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Mathematical modelling to assess the feasibility of Wolbachia in malaria vector biocontrol

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ABSTRACT

Releasing mosquitoes transinfected with the endosymbiotic bacterium Wolbachia is a novel strategy for interrupting vector-borne pathogen transmission. Following its success in controlling arboviruses spread by *Aedes aegypti*, this technology is being adapted for anopheline malaria vectors. However, antagonistic interactions between Wolbachia and naturally resident Asaia bacteria in malaria vectors have been demonstrated experimentally, potentially jeopardising Wolbachia biocontrol. We developed the first mathematical model accounting for interspecific competition between endosymbionts to assess the feasibility of this novel strategy for controlling malaria. First, Asaia prevalences among natural mosquito populations were compared with simulations parametrized with rates of Asaia transmission reported from laboratory studies. Discrepancies between projections and natural Asaia prevalences indicated potential overestimation of Asaia transmissibility in artificial laboratory settings. With parametrization that matches natural Asaia prevalence, simulations identified redundancies in Asaia's many infection routes (vertical, sexual and environmental). This resilience was only overcome when Wolbachia conferred very high resistance to environmental infection with Asaia, resulting in Wolbachia fixation and Asaia exclusion. Wolbachia's simulated spread was prevented when its maternal transmission was impeded in coinfected mosquitoes and the pre-control Asaia prevalence was beyond a threshold of 60–75%. This theoretical assessment highlights critical next steps in laboratory experiments to inform this strategy's feasibility.

1. Introduction

Malaria remains a global health priority (WHO, 2021). Control is primarily dependent on insecticides delivered either through insecticide impregnated bednets or indoor residual spray (World Health Organization, 2021). Resistance to the four main chemical classes used for insecticides has emerged and is widespread across sub-Saharan Africa where the brunt of disease burden is experienced (WHO, 2021; The World Malaria Report, 2020). In 2021, the WHO renewed its call for novel vector control methods to improve the effectiveness and sustainability of current control programmes (World Health Organization, 2021). One novel strategy is the biocontrol of malaria using *Anopheles* mosquitoes transinfected with an endosymbiotic bacteria called Wolbachia.

For several years, dengue control programmes have benefited from two desirable properties of Wolbachia infection in the vector *Aedes aegypti*: it blocks pathogen transmission (Walker et al., 2011), and it induces cytoplasmic incompatibility (CI), a reproductive phenotype that allows this endosymbiont to spread through wild mosquito populations (Hoffmann et al., 2011). Field trials in Indonesia showed a reduction in dengue of 77% following the release of Wolbachia infected mosquitoes (Utarini et al., 2021). A decade after its release in northern Queensland, Wolbachia remains in the local population evidencing the stability and longevity of this strategy (Ryan et al., 2020). Attempts to translate this success from arbovirus to malaria vectors have been hampered by difficulties with producing stable Wolbachia transinfected lines of *Anopheles* mosquitoes (Chrostek and Gerth, 2019).

Over the past 10 years, a growing number of studies have isolated Wolbachia DNA from diverse natural anopheline populations. Finding natural Wolbachia infections among malaria vectors debunks previous beliefs that anopheline vectors are intrinsically resistant to these endosymbionts. Further, Wolbachia transinfection success is influenced by the relatedness of donor and recipient host. To date, stable transinfection has only been achieved with the Asian malaria vector *An. stephensi* (Bian et al., 2013). Very recently, fluorescent in situ hybridization localized a heavy Wolbachia infec-

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tion in the ovaries of *An. moucheti* (Walker et al., 2021), an important vector in forested and degraded forest areas of equatorial Africa. The further findings that these natural Wolbachia infections were maternally transmitted and that they possess the capacity to induce the CI phenotype offer rekindled hope in adapting this biocontrol strategy to major African malaria vector species.

Wolbachia is not the only endosymbiont being investigated for malaria vector biocontrol. Others include Serratia (Soenens and Imperial, 2020) and Asaia (Favia et al., 2007). Like Wolbachia, Asaia has been isolated from different species of malaria vectors across Africa. Its anti-*Plasmodium* ability is less studied than that of Wolbachia but early indication is that Asaia is a natural effector for mosquito immune priming (Cappelli et al., 2019). In studies where it is sought, Asaia is often found at very high prevalence, and this is attributed to several aspects of its biology. Asaia is not only maternally transmitted but is also transmitted through copulation (only male-to-female transmission has been demonstrated thus far) and environmental contamination, often through other ovipositing mosquitoes or the hatching of their larvae (Favia et al., 2007).

While the existence of multiple candidate endosymbionts could be perceived as an advantage for developing this biocontrol strategy, their co-existence may be problematic. Studies examining interactions between Wolbachia and Asaia have indicated that they act antagonistically (Rossi et al., 2015; Hughes et al., 2014). A reciprocal negative interference has been documented for the colonisation of the gonads and salivary glands (Rossi et al., 2015). It remains unknown how the feasibility of a control programme deploying Wolbachia-infected mosquitoes might be affected by naturally occurring antagonistic endosymbionts such as Asaia. Through mathematical modelling, this study sought to provide the first indication of the extent to which Asaia may jeopardise the success of malaria biocontrol using Wolbachia. Analysis of the co-infection model also allowed for the identification of dominant traits among endosymbiont interactions that are likely to be most influential in the success or failure of a Wolbachia programme for controlling malaria transmission.

