Impact of population age structure on *Wolbachia* transgene driver efficacy: ecologically complex factors and release of genetically modified mosquitoes

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Abstract

Wolbachia symbionts hold theoretical promise as a way to drive transgenes into insect vector populations for disease prevention. For simplicity, current models of *Wolbachia* dynamics and spread ignore ecologically complex factors such as the age structure of vector populations and overlapping vector generations. We developed a model including these factors to assess their impact on the process of *Wolbachia* spread into populations of three mosquito species (*Anopheles gambiae*, *Aedes aegypti* and *Culex pipiens*). Depending on the mosquito species, *Wolbachia* parameters, released mosquito life stage and initial age structure of the target population, the number of *Wolbachia*-infected mosquitoes that we predict would need to be released ranged from less than the threshold calculated by the simple model to a 10–30-fold increase. Transgenic releases into age-structured populations, which is an expectation for wild mosquitoes, will be difficult and depending on the circumstances may not be economically or logistically feasible due to the large number of infected mosquitoes that must be released. Our results support the perspective that understanding ecological factors is critical for designing transgenic vector-borne disease control strategies.
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1. Introduction

The lack of vaccines or clinical cures for many vector-borne diseases and the evolution of insecticide resistance by vector arthropods and drug resistance by parasites have stimulated a large-scale effort to prevent these diseases by genetic modification of vector arthropods (Beaty, 2000; Hemingway and Ranson, 2000; Ito et al., 2002). Most strategies to control vector-borne diseases by release of genetically modified mosquitoes require the spread of transgenic traits to high frequency in the population, a concept known as population replacement (James, 2000). Due to lower expected fitness of individuals carrying a transgene, spread of the trait is not expected to occur spontaneously. Rather, the transgene of interest must be actively driven into the population in spite of fitness costs (Turelli and Hoffmann, 1999) and the goal would be to do this rapidly, in an epidemiologically rather than evolutionarity relevant time period.

Maternally inherited *Wolbachia* symbionts are under theoretical consideration as a possible transgene driver. In mosquitoes, *Wolbachia* is associated with cytoplasmic incompatibility (CI), i.e. reduced egg hatch when uninfected females mate with infected males. Matings between infected females and infected or uninfected males are fertile. Consequently, infected females have a reproductive advantage, allowing *Wolbachia* to spread rapidly through host populations in spite of induced fitness costs. As *Wolbachia* spreads, linked traits (either inserted into the *Wolbachia* genome or carried on a cytoplasmic factor) will "hitch-hike" along with the symbiont and spread into the population (Turelli and Hoffmann, 1999).

Three kinds of information are critical for using *Wolbachia* in an applied manner to prevent disease: (1) the unstable equilibrium; that is, the introduction threshold of *Wolbachia*-infected individuals that must be surpassed for infection to spread and become established in the population, (2) the stable equilibrium frequency that infection will ultimately reach, and (3) how long the invasion will take to reach an equilibrium frequency from a given introduction level. Of these, predictions of introduction thresholds are most critical from an applied perspective because threshold values will determine whether the transgenic release will be logistically and economically feasible. Mathematical models, parameterized using field data, are essential for estimating thresholds (Turelli and Hoffmann, 1999).

For simplicity, current "Turelli–Hoffmann" (TH) models of *Wolbachia* spread make simplifying assumptions. Although these assumptions make model calculations more tractable, predictions based on these models may not be accurate representations of natural populations in which simplifying assumptions are not supported. Two important assumptions of TH models are (1) no population age structure (single age class) and (2) discrete, non-overlapping generations. We developed a matrix model of *Wolbachia* spread that includes age structure and overlapping generations to assess the impact of these factors on the dynamics of *Wolbachia* spread and on applied predictions for release of genetically modified mosquitoes.

