yakuba species complex do not induce cytoplasmic incompatibility but fully rescue the wRi modification. Genetics 167:827–834.
Zéle, F., A. Nicot, A. Berthomieu, M. Weill, O. Duron, and A. Rivero 2014. Wolbachia increases susceptibility to Plasmodium infection in a natural system. Proceedings of the Royal Society B-Biological Sciences 281:20132837.
Zhang, G. M., M. Hussain, S. L. O’Neill, and S. Asgari 2013. Wolbachia uses a host microRNA to regulate transcripts of a methyltransferase, contributing to dengue virus inhibition in Aedes aegypti. Proceedings of the National Academy of Sciences of the USA 110:10276–10281.

Supporting Information

Additional Supporting Information may be found in the online version of this article:
Appendix S1. Stable Wolbachia infections produced through microinjection, their effects on host reproduction and fitness, and potential blocking effectiveness where demonstrated.