2. Methods

A stage-structured model of *Anopheles* mosquitoes adapted from White et al. (White et al., 2011) is used to investigate the feasibility of Wolbachia spreading into a mosquito population when Asaia is also present. A small number of studies detailing Wolbachia spread into anopheline populations already exist. Shaw et al. (Shaw et al., 2016) use a time-delay model to describe how a certain level of fixed Wolbachia prevalence may reduce malaria prevalence in human populations. Onyiaji et al. (Ebube Onyiaji et al., 2018) also use a simple, Ross-Macdonald related model which includes Wolbachia, and present the results of a local stability analysis. To the best of our knowledge, the current study is the first to include anopheline stage structure in simulating Wolbachia spread. This was deemed a necessary inclusion because of the different mosquito life stages that are known to be susceptible to Asaia infection. After including both Wolbachia and Asaia in the equations describing mosquito population dynamics ('*Mathematical model*' below), a range of interspecific interactions between the endosymbionts are explored through numerical simulation ('*Computational experiments*' below) to gauge their potential impact on a malaria biocontrol strategy using Wolbachia.

2.1. Mathematical model

The rates of change in the number of uninfected, Wolbachia-infected, Asaia-infected, and doubly infected eggs (respectively, O, O_W, O_A and O_D) Table 1 are determined from the following equations:

$$\frac{dO}{dt} = \phi f^n \frac{m^n}{m} - (\lambda_O + \mu_O)O$$

$$\frac{dO_W}{dt} = \phi f^w - (\lambda_O + \mu_O)O_W$$

$$\frac{dO_A}{dt} = \phi\left(f^a \frac{m^a + m^n}{m} + f^n \frac{m^a}{m}\right) - (\lambda_O + \mu_O)O_A$$

$$\frac{dO_D}{dt} = \phi\left(f^d + f^w \frac{(1-\sigma)m^a + (1-\sigma)m^d}{m}\right) - (\lambda_O + \mu_O)O_D$$

Where,

$$f^n = F + \omega F_W + \alpha F_A + \alpha\omega F_D$$

$$m^n = M + \sigma(M_W + cM_{W,r}) + \beta M_A + \beta\sigma M_D$$

$$f^w = (1-\omega)F_W + \alpha(1-\omega)F_D$$

$$f^a = (1-\alpha)F_A + (1-\alpha)\omega F_D$$

$$m^a = (1-\beta)M_A + (1-\beta)\sigma M_D$$

$$f^d = (1-\alpha)(1-\omega)F_D$$

$$m = M + M_W + cM_{W,r} + M_A + M_D$$

ϕ is the daily rate of egg oviposition per adult female, '*F*' (with subscript matching that of eggs). Adult males are denoted by '*M*' and newly released, Wolbachia-infected males ($M_{W,r}$) are assumed to have reduced mating competitiveness ($0 < c < 1$) relative to wild-borne males. λ is the rate of maturation to the next insect life stage and μ is the mortality rate. Maternal transmission of both Wolbachia and Asaia is imperfect, with failed transmission occurring at respective rates ω and α. For Asaia, paternal transmission of the bacterium has also been documented and this occurs at rate β. Cytoplasmic incompatibility occurs when Wolbachia-infected males mate with Wolbachia-uninfected females, resulting in nonviable offspring. However, this has been documented to fail in a small proportion of matings, and this failure rate is denoted by σ.

The rates of change in the number of uninfected, Wolbachia-infected, Asaia-infected and doubly infected larvae (respectively, L, L_W, L_A and L_D) are determined from the following equations:

$$\frac{dL}{dt} = \lambda_O(1-\varepsilon)O - L\left(\lambda_L + \mu_L(1+\gamma)\frac{l}{K}\right)$$

$$\frac{dL_W}{dt} = \lambda_O(1-\varepsilon)O_W - L_W\left(\lambda_L + \mu_L(1+\gamma)\frac{l}{K}\right)$$

$$\frac{dL_A}{dt} = \lambda_O(O_A + \varepsilon O) - L_A\left(\lambda_L + \mu_L(1+\gamma)\frac{l}{K}\right)$$

$$\frac{dL_D}{dt} = \lambda_O(O_D + \varepsilon O_W) - L_D\left(\lambda_L + \mu_L(1+\gamma)\frac{l}{K}\right)$$

Where, γ controls the strength of density dependence in larval survival; l is the sum of all (uninfected and infected) larvae; and, ε is the rate at which Asaia in the aquatic environment infects aquatic mosquito stages. Asaia persists in the environment in the absence of mosquitoes and has been isolated from numerous flowering plants in the tropics (Yamada et al., 2000; Katsura et al., 2001;

Table 1
Model parameters and variables with definitions and values.