2. Methods

The framework of our model included 10 immature age classes (eggs, larvae and pupae), and 30 adult age classes. We assumed that males and females had similar daily survival rates. We conducted separate simulations for three different mosquito vector species (*Anopheles gambiae*, *Aedes aegypti* and *Culex pipiens*) using life table data (daily survival and fecundity) estimated from published reports. Daily survival rates (p_x) for each species were: *An. gambiae*, 0.85 (Bockarie et al., 1995); *Ae. aegypti*, 0.89 (McDonald, 1977); and *Cx. pipiens*, 0.9 (Macdonald et al., 1968). Daily fecundity (m_x) values for *An. gambiae* (Gary and Foster, 2001), *Ae. aegypti* (Harrington et al., 2001) and *Cx. pipiens* (Gómez et al., 1977) are shown in Fig. 1. For *Wolbachia* parameters, F, relative fecundity of infected vs. uninfected females; μ, % uninfected offspring produced from an infected female; H, relative hatch rate of an incompatible vs. compatible cross, and $S_h = (1 - H)$. We assumed that CI does not change with male age, because (1) in previous analyses we concluded that this is not an important factor in age-structured populations and does not greatly affect predictions of *Wolbachia* equilibrium values (Rasgon et al., 2003), and (2) we have found no
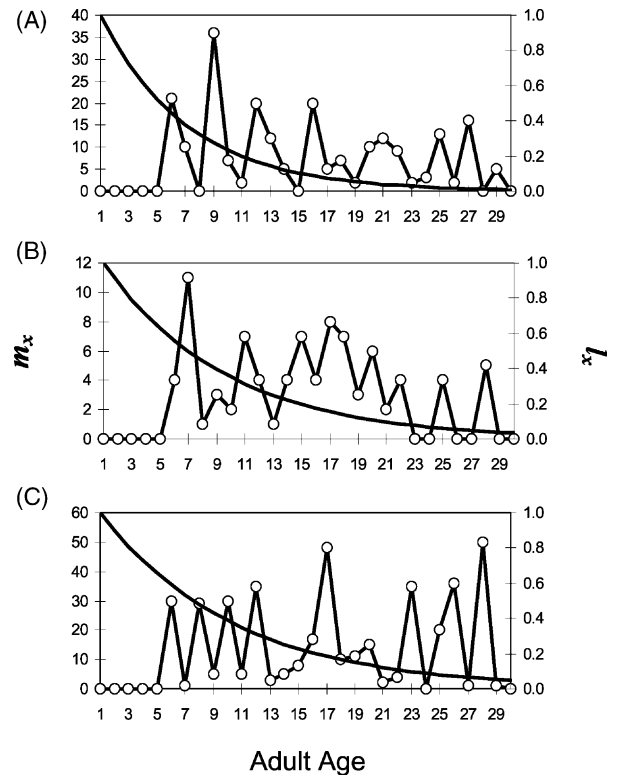

Fig. 1. Cumulative survival (l_x) and daily fecundity (m_x) data used in the parameterization of the model for simulations. Solid line = l_x, circles = m_x. (A) *Anopheles gambiae*; (B) *Aedes aegypti*; (C) *Culex pipiens*.

empirical evidence for significant reductions in CI expression with male age in *Cx. pipiens* complex mosquitoes (Rasgon and Scott, 2003). $N_{x,t}$ denotes the number of mosquitoes in age class x at generation t. I and U refer to infected and uninfected mosquitoes, respectively and ω denotes the oldest age class.

2.1. Infected

$$
\begin{pmatrix}
{}^I B_0 & {}^I B_1 & \cdots & {}^I B_{\omega-1} & {}^I B_\omega \\
{}^I p_0 & 0 & \cdots & 0 & 0 \\
0 & {}^I p_1 & \cdots & 0 & 0 \\
\vdots & \vdots & \ddots & \vdots & \vdots \\
0 & 0 & 0 & {}^I p_{\omega-1} & 0
\end{pmatrix}
\times
\begin{pmatrix}
{}^I N_{0,t} \\
{}^I N_{1,t} \\
{}^I N_{2,t} \\
\vdots \\
{}^I N_{\omega,t}
\end{pmatrix}
$$
$$
=
\begin{pmatrix}
{}^I N_{0,t+1} \\
{}^I N_{1,t+1} \\
{}^I N_{2,t+1} \\
\vdots \\
{}^I N_{\omega,t+1}
\end{pmatrix},
\tag{1}
$$

