Modulating malaria with *Wolbachia*

New control strategies for malaria are urgently required. One current area of investigation is blocking the transmission of the malaria parasite through its mosquito vector. Bian *et al.*[1] highlight the potential use of *Wolbachia*, symbiotic bacteria that infect insects, in control of malaria transmission. The authors describe the stable establishment of *Wolbachia* infection in *Anopheles stephensi*, an important malaria vector, and show that seeding of infected female *A. stephensi* into a population of uninfected mosquitoes resulted in invasion of *Wolbachia* into the uninfected population. Moreover, *Wolbachia* infection rendered *A. stephensi* refractory to infection with the malaria parasite. We asked four experts to comment on the implications of this study.

Gerry F Killeen

This report represents an exciting opportunity to develop an innovative new approach to combating malaria. Even more encouragingly, more advanced, equivalent technologies to target *Aedes* mosquito vectors of dengue virus have already performed admirably in preliminary field trials[2]. New approaches for malaria really are needed: there is a vast gap between what we can achieve with existing control tools and what we need to eliminate malaria from its tropical strongholds, and this gap remains essentially unchanged since the days of the Global Malaria Eradication Programme half a century ago[3].

Like all studies, however, the work of Bian *et al.*[1] has its limitations and substantial obstacles to further development of *Wolbachia* as a means to control malaria remain. First, even a tiny number of sporozoites (the infective form of the malaria parasite) can infect a human victim, so it is the prevalence of sporogonic-stage parasites in mosquito populations that determines malaria transmission intensity, rather than the density of those oocysts or sporozoites in individual mosquitoes. Thus, even though remarkably modest effects on sporozoite prevalence can eliminate parasite populations[4], the supplementary material of Bian *et al.*[1] notably indicates that *Wolbachia* infection had negligible impact on this more epidemiologically relevant outcome. Second, the rate at which vectors are infected by malaria parasites is a less important determinant of transmission than the longevity of the mosquito, which must live long enough for the development and delivery of mature sporozoites to occur. It is therefore important that subsequent studies carefully examine the effect of *Wolbachia* on vector survival, which may well turn out to be the most important mode of action for this promising biological weapon against malaria transmission.

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The introduction of *Wolbachia*, a maternally transmitted symbiotic bacterium, into mosquitoes can suppress the replication of several viruses that infect humans, greatly reducing disease transmission[2,5]. Despite extensive research, the stable introduction of *Wolbachia* into anopheline mosquitoes to test its potential to block malaria transmission has proved challenging. However, an isogenic female line of the malaria vector *A. stephensi* that maternally transmits *Wolbachia* with an efficiency of 100% and that has been stable for many generations has recently been established, and experiments with caged mosquitoes indicated that the bacteria can spread into mosquito populations[1].

"It will be crucial to identify *Wolbachia* strains that are more effective at suppressing *Plasmodium* infection and to establish lines in other anophelines, such as *Anopheles gambiae*."

Although *Wolbachia* infection significantly reduced the susceptibility of mosquitoes to *Plasmodium falciparum* infection[1], the observed effect is probably too modest to suppress malaria transmission in the field. *Wolbachia* is much more effective at suppressing viral replication than *Plasmodium* infection, probably because *Wolbachia* and viruses are both intracellular pathogens, whereas *Plasmodium* develops extracellularly

Matthew B Thomas

Challenges in implementing conventional tools to control disease vectors are creating a pressing need for new interventions[6]. For a number of years, there has been interest in innovative approaches that involve the transformation of mosquitoes to render them ineffective at transmitting malaria parasites. The recent paper by Bian *et al.*[1] demonstrating the stable transinfection of a key Asian malaria vector (*A. stephensi*) with a maternally transmitted symbiotic bacteria (*Wolbachia*), represents a substantial step in this direction. *Wolbachia* not only induces refractoriness to the human malaria parasite *P. falciparum* but also alters normal patterns of Mendelian inheritance through a process of cytoplasmic incompatibility to facilitate its own spread through mosquito populations.

"It is therefore important that subsequent studies carefully examine the effect of *Wolbachia* on vector survival, which may well turn out to be the most important mode of action for this promising biological weapon."