Symbol	Definition (units)	Values	Ref
O	Eggs (number)	dynamic	
L	Larvae (number)	dynamic	
P	Pupae (number)	dynamic	
M	Male adults (number)	dynamic	
F	Female adults (number)	dynamic	
ϕ	Rate of eggs oviposition (per female per day)	21.19	(White et al., 2011)
λ_O	Rate of egg-to-larva maturation (per day)	0.15	(White et al., 2011)
λ_L	Rate of larva-to-pupa maturation (per day)	0.27	(White et al., 2011)
λ_P	Rate of pupa-to-adult maturation (per day)	1.56	(White et al., 2011)
μ_O	Rate of egg mortality (per day)	0.034	(White et al., 2011)
μ_L	Rate of larval mortality (per day)	0.035	(White et al., 2011)
μ_P	Rate of pupal mortality (per day)	0.25	(White et al., 2011)
μ_A	Rate of adult mortality (per day)	0.12	(White et al., 2011)
ω	Failure of Wolbachia to maternally transmit (proportion)	0.01	(Walker et al., 2011)
α	Failure of Asaia to maternally transmit (proportion)	0.4	(Favia et al., 2007)
β	Failure of Asaia to paternally transmit (proportion)	0.4	(Damiani et al., 2008)
σ	Failure of cytoplasmic incompatibility (proportion)	0.012	(Bian et al., 2013)
γ	Strength of density dependence (unitless)	13.25	(White et al., 2011)
δ	Rate of sexual transmission of Asaia (per mating per Asaia male)	0.5	(Favia et al., 2007)
ε	Rate of Asaia infection from aquatic environment (per day)	adjusted	*

* The only environmental infection rate we could identify from the literature was reported by Favia et al. (Favia et al., 2007) in which Asaia were artificially introduced into larvae breeding water at unknown concentration, resulting in half of the exposed mosquito larvae being colonised 24 h later. This parameter is adjusted to model different prevalence levels.

Moore et al., 2002; Yukphan et al., 2004) and others have implicated plants as an important source of anopheline infection with Asaia (Bassene et al., 2020). It is unknown whether the main source of Asaia infection during the aquatic mosquito stages is local flora or if it comes from other mosquitoes sharing the same breeding site. We explore both scenarios by first setting ε at a fixed rate and second, by incorporating it as a simple linear function of Asaia prevalence among the local mosquito population.

The rates of change in the number of uninfected, Wolbachia-infected, Asaia-infected, and doubly infected pupae (respectively, P, P_W, P_A and P_D) are determined from the following equations:

$$\frac{dP}{dt} = \lambda_L(1-\varepsilon)L - P(\lambda_P + \mu_P)$$

$$\frac{dP_W}{dt} = \lambda_L(1-\varepsilon)L_W - P_W(\lambda_P + \mu_P)$$

$$\frac{dP_A}{dt} = \lambda_L(L_A + \varepsilon L) - P_A(\lambda_P + \mu_P)$$

$$\frac{dP_D}{dt} = \lambda_L(L_D + \varepsilon L_W) - P_D(\lambda_P + \mu_P)$$

And, the rates of change in the number of uninfected, Wolbachia-infected, Asaia-infected and doubly infected adults are determined from the following equations:

$$\frac{dM}{dt} = 0.5\lambda_P P - \mu_M M$$

$$\frac{dM_W}{dt} = 0.5\lambda_P P_W - \mu_M M_W$$

$$\frac{dM_{W,r}}{dt} = released - \mu_M M_{W,r}$$

$$\frac{dM_A}{dt} = 0.5\lambda_P P_A - \mu_M M_A$$

$$\frac{dM_D}{dt} = 0.5\lambda_P P_D - \mu_M M_D$$

$$\frac{dF}{dt} = 0.5\lambda_P P - \delta F\frac{M_A + M_D}{m} - \mu_F F$$

$$\frac{dF_W}{dt} = released + 0.5\lambda_P P_W - \delta F_W\frac{M_A + M_D}{m} - \mu_F F_W$$

$$\frac{dF_A}{dt} = 0.5\lambda_P P_A + \delta F\frac{M_A + M_D}{m} - \mu_F F_A$$

$$\frac{dF_D}{dt} = 0.5\lambda_P P_D + \delta F_W\frac{M_A + M_D}{m} - \mu_F F_D$$

Where, '*released*' denotes the pulsed release of Wolbachia-infected adults. Unless stated otherwise, fortnightly releases of both females and males continuing for 6 months are simulated. Whereas the newly released Wolbachia-infected males are tracked separately from wild-born Wolbachia-infected males because of their reduced mating competitiveness, no such disadvantage has been shown for laboratory-reared Wolbachia-infected females. Finally, Asaia-infected males have been shown to sexually transmit Asaia to females, and this is assumed to occur at rate δ.

2.2. Computational experiments

There are no previously published models of Asaia dynamics in anopheline populations. The first set of experiments sought to understand the relationship between equilibrial infection prevalence and the parameters governing Asaia dynamics: maternal and paternal transmission, sexual transmission, and the rate of infection through environmental contamination. The aim was to determine whether Asaia prevalence recorded in wild anopheline populations were concordant with the Asaia transmissibility rates recorded in laboratory experiments.

Next, a global sensitivity analysis was conducted to determine the key Wolbachia- and Asaia-associated parameters underlying a successful Wolbachia deployment programme, where 'success' was measured by the equilibrial prevalence of Wolbachia-infected mosquitoes after releases ceased.