where

$$
{}^I B_x = (m_x)(1 - \mu)F.
\tag{2}
$$

2.2. Uninfected

$$
\begin{pmatrix}
{}^{U}B_o & {}^{U}B_1 & \cdots & {}^{U}B_{\omega-1} & {}^{U}B_\omega \\
{}^{U}p_0 & 0 & \cdots & 0 & 0 \\
0 & {}^{U}p_1 & \cdots & 0 & 0 \\
\vdots & \vdots & \ddots & \vdots & \vdots \\
0 & 0 & 0 & {}^{U}p_{\omega-1} & 0
\end{pmatrix}
\times
\begin{pmatrix}
{}^{U}N_{0,t} \\
{}^{U}N_{1,t} \\
{}^{U}N_{2,t} \\
\vdots \\
{}^{U}N_{\omega,t}
\end{pmatrix}
$$

$$
=
\begin{pmatrix}
{}^{U}N_{0,t+1} + U \\
{}^{U}N_{1,t+1} \\
{}^{U}N_{2,t+1} \\
\vdots \\
{}^{U}N_{\omega,t+1}
\end{pmatrix},
\tag{3}
$$

where

$$
{}^{U}B_x = (m_x)(1 - S_h W_x). \tag{4}
$$

The model assumes that females mate only once in their lifetime. Thus, CI expression during each oviposition cycle throughout each female's life is a function of the infection frequency among males of mating age at the time that the female in question mated, denoted W_X. U is the number of uninfected eggs produced by infected mothers due to imperfect transmission and takes the value

$$
U = \sum_{x=1}^{\varpi}(m_x)({}^{I}N_{x,t})(\mu F[1 - W_X] + \mu F H[W_X]). \tag{5}
$$

We modeled releases of adult mosquitoes into the population, because adult releases allow for adequate insect dispersal (Pal and LaChance, 1974). For standardization, we initially modeled releases into a population at a stable age distribution and then varied this assumption in later analyses. To assess the impact of introducing different life stages of mosquitoes, we modeled releases of infected mosquitoes as (1) newly emergent teneral adults or (2) 6-day-old gravid females and similarly aged males. For each simulation, we (1) calculated the ratio of released infected to wild mosquitoes necessary to surpass introduction thresholds required for infection invasion and (2) determined the stable equilibrium frequency that infection will ultimately reach. For comparison between our approach and that of used in earlier studies (Turelli and Hoffmann, 1999), we calculated the relative increase in the number of released transgenic mosquitoes in our analyses compared to predictions from the TH model.

3. Results

In all simulations for all three species, population age structure and overlapping generations had no effect on predicted stable equilibrium levels. Predictions from both the TH model and the age-structured model were identical.

The only situation where introduction threshold predictions between the TH and age-structured models were identical was where there were no fitness effects due to infection or transgenic manipulation ($F = 1.0$) and *Wolbachia* vertical transmission was 100% ($\mu = 0$) for any value $H < 1$. In this situation, infection is predicted to spread from any introduction above 0. If there is any fitness effect due to infection or the transgene, and/or *Wolbachia* transmission is less than perfect, predicted introductions of transgenic mosquitoes into age-structured populations were significantly increased compared to predictions of the TH model.

3.1. Releases of teneral adults

If mosquitoes are introduced as newly emergent teneral adults, the number of infected individuals that must be released for a successful invasion is increased from 5- to 10-fold as compared to predictions of the TH model, depending on the values for *Wolbachia* transmission, CI, and fitness effects. In general, results for each species were similar (Fig. 2A). The release of individuals into a single age class results in a "pulse" of reproduction that travels temporally across the various age classes. Each temporal pulse in turn creates a new pulse as newly produced mosquitoes begin reproducing. These overlapping reproductive pulses result in oscillations in infection frequency over time. Oscillations damp out as infection becomes stratified equally across all age classes (Fig. 3A). If infection frequency in all age classes at the time of oscillation damping is above the introduction threshold predicted by the TH model, infection will spread into the population. If, at this point, frequency is below the TH threshold, infection will be lost.

