Wolbachia Can Enhance Plasmodium Infection in Mosquitoes: Implications for Malaria Control?

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The symbiotic bacterium Wolbachia is an attractive agent for vector-borne pathogen control. It has long been studied for its ability to manipulate host reproduction and spread into arthropod populations [1]. These properties, coupled with the recently identified ability to inhibit diverse pathogens [2–6], open avenues for its use in controlling vector-borne disease. Numerous Wolbachia-based control strategies are being investigated [reviewed in (7–9)], with some studies having progressed to field trials [10,11]. However, a worrying trend is emerging whereby Wolbachia infections have been demonstrated to enhance rather than suppress pathogens in some systems [12–18]. Plasmodium parasites, which are the causal agent of malaria, seem particularly prone to Wolbachia-mediated pathogen enhancement [13–16].

Wolbachia-based strategies have been proposed to control malaria [19]. Anopheles mosquitoes (the vectors of human malaria parasites) are not naturally infected by Wolbachia [20,21], but artificial transfer of this bacterium between species can be accomplished in the laboratory (reviewed in [22]). Pathogen interference phenotypes appear to be most prominent when Wolbachia is transferred into a novel host [16,23]. Given that Anopheles are for the most part naturally uninfected by Wolbachia (but see [24]), they can be considered an open niche for infection and a prime mosquito genus for Wolbachia-based control strategies. However, the main impediment for developing a control strategy is the difficulty in creating a stable artificial infection in Anopheles [19]. While examining Plasmodium interference in a stably infected host is the gold standard, a more convenient system is to transiently infect mosquitoes by intrathoracic microinjection. Using this system, the infection persists during the lifetime of the transfected individual but is not transmitted to its offspring. Transient infection allows the rapid assessment of Wolbachia-host interactions without the need for generating stable artificial infections [5]. It is uncertain how representative transient infections are of stable inherited associations; however, similarities in tissues tropism and fitness costs incurred upon the host between stable and transiently infected Anopheles mosquitoes are evident [5,14,25]. Furthermore, both types of infection have been shown to inhibit the human malaria parasite Plasmodium falciparum [5,25]. However, studies using transient infection models have found that Wolbachia can enhance certain Plasmodium species [13,14].

The Plasmodium interference phenotype is therefore not universal, but context dependent. While P. falciparum is suppressed by the wAlbB strain of Wolbachia from Aedes albopictus [5,25], transient infections have shown the opposite effect on rodent malaria parasites. Anopheles gambiae transiently infected with wAlbB exhibited enhanced P. berghei development at the oocyst stage [14]. Similarly, wAlbB increased the number of P. yoelii oocysts in An. stephensi, although the phenotype was modulated by temperature [13]. At a temperature optimal for parasite development, Wolbachia increased parasite intensity compared to uninfected controls, but at warmer temperatures, Wolbachia inhibited Plasmodium development [13].

While P. falciparum is a major parasite in sub-Saharan Africa, four other parasites also cause human malaria worldwide: P. malariae, P. ovale, P. knowlesi, and P. vivax (the etiological agent of the most prevalent form of relapsing malaria). To our knowledge, the effect of Wolbachia on these other human Plasmodium parasites is unknown. The question is relevant for two reasons. First, the precedent that a particular Wolbachia strain can inhibit one parasite yet enhance another has already been documented [5,14], indicating that effects on parasites can be species-specific. Troublingly, P. malariae, P. ovale, P. knowlesi, and P. vivax are phylogenetically more closely related to rodent malaria parasites, which are enhanced by Wolbachia infections [13,14], than they are to P. falciparum (Figure 1) [26,27]. Second, many human Plasmodium parasites occur in sympathy and are transmitted by the same vectors. A case in point is P. falciparum and P. vivax, both of which occur in sympathy over large stretches of the Asian continent where they are both transmitted by An. stephensi [28,29]. Any potential control strategy devised in regions where more than one parasite species occurs needs to thoroughly investigate the effect of Wolbachia on all parasite species transmitted by the vector, as well as other pathogens such as filarial worms or arboviruses (both as single infections and in the context of coinfections) to ensure that Wolbachia-infected mosquitoes do not inadvertently enhance transmission of secondary pathogens.

While difficult, forecasting the long-term evolutionary response in this tripartite relationship between Wolbachia, Plasmodium, and Anopheles is very important. Natural Wolbachia-mosquito associations in which the symbiont and the host have tightly coevolved exist and may provide powerful models for studying the long-term evolutionary effects of Wolbachia.
infections. The evidence currently available suggests that natural Wolbachia infections can also enhance malaria parasite development within the mosquito. *Aedes fluviatilis* naturally infected with the wFlu Wolbachia strain had a significantly higher number of *P. gallinaceum* oocysts compared to an *Ae. fluviatilis* line which had been cleared of the Wolbachia infection [15]. *Ae. fluviatilis* is not, however, a natural vector of *P. gallinaceum*, and it is well known that the outcome of experiments using such laboratory models can differ significantly from those of natural mosquito–*Plasmodium* combinations (e.g., Boete [30]). Recent studies carried out in *Culex pipiens* mosquitoes, which are naturally infected with the wPip Wolbachia strain and transmit the avian malaria parasite *P. relictum*, have also demonstrated *Plasmodium* enhancement. In this natural system, Wolbachia protects the mosquito host against the detrimental fitness effects incurred by *Plasmodium* infection [31] and increases the susceptibility of *C. pipiens* to *P. relictum*, with wPip-infected mosquitoes having a higher prevalence of *Plasmodium* sporozoites in the salivary glands [16]. These studies show that the