Whereas some have reported no evidence for antagonistic interactions between Wolbachia and Asaia (Straub et al., 2020),

others have shown evidence for antagonisms (Segata et al., 2016; Jeffries et al., 2018). These antagonisms included: reduced susceptibility of Wolbachia-infected insects to Asaia infection through environmental contamination and copulation (Rossi et al., 2015); reduced maternal and paternal transmission of Asaia for doubly infected insects (Rossi et al., 2015); and, reduced maternal transmission of Wolbachia for doubly infected insects (Hughes et al., 2014). The equations showing the addition of these interactions can be found in the supplementary materials. The sensitivity of a successful Wolbachia deployment programme to dominant parameters identified in the global sensitivity analysis was explored with and without negative interspecific interactions.

3. Results

3.1. Asaia prevalence in the absence of Wolbachia

There are many routes of Asaia infection: maternal and paternal transmission, through environmental contamination with Asaia, and sexual transmission (from male to female). Intuitively, with so many opportunities for infection, simulations within much of the explored parameter spaces resulted in endemically stable Asaia prevalences. Fig. 1 shows the conditions required for incomplete Asaia penetration of the mosquito population. With vertical transmission rates set to the only estimates found in the literature (Favia et al., 2007; Damiani et al., 2008), Asaia infection stabilised at very high prevalence regardless of a total absence in sexual or environmental transmission. Reducing the vertical transmission rate of Asaia resulted in reduced prevalence, and in conjunction with very low levels of sexual and environmental transmission, conditions could be obtained whereby Asaia prevalence was considerably reduced, including where it failed to establish in the mosquito population.

There are limited data informing the Asaia prevalence among wild anopheline populations. One recent study sampled malaria vectors from Guinea, DRC, Ghana, Uganda and Madagascar and showed the full range of prevalence (<5% to 100%) among Asaia-positive collection sites (Jeffries et al., 2018). Among sites where Asaia was present but Wolbachia absent (i.e. Asaia's prevalence was unlikely to be affected by Wolbachia interference locally), re-analysis of the data from (Jeffries et al., 2018) shows an average Asaia prevalence of 52% but with high variability (standard deviation = 32%). Variation in natural Asaia infection prevalence has been attributed to heterogeneities in environmental exposure (Kang et al., 2019). To assess the feasibility of this, rates of transmission from the natural environment (an aspect of Asaia transmission for which data could not be found) were varied (0–1) and the resultant Asaia prevalence recorded. The large Asaia prevalence range recorded from field-caught mosquitoes could not be achieved using the model parameters informed through laboratory studies – only a 14% range in prevalence was achievable (spanning 86–100%, Fig. 2). Both vertical and sexual transmission limited the model's ability to generate low equilibrial Asaia levels. Fig. 2 shows how either vertical or sexual transmission (or both) had to be substantially modified from rates recorded in laboratory studies for a wide range of prevalence to be achievable. Going forward, two modified parameter sets that facilitate the complete range of prevalence recorded from wild populations are explored in parallel: i) set1 which includes a 1.75-fold increase in vertical transmission failures combined with a 10% sexual transmission risk, and ii) set2 which includes a 2-fold increase in vertical transmission failures combined with a 30% sexual transmission risk.

3.2. Wolbachia-Asaia coinfection model

The influence of all parameters governing both Asaia and Wolbachia transmission on the success of a Wolbachia deployment programme was assessed using the total Sobol index produced from a global sensitivity analysis (details in the supplementary materials). The total Sobol index is the contribution to the output variance including all variance caused by its interactions with other input parameters (Sobol′, 2001). The prevalence of Wolbachia-infected mosquitoes after the system re-equilibrated following Wolbachia deployment was used as the metric of success. Fig. 3 shows that parameters governing Asaia transmission dominated those governing Wolbachia's transmission, with the rate at which Asaia maternally transmits being the key parameter in determining Wolbachia's success. Proportional changes to failure of Wolbachia to maternally transmit and failure of cytoplasmic incompatibility had negligible impact on Wolbachia's success because, in accordance with the literature, the baseline rates for these parameters are so low (Walker et al., 2011; Chrostek and Gerth, 2019).