3.2. Releases of gravid adults

If mosquitoes are introduced as gravid adults, the number of infected individuals that must be released for a successful invasion is increased from 1.2- to 2.75-fold compared to predictions of the TH model, depending on the values for *Wolbachia* transmission, CI, fitness effects. Results for all three species were similar (Fig. 2B). Infection oscillations and damping are similar to those experienced from teneral releases (Fig. 3B). Again, if frequency at the time of oscillation damping is above the predicted TH threshold, infection will spread.

3.3. Populations not at the stable age distribution

To this point in our analyses, we assumed that introductions were made into populations with a stable age distribution. The initial age structure of the population, however, can have a large positive or negative effect on
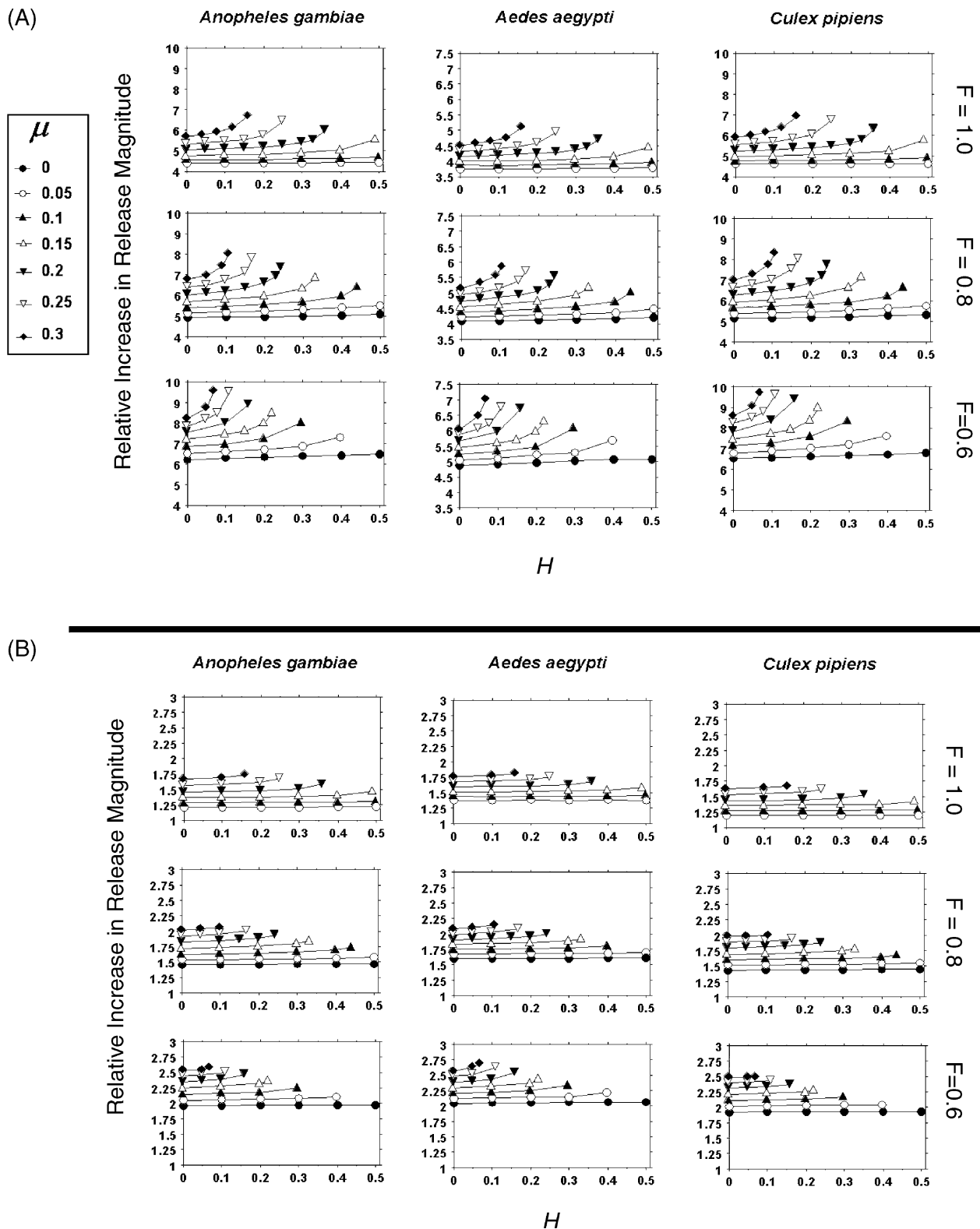
Fig. 2. Relative increase in *Wolbachia* introduction thresholds in age structured populations relative to predictions of the Turelli–Hoffmann model (stable age distribution). *F*, relative fecundity of infected vs. uninfected females; *H*, relative hatch rate of an incompatible vs. compatible cross and μ, % uninfected offspring produced from an infected female. (A) Teneral release; (B) gravid release.