As with many lab-based mosquito studies, the experiments of Bian *et al.*[1] were conducted at constant 27 °C. However, mean temperatures in malaria transmission environments range from 18 to 34 °C, and daily temperature variation of >10 °C is common. Studies in other insects show that growth, dissemination, vertical transmission, fitness effects and the extent of cytoplasmic incompatibility of *Wolbachia* can vary considerably with temperature[7]. Mosquito immune responses to *Wolbachia* and malaria parasites are likely to also vary with temperature[7], potentially influencing how effectively *Wolbachia* blocks transmission of the parasite. Indeed,

and its direct interactions with *Wolbachia* are transient. Moving forward, it will be crucial to identify *Wolbachia* strains that are more effective at suppressing *Plasmodium* infection and to establish lines in other anophelines, such as *Anopheles gambiae*, the main malaria vector in Africa. This would require a more effective strategy to generate stable lines than the one described in the recent study[1] so that a large number of *Wolbachia* strains can be screened. Anopheline mosquitoes have a complex population structure, especially in Africa, and have much more reproductive isolation than culicine mosquitoes, and this could limit the spread of *Wolbachia*. Despite these challenges, *Wolbachia* is an attractive system to disrupt malaria transmission because it is naturally present in many insect species but does not infect vertebrates; it is vertically transmitted over many generations; it induces cytoplasmic incompatibility that drives the symbiont into natural populations and prevents the dampening effect of immigration from neighboring *Wolbachia*-free mosquito populations; and once *Wolbachia* infection is established, it does not require continuous intervention. Thus, *Wolbachia* could drive the beginning of the end of malaria transmission.

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previous studies conducted under cooler conditions using a rodent species of malaria showed that the same strain of *Wolbachia* used by Bian *et al.*[1] facilitated malaria infection rather than blocking it[8]. Whether this is a temperature effect or a mosquito–parasite species interaction effect is unclear, but the implications could be far reaching, as there are several species of human malaria parasite that often occur concurrently across diverse environments.

Blocking parasite transmission combined with a built-in drive mechanism for replacing the infected vector population represents a potentially powerful tool for controlling vector-borne disease. Translation beyond the current proof-of-principle studies requires an understanding of how mosquitoes, *Wolbachia* and malaria parasites interact in the real world.

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Brian Greenwood

The clinical burden of malaria has recently been reduced substantially in many malaria-endemic areas as a result of the widespread deployment of more effective treatment and preventative measures, but the infection remains a major threat[9], and new and innovative approaches to malaria control are needed. One novel approach under investigation is the infection of mosquito vectors with bacteria that inhibit the development of the malaria parasite in the stomach of the mosquito. Species being investigated include *Pantoea agglomerans*[10], which naturally infects anopheline mosquitoes, and *Wolbachia*[1], which probably does not. However, Bian *et al.*[1] recently reported that a stable *Wolbachia* infection can be introduced into *A. stephensi*, an important malaria vector in Asia, by microinjecting early-stage *A. stephensi* embryos with the bacteria, which are passed to the next generation through the female germ cell line. Importantly, *A. stephensi* infected with *Wolbachia* are partially resistant to infection with the malaria parasite *P. falciparum*. Only a modest reduction was seen in the number of *P. falciparum* oocysts in the stomach of

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Wolbachia-infected mosquitoes, not the almost complete inhibition of development that would be needed to make a mosquito noninfectious and to stop malaria transmission. However, in the experimental system used by the authors, mosquitoes infected with the malaria parasite carried a much larger number of oocysts than is usually found in naturally infected mosquitoes[1]. Thus, the inhibitory effect of *Wolbachia* infection in the wild might be greater than in the laboratory, but would it be high enough to interrupt malaria transmission?

A major challenge for all approaches to malaria control that involve generating mosquitoes with a reduced susceptibility to malaria infection is how to replace the countless number of wild mosquitoes found in many malaria-endemic areas with modified vectors. Bian *et al.*[1] achieved this by seeding a laboratory mosquito population with a percentage of *Wolbachia*-infected females as low as 5%, but could this be accomplished in the field? Perhaps the next step should be an attempt to achieve the same result in a biosphere that has an environment that more closely resembles the situation in a malaria-endemic area, rather than a laboratory cage.

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