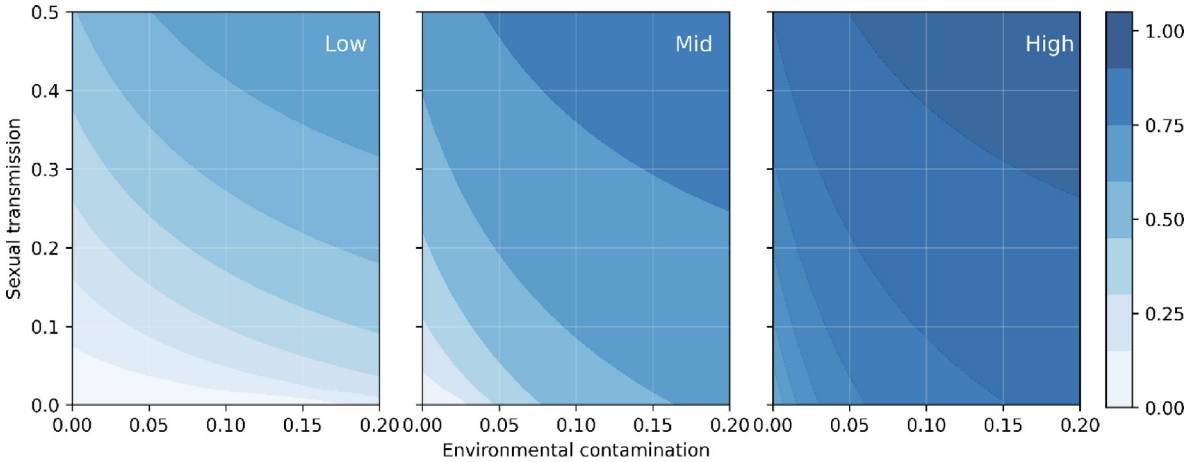

Fig. 1. Prevalence of Asaia in a mosquito population as a function of the rates of infection from sexual transmission (male-to-female) and from environmental contamination of breeding sites. Results are shown for three assumed rates of vertical transmission of Asaia (top-right of plots): 'Low', 'Mid' and 'High' vertical transmission rates resulted 200%, 150% and 100% the vertical transmission failure rate identified in the literature. Results were produced for fixed environmental contamination rate but similar results were produced when this rate was weighted by the prevalence of Asaia among local mosquito population (Supplementary Fig. 1).

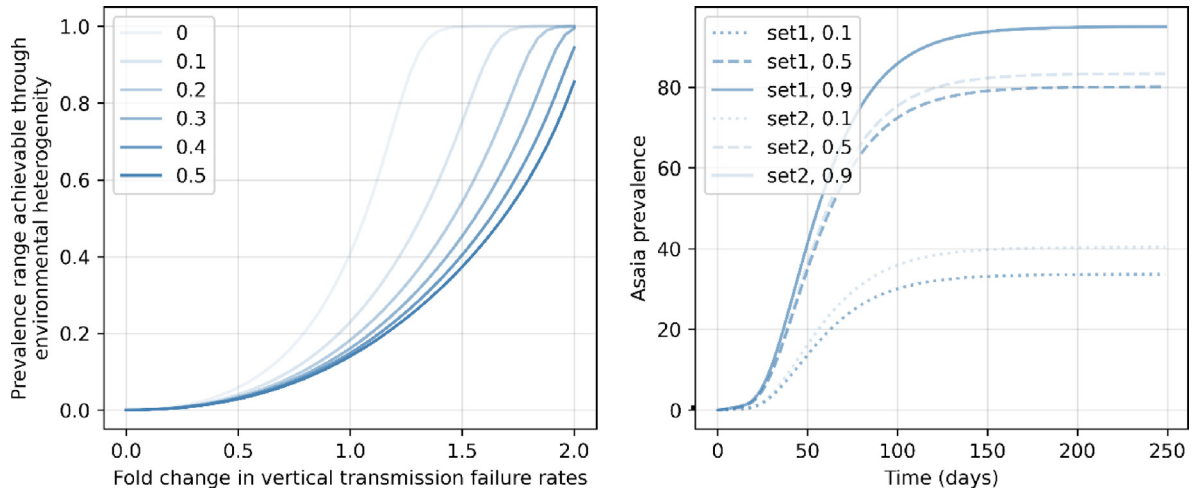

Fig. 2. A) Range in equilibrial Asaia prevalence achievable from adjusting environmental infection rate. The darkening lines denote the sexual transmission rate (male-to-female) with values in the legend. When vertical transmission failure rates are low (left on the x-axis), Asaia penetration of the mosquito population is complete, and its prevalence is not impacted by reduced sexual transmission or environmental contamination. When vertical transmission failure rates are high (right on the x-axis), Asaia prevalence becomes sensitive to sexual transmission and environmental contamination rates. B) Asaia spread dynamics for two alternative parameter sets ('set1' or 'set2') that allow for environmental contamination (proportions in legend) to dictate the equilibrial prevalence level.

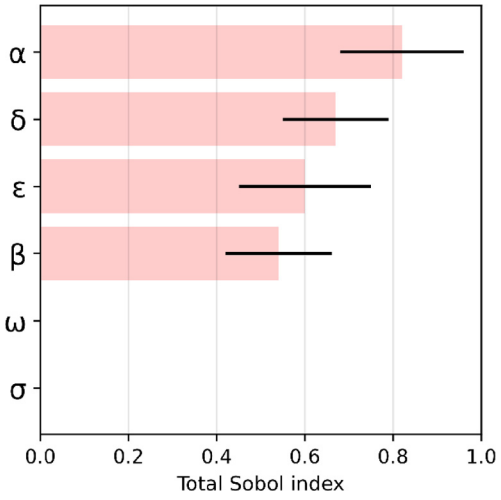

Fig. 3. Global sensitivity analysis of equilbrial prevalence of Wolbachia-infected adults to all parameters governing Asaia and Wolbachia transmission. Parameters include: α – failure of Asaia to maternally transmit, δ – rate of sexual transmission of Asaia, ε – rate of Asaia infection from aquatic environment, β – failure of Asaia to paternally transmit, ω – failure of Wolbachia to maternally transmit and σ – failure of cytoplasmic incompatibility.