the size of the required introduction. In Fig. 4A, we show simulation results for *Cx. pipiens* as an example,

where $F = 0.95$, $\mu = 0.05$ and $H = 0.1$; results for other species and *Wolbachia* parameters were qualitat-
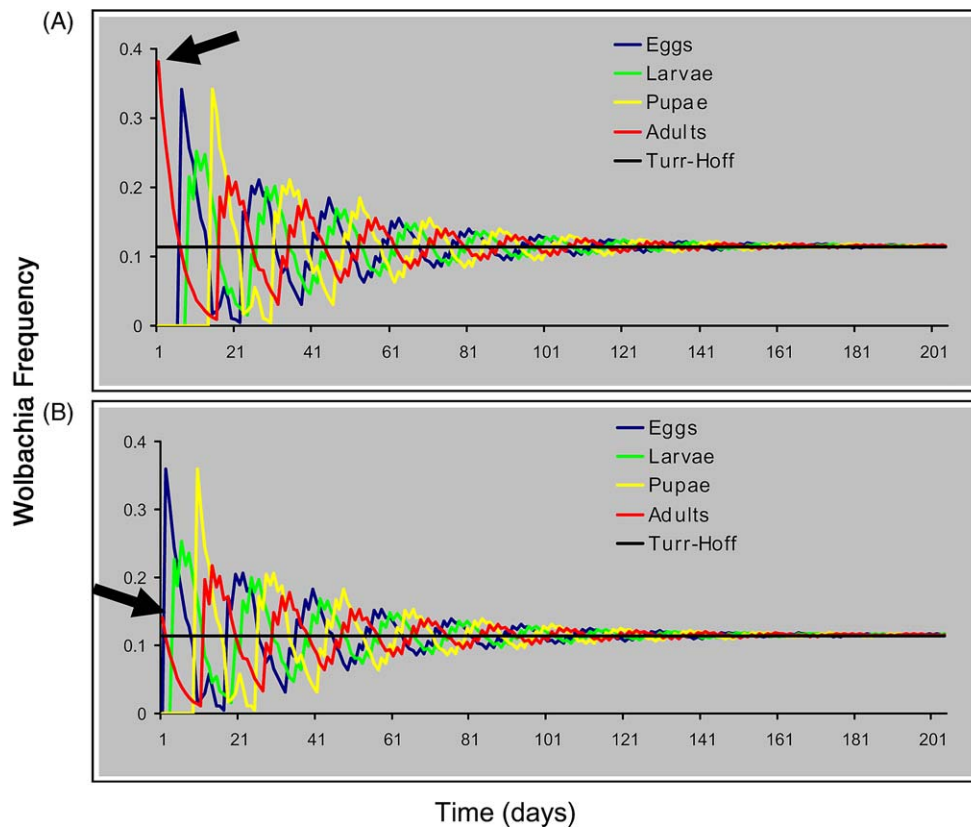
Fig. 3. Infection dynamics of *Wolbachia* infection among different mosquito life stages (data from *Culex pipiens*). Infection was introduced into a population at the stable age distribution. Simulations were conducted using a "minimal release" strategy, i.e., the minimum release required for infection to surpass the TH threshold. If infection surpasses this threshold it will drive into the population and reach a stable equilibrium frequency of 99.4%. *Wolbachia* parameters are: $\mu = 0.05$, $H = 0.1$, $F = 0.95$. Arrows indicate the frequency of infected adults that must be released in order for infection to surpass the Turelli–Hoffmann threshold at equilibrium (black line). (A) Teneral release; (B) gravid release.