![Figure 1. Representative phylogenetic dendrogram of *Plasmodium* parasites, their vertebrate hosts, and the influence of Wolbachia infection on parasite development within the mosquito vector. The protective effect of Wolbachia is variable and dependent on the Wolbachia strain and the insect host background, suggesting that complex tripartite interactions influence the effect on *Plasmodium*. The type of association between Wolbachia with the vector may also influence *Plasmodium*. Only one human malaria parasite (*P. falciparum*) has been assessed, while the effect of Wolbachia infection on the other four human parasites is unknown. Arrows indicate suppression (green), enhancement (red), or no effect (grey) of *Plasmodium*. The type of association within the host is depicted by symbols (target: natural infection, square: stable artificial infection, diamond: transient artificial infection). Numbers indicate: (1) the phenotype is temperature sensitive, (2) Wolbachia infection also increases insect life span [31], which has implications for pathogen transmission. Phylogeny was reconstructed based on work from Carlton et al. [26] and Martinsen et al. [27]. doi:10.1371/journal.ppat.1004182.g001](#)
Plasmodium-inhibiting properties of Wolbachia are far from universal; certain mosquito-Wolbachia-Plasmodium combinations and experimental conditions transform Wolbachia-infected mosquitoes into better vectors of malaria parasites. This is worrisome for the general implementation of Wolbachia-based control strategies. Given that Wolbachia-based control strategies will use stable transinfected mosquitoes, the key question is whether stable and natural infections will behave in the same way. The stable transfer of Wolbachia into the host likely alters many aspects of host homeostasis, as evidenced by the novel phenotypes induced by infection [32–34], and as such, these associations likely differ from natural associations where Wolbachia and its host have coevolved. Another question is whether stable artificial infections will evolve over time. Theory and empirical studies show that these maternally transmitted bacteria will tend to evolve towards mutualistic associations with their host [35–39]. However, the evolutionary outcomes of pathogen interference or enhancement are harder to predict. A more complete mechanistic understanding of how Wolbachia infection modulates Plasmodium parasites is critical to address these important evolutionary questions and to evaluate if they are likely to occur in timescales relevant for disease control.

To date, two stable artificial Wolbachia transfections have been assessed for their effect on Plasmodium. First, an Aedes aegypti line infected with wMelPop had inhibited P. gallinaceum infection [4]; Ae. aegypti is not, however, the natural vector of this parasite. Second, and more recently, the wAlbSB strain was stably transferred into An. stephensi, one of the main vectors of human malaria in Asia [25]. This groundbreaking work demonstrated that stable artificial infections in epidemiologically relevant malaria vectors are feasible, and that P. falciparum can be inhibited by Wolbachia within its natural vector. If the severe fitness effects induced by Wolbachia in Anopheles can be overcome [25], then this approach is highly promising.

The work by Bian and colleagues [25] dramatically enhances the prospect for the use of Wolbachia in a malaria control strategy, but many questions still remain. What are the effects of Wolbachia on the other four species of Plasmodium parasites that infect humans? How relevant are transient infection models? Do some strains of Wolbachia enhance pathogens in a field context? What are the long-term evolutionary consequences of novel Wolbachia-host associations on Plasmodium development within the insect host? What are the mechanisms behind pathogen interference and enhancement of Wolbachia on Plasmodium parasites, and are the mechanisms of enhancement seen in rodent and avian model systems relevant to human malaria parasites? How influential are environmental variables on pathogen inhibition phenotypes? While many of these questions may be difficult to answer in the short term, assessing the relevance of transient infections would seem within the grasp of the scientific community. Although challenging, understanding the evolutionary consequences of novel Wolbachia associations on pathogen transmission and identifying the mechanisms behind Wolbachia modulation of Plasmodium is critical for developing effective control strategies and assessing their long-term feasibility. Insights from non-Anopheline systems where Wolbachia naturally infects the vector may be useful in this regard [16,31,39].

In conclusion, Wolbachia-based control of vector-borne pathogens is a promising novel strategy that has many advantages over other conventional and contemporary control methods. The development of a stable infection in Anopheles means the prospect of Wolbachia-based control of malaria can now be entertained [25], but many important questions need to be resolved before this idea can become a reality. While the concerns raised here focus on Plasmodium, these issues are relevant for Wolbachia control of any vector-borne pathogen [18]; we suggest that transinfected mosquitoes intended for release into nature should be assessed for inhibition (or lack thereof) of all relevant pathogens circulating in the system.

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