Using parametrization that allowed for the wide range in Asaia prevalence to be driven by environmental transmission levels (i.e., set1 and set2), the importance of the endemically stable level of Asaia prior to Wolbachia deployment was tested. When there is no negative interference between Wolbachia and Asaia (Straub et al., 2020), the prevalence of singly Wolbachia-infected mosquitoes matches the prevalence of Asaia-uninfected mosquitoes that existed prior to Wolbachia release, and the remaining mosquitoes are coinfected (Fig. 4). In other words, Wolbachia invades successfully, infecting everything including the mosquito sub-population that was infected with Asaia.

Finally, the reported negative interactions between Wolbachia and Asaia were incorporated singly and in combination to determine their consequences to successful Wolbachia deployment. A reduced susceptibility of Wolbachia-infected mosquitoes to both sexual and vertical transmission of Asaia had intuitive results: they favoured Wolbachia mono-infection (Fig. 5). Reduced susceptibility of Wolbachia-infected mosquitoes to environmental infection had a more pronounced benefit to a Wolbachia mono-infection and indicated that there was a threshold susceptibility reduction beyond which Wolbachia competitively excluded Asaia from the local mosquito population. Combined susceptibility reductions for all Asaia infection pathways had qualitatively similar effects to a reduced susceptibility to environmental infection but favoured Wolbachia mono-infection to a greater degree.

Owing to reports of reduced maternal transfer of Wolbachia among coinfected mosquitoes (Hughes et al., 2014), the significance of this feature was explored in simulations accounting for all reported interspecific interactions (Suppl Figs. 3 and 4). A 50% reduction in Wolbachia's maternal transfer only had marginal impact when Asaia prevalence was very high prior to Wolbachia deployment (Suppl Fig. 3). Here, Wolbachia could not fully penetrate the mosquito population and a small prevalence of Asaia-only infected mosquitoes remained. However, when 100% of Wolbachia's maternal transfer was blocked in coinfected mosquitoes, there was a threshold initial Asaia endemic prevalence beyond which Wolbachia failed to establish a stable prevalence (Suppl Fig. 4). This blocked spread of Wolbachia was only overcome when Wolbachia infection severely reduced Asaia infection through the environmental contamination route.

4. Discussion

Wolbachia is currently under development as a potential biocontrol strategy for malaria vectors. Transinfection of this pathogen-blocking endosymbiotic bacterium into *Aedes* spp. has had considerable success in the control of dengue (Utarini et al., 2021). However, the presence of Asaia, a potentially antagonistic resident endosymbiont, among anopheline mosquitoes may represent a potential hurdle in the translation of these successes to malaria control. Using data from laboratory experiments and from surveys of endosymbionts in mosquitoes caught in the field, this study sought to assess the feasibility of a successful Wolbachia biocontrol programme for malaria.

Asaia infection in anopheline mosquitoes has only yielded scarce data mostly from laboratory experiments that do not attempt to simulate natural settings. For example, to test the

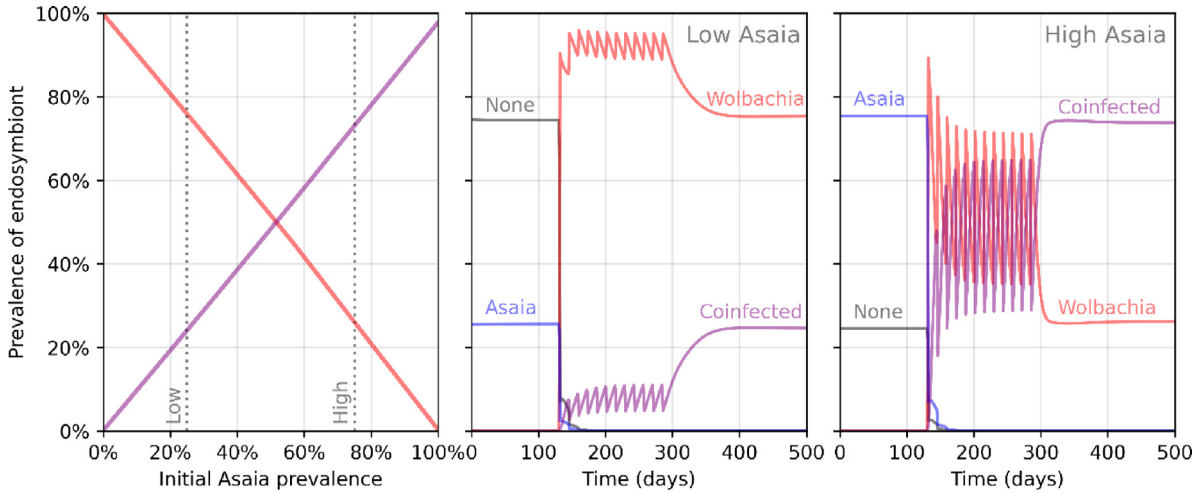

Fig. 4. Endemic equilibrial Wolbachia prevalence following releases into populations with simulations initiated with differing endemically stable levels of Asaia (left) and, examples of the associated dynamics (middle and right). These results were produced using set2 parameters but set1 parameters gave very similar results (Supplementary Fig. 2).