ively similar. We simulated two deviations from the stable age distribution resulting from (1) an extended bout of adult control that temporarily eliminated the adult population without affecting the immature population and (2) a mass emergence event where the entire population was clustered within the four youngest adult age classes (Fig. 4A). Our results indicate that altering the population age structure by reducing the adult population density prior to release of transgenic mosquitoes results in the lowest relative release levels. If performed in conjunction with a gravid release, adult population reduction could potentially result in predicted introduction levels below those of the TH model. If, however, a release was conducted after a mass emergence event, release magnitudes could potentially be increased by almost 30-fold compared to predictions of the TH model (Fig. 4B).

4. Discussion

We did not detect any difference in predicted stable *Wolbachia* equilibrium levels between the TH model

and the age-structured model. Therefore, if one's study goal is to determine the stable equilibrium frequency for a particular *Wolbachia* strain, TH models are adequate for the task and should be used due to their mathematical tractability. The predictive ability of the TH model for *Wolbachia* stable equilibrium levels has been validated in natural populations for several species of *Drosophila* (Turelli and Hoffmann, 1995; Hoffmann et al., 1998) and for *Cx. pipiens* complex mosquitoes (Rasgon and Scott, 2003).

In almost all cases, however, the TH model underestimates the number of transgenic mosquitoes that must be released into an age-structured population for a successful population replacement event. Thus, while the TH model is useful for describing the overall dynamics of the system, it is inadequate for making applied predictions for *Wolbachia*-based vector-borne disease control strategies and highlights the need for including ecologically realistic parameters in predictive modeling efforts.

Seemingly uncomplicated factors, such as the life stage of released insects, can have a large impact on the numbers of transgenic mosquitoes that must be
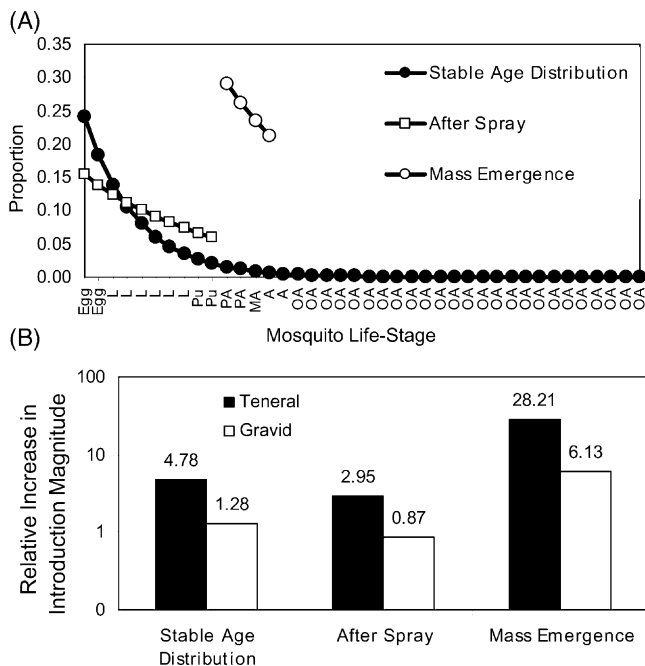
(A)

(B)

Fig. 4. Deviation from the stable age distribution and effect on *Wolbachia* introduction thresholds (data from *Culex pipiens*). *Wolbachia* parameters are as stated in Fig. 3. (A) Age distributions used in simulations (see text for derivations). E, egg; L, larvae; P, pupae; Pr, pre-mating adult; MA, mating adult; Po, post-mating adult; OA, ovipositional adult. (B) Predicted introduction threshold relative to predictions of the Turelli–Hoffmann model.