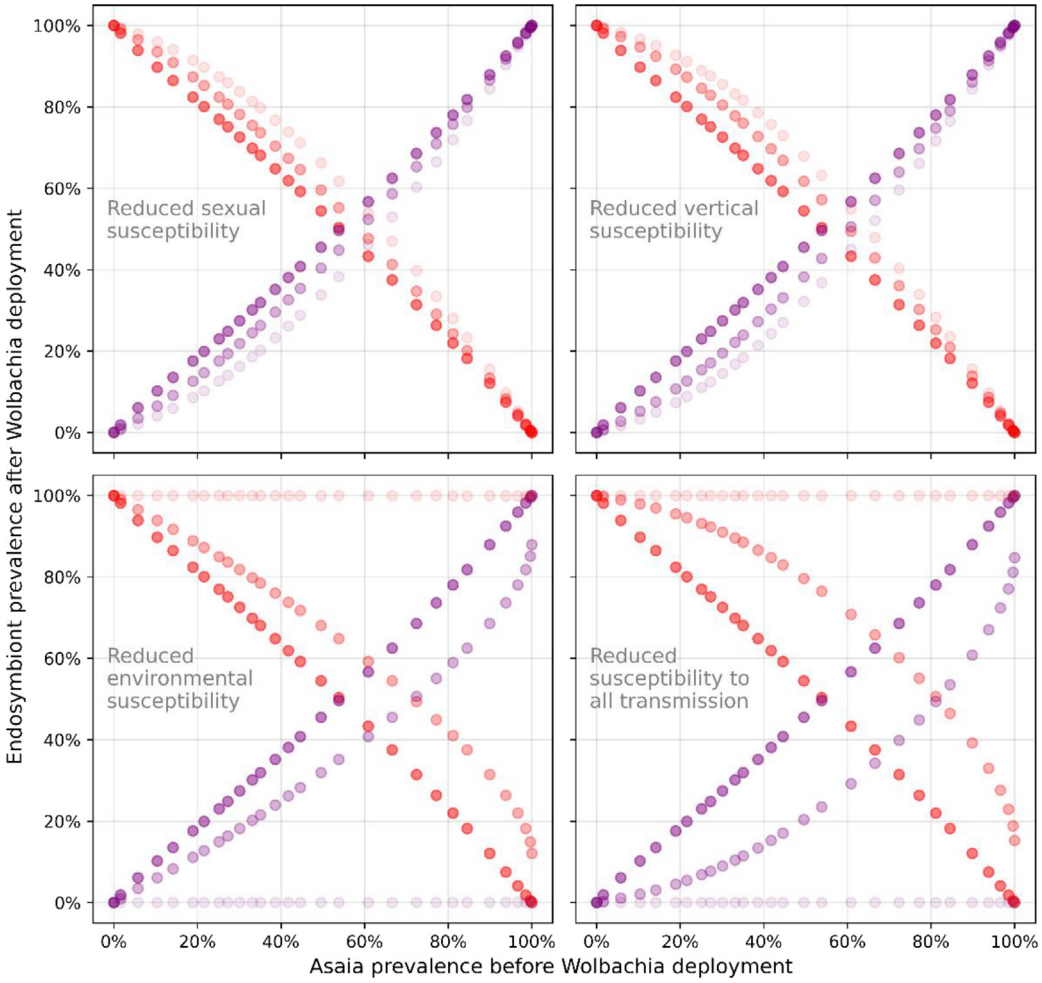

Fig. 5. The impact of reduced susceptibility of Wolbachia-infected mosquitoes to the various routes of Asaia infection singly (top-left, top-right, bottom-left) and combined (bottom-right). Dark markers delimit the baseline parametrization with lighter markers indicating 50% and 100% reduced susceptibility.

potential for Asaia in the larval pools infecting aquatic stages of mosquitoes, the water was spiked with an undisclosed concentration of Asaia that may differ markedly from what is experienced by larvae in natural settings (Favia et al., 2007). While these experiments are critical for informing proof-of-principal, caution must be exercised in converting transmissibility in this artificial setting to real-life contexts. For this reason, we first assessed whether the rates identified in artificial lab settings for the various routes

of transmission could potentially yield the wide range of Asaia prevalences recorded from distinct, wild mosquito populations even in the absence of Wolbachia. Critically, we showed that without drastic alteration (reductions) in Asaia's transmissibility, simulations invariably resulted in this endosymbiont rapidly reaching fixation (or, near-fixation). This indicates that the rates of transmission recorded in the lab could represent considerable overestimates. Global sensitivity analysis showed that it was these parameters governing Asaia transmission that dominated those governing Wolbachia transmission in determining success of a Wolbachia biocontrol strategy.

Of the various Asaia transmission routes, sexual and vertical transmission rates were considered to be less likely to vary substantially among separate mosquito populations. Therefore, modified (reduced) transmission rates that allowed for the natural prevalence range were used and the variability in prevalence was assumed to be driven by heterogeneous environmental infection rates. Intuitively, when there were no negative interactions assumed between the endosymbionts (i.e., following reports from Straub et al. (Straub et al., 2020), release ratios exceeding the threshold for Wolbachia's spread resulted in its fixation among a mosquito population already harbouring Asaia. Asaia's prevalence remained unchanged, but these mosquitoes became co-infected. Incorporating the antagonistic effects of Wolbachia on Asaia reported by Rossi et al. (Rossi et al., 2015) and Hughes et al. (Hughes et al., 2014) also had largely intuitive results: they reduced the proportion of co-infected mosquitoes (with a concomitant increase in the singly Wolbachia-infected proportion) after Wolbachia's deployment. How important this distortion in mono- versus co-infection is in limiting arbovirus transmission is unknown but we speculate that presence of negative interspecific competition between the endosymbionts may mitigate at least some of the arbovirus blocking within co-infected mosquitoes. For example, Rossi et al. (Rossi et al., 2015) showed that among coinfected mosquitoes, the density of Wolbachia in the salivary glands were reduced.

An intrinsic redundancy in Asaia transmission routes was shown in simulations where each rate was halved or eliminated but Asaia infection persisted. This resilience was only overcome when Wolbachia conferred complete resistance to environmental infection with Asaia resulting in Wolbachia's fixation and Asaia's complete exclusion. Both endosymbionts occupy the same niches within larval and adult mosquitoes and Rossi et al. (Rossi et al., 2015) have shown for *Anopheles* and other mosquitoes that resident Wolbachia infection precludes subsequent colonisation by Asaia. Should this equate to a very high level of resistance to infection by Asaia from the environment, not only does this facilitate fixation of Wolbachia mono-infection but it also implies that a mosquito population with high prevalence of Wolbachia will be renitent to Asaia (re-)invasion.

A single study describes reduced maternal transmission of Wolbachia infection in mosquitoes also infected with Asaia (Hughes et al., 2014). Simulations accounting for bidirectional antagonisms yield a qualitatively distinct outcome following Wolbachia deployment whereby Wolbachia fails to spread into the wild population and Asaia mono-infection retains its pre-control prevalence. This outcome is more likely when Asaia has higher prevalence pre-control. Contingent on the strength of Asaia resistance in Wolbachia infected mosquitoes and the route(s) of transmission blocked, between a 60–75% endemic Asaia prevalence is generally sufficient to preclude a successful Wolbachia campaign when Asaia eliminates Wolbachia's ability to maternally transmit. An exception again occurs when Wolbachia infection provides very high protection against environmental Asaia infection – in which case Wolbachia still over-rides Asaia.

To our knowledge this is the first mathematical model of Asaia infection in disease vectors and the first Wolbachia model that includes interspecific interactions between species of endosymbiont. Thus, it is particularly important to acknowledge several limitations. There are very few studies of Asaia in malaria vectors. Assumed traits of Asaia infection biology were often gleaned from singular observations as a consequence. All modes of Asaia transmission have only been demonstrated in laboratory settings and usually in experimental set-ups that diverge markedly from natural settings. We could not ignore this uncertainty; we introduced each infection mode systematically, exploring a wide range of rates to ascertain findings which were widely, if not generally, applicable. There are no stable lines of Wolbachia transinfected African malaria vectors, so we had to resort to findings reported for the Asian vector species *An. stephensi*. Further, there is discord in the literature over the evidence for antagonisms between endosymbionts. Again, we could not ignore these opposing findings and instead explored all scenarios: no interactions, uni- and bi-directional antagonisms. Consequently, results are caveated; but these caveats highlight future experiments that comprise the essential next steps in assessing the feasibility of a Wolbachia biocontrol strategy for malaria vectors.

First, laboratory experiments that build on the proofs-of-principal by better emulating natural conditions to find the rates of Asaia transmission via the alternative pathways is an obvious next step. We could not reconcile the data from initial laboratory studies with the wide range of natural Asaia prevalences and we suspect that this may be because these infection rates are currently overestimated. Second, reproducible demonstration of Asaia-blocking potential in Wolbachia infected mosquitoes at all life stages is fundamental. Results from our computational experiments suggest that generating a solid evidence base to substantiate and quantify resistance to environmental Asaia infection is particularly important. We cannot yet do this for African malaria vectors so in the first instance these experiments could use *An. stephensi.* Third, our results showed that Asaia co-infection altering maternal transmission rates of Wolbachia could seriously jeopardise a biocontrol strategy, highlighting the need to quantify this antagonism.

By no means do these experiments encompass all that are needed to address feasibility of a Wolbachia biocontrol strategy for malaria vectors, but they do identify achievable targets representing early checks necessary prior to initiating more complicated investigations e.g. arbovirus-blocking potential among co-infected mosquitoes; differential impacts of temperature on the endosymbionts. Finally, by accounting for the system uncertainties for this burgeoning technology, the model produced in this work provides a very flexible framework for future feasibility assessments when more refined experimental data become available.

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CRediT authorship contribution statement

Saige Andreychuk: Formal analysis, Investigation, writing – original draft. **Laith Yakob:** Conceptualization, Methodology, supervision, Writing – review & editing.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jtbi.2022.111110.

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