released. Increases in predicted *Wolbachia* introduction thresholds in age-structured populations are due primarily to mortality experienced by released infected mosquitoes prior to the onset of oviposition and the production of new uninfected individuals from the standing population of uninfected larvae at the time of introduction. Released teneral mosquitoes must live for at least one week to have time to mate, obtain a blood meal, and develop eggs. To compensate for the effects of daily mortality, more individuals must be initially released to ensure that an adequate number are alive when oviposition begins.

To reduce the number of individuals that must be released for infection to spread, it is necessary to reduce the mortality period experienced by released infected mosquitoes between the times of release and oviposition. One way to accomplish this is to release infected mosquitoes as gravid adults. In this situation, released mosquitoes will be ready to oviposit immediately after release, allowing for more rapid invasion of infection into the next generation and consequently lower required introduction levels.

Teneral releases, although conceptually simple, require large relative releases to be effective. For vector species that have large effective population sizes, such as *Cx. pipiens* complex mosquitoes (Rajagopalan et al., 1976; Reisen et al., 1991), releases of teneral mos-

quitoes may not be economically or logistically feasible due to the magnitude of the required introduction, although the size of individual releases could possibly be reduced by carrying out multiple releases. Releases of teneral adults might be easier for vector species that have lower population densities, such as *Ae. aegypti* (Focks et al., 1981). Dramatic, seasonal fluctuations in population density in such species as *Cx. pipiens* complex mosquitoes (Reisen et al., 1992) and *An. gambiae* (Minakawa et al., 2001) might also be exploited to reduce the number of mosquitoes needed for a successful release. In all cases, *Wolbachia* is a more efficient transgene driver if gravid adults are released as opposed to tenerals. It is operationally much more difficult, however, to release gravid adults. To produce gravid adults, females must be allowed to mate and blood-feed in the laboratory before release. Some species may not mate easily under laboratory conditions and may even require force mating (Clements, 1999) or may not blood-feed easily for mass-rearing efforts. Thus, there is a trade-off between the most effective release strategy (gravid) and the most logistically feasible one (teneral). The choice of strategy will vary depending on the biology of the species of interest.

Our initial simulations assumed that the vector population was at the predicted stable age distribution, which is based on the mortality and survival trajectories of the insects. Natural populations, however, may not be at the stable age distribution. Alteration of the initial age distribution can greatly affect predictions of *Wolbachia* introduction thresholds. Relative to predictions of the TH model, reducing the adult population (for instance with an adulticide campaign) before releasing infected mosquitoes results in the lowest relative release levels. Conducting releases after a mass emergence event results in the highest relative release levels. However, during these kinds of analyses, it is important to take into account the absolute size of the population at the time of the initial release. Although it is true that releasing during a mass emergence event could require large relative releases, the absolute population size may be small and thus result in a logistically low release level in terms of absolute numbers.

Results from our study support the notion that a thorough understanding of the ecological parameters that govern vector populations is critical for implementing vector-borne disease control using genetically modified arthropods (Scott et al., 2002). Although we have focused on *Wolbachia*-based systems, it is likely that similar principles will be important for any type of transgenic mosquito release. It is important to note that population age structure is just one ecologically complex factor that will affect the success of transgenic introductions and, as such, it highlights the complexity of natural systems that must be taken into account. There are many other factors that are likely to have

significant effects on the success of transgenic strategies. Those factors include, but are not limited to, vector population regulation (Dobson et al., 2002), vector metapopulation dynamics (Schofield, 2002; Rasgon, 2003), assortative mating, and stability of transgene constructs under field conditions. Now that the molecular techniques underpinning genetically modified arthropods have been developed, successful application of this technology will rely on a thorough understanding of insect vector ecology (Scott et al., 2002). Combining molecular science with ecological studies will result in the development of novel, cost-effective, and efficient vector-borne disease control strategies for the 21st century.

Acknowledgements

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