Title: Dual effects of insect fecundity overdispersion on the *Wolbachia* establishment and the implications for epidemic biocontrol

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Abstract

The utilization of the most prevalent endosymbionts *Wolbachia* spp. to tackle insect-borne viral diseases is growing rapidly. Understanding how and how often *Wolbachia* establish in a local population is fundamental to replacement releases but remains unclear. Previous models make the prediction of poor performance of *Wolbachia* at low frequencies that contradicts the natural ubiquity of those endosymbionts, and the prediction of almost certain fixation of *Wolbachia* at high frequencies that cannot explain the large fluctuations and collapses of infection in field releases. Here, we investigated whether those paradoxes can be reconciled by the stochasticity originating from fecundity overdispersion within host insects. We first reanalyzed published datasets and showed that fecundity was mostly overdispersed in insects. To understand the effects of host fecundity variation on *Wolbachia* establishment, we further constructed a model accounting for cytoplasmic incompatibility and fecundity cost on infected hosts. Based on the empirical results of fecundity overdispersion, the model predicted not only a biologically relevant probability for *Wolbachia* to establish from a single infection, but also a large uncertainty of fixation at high frequencies. These findings will enable a better understanding of endosymbiont-insect dynamics and help design sustainable strategies to control arboviral diseases.

Keywords: arbovirus; insect endosymbiont; bistable dynamics; stochastic process; negative binomial distribution

Key message

- *Wolbachia* has been widely used to control arboviruses, but how fecundity variation of the vector impacts *Wolbachia* establishment remains unknown.

- Fecundity overdispersion was observed in most studied insects, and it may help *Wolbachia* establish from low frequencies but reduce the certainty of fixation at high frequencies.

- Fecundity variation should be considered in *Wolbachia*-based vector replacement strategies, and more transfected individuals may need to be released across a longer period than is currently used.

Introduction

Ubiquitous arthropod-borne viruses (arboviruses) have been posing threats to human health for a long time. The pandemic of dengue virus infection has spread to five continents at an unprecedentedly high rate of 390 million cases per year, placing over 3 billion people at risk of the disease (Bhatt et al. 2013; Brady and Hay 2020). Since the first noteworthy epidemic on Yap Island in Micronesia in 2007, Zika virus has caused massive outbreaks throughout South America, Central America, and the Caribbean (Cugola et al. 2016; Fauci and Morens 2016). Besides the developments of effective vaccines and therapeutics against pathogenic arboviruses, control of their vector populations also plays a critical role in epidemic prevention (Ritchie et al. 2018). However, suppressing the vectors through chemical insecticides is challenged by the evolution of resistance (Moyes et al. 2017) and criticism on the damages to ecosystems caused by the chemicals (Pance 2018). Recently, more effective, natural, and self-sustaining biocontrol strategies using endosymbionts have been proposed and developed (Dorigatti et al. 2018; Ghosh et al. 2018; Ross et al. 2019).

Wolbachia spp. are probably the most abundant endosymbiotic intracellular bacteria infecting more than half of the million-plus species of insects (Hilgenboecker et al. 2008; Zug et al. 2012). Some *Wolbachia* strains have been recognized to effectively inhibit the proliferation and transmission of various RNA arboviruses in their host insects (Hedges et al. 2008; Teixeira et al. 2008), probably through competition over resources and innate immune priming (Terradas and McGraw 2017). However, *Wolbachia* do not naturally occur in some arboviral vectors including the primary vector of Zika, dengue, malaria, and

75   chikungunya (i.e. the *Aedes aegypti* mosquito). A promising avenue for tackling this issue

76   is release of transinfected vectors, which aims at population replacement with virus-

77   blocking *Wolbachia*. So far, introduction of *w*Mel *Wolbachia* strains from *Drosophila*

78   *melanogaster* into *A. aegypti* is the leading transinfection mode, with operational releases

79   in ten countries by the World Mosquito Program (Ritchie et al. 2018). These *w*Mel-releases

80   have resulted in a dramatic reduction (up to 97%) of dengue incidence in the project sites

81   where *Wolbachia* have established at high frequency, in contrast to only a moderate

82   reduction (*c*. 50%) in hot areas such as Brazil (https://www.worldmosquitoprogram.org/).

83   In light of large reduction in invasiveness and virus-blocking capacities for *w*Mel at high

84   temperatures in contrast to *w*AlbB from *A. albopictus* which is much less susceptible to

85   similar temperatures (Ant et al. 2018; Ross et al. 2017), *w*AlbB may be well suited for

86   population replacement in hot tropical environments. Recently, *A. aegypti* mosquitoes

87   carrying *w*AlbB were released at six sites in Kuala Lumpur, Malaysia (Nazni et al. 2019).

88   The strain has been successfully established and maintained at high frequency at some sites,

89   but large fluctuations of *Wolbachia* frequency were also observed (Nazni et al. 2019).

90   A key driver for *Wolbachia* replacement is the induction of cytoplasmic incompatibility

91   (CI), which elevates the mortality of embryos of matings between infected males and

92   uninfected females or females carrying an incompatible *Wolbachia* strain (Hoffmann and

93   Turelli 1997; Laven 1956). Because of maternal transmission of *Wolbachia*, CI provides

94   infected females with a frequency-dependent fitness advantage (Caspari and Watson 1959).

95   Successful establishment of *Wolbachia* then depends on CI outweighing possible fitness

96   cost on infected hosts and outweighing incomplete maternal transmission of infection

97   (Hancock et al. 2011; Turelli 1994). This creates bistable frequency dynamics under which

98   the infection frequency must exceed a threshold for *Wolbachia* to spread rather than to be

99   lost from a population (Caspari and Watson 1959). Other dynamics, such as Fisherian

100   dynamics under which *Wolbachia* will always spread throughout the host populations even

101   with very low initial numbers in the absence of CI (Barton and Turelli 2011), are seldom

102   reported or utilized in arbovirus control (but see Kriesner et al. 2013).

103   However, large fluctuations in infection frequency and thereby obstructions of

104   *Wolbachia* invasion confronting field replacement releases are beyond the prediction of the

105   model. In the *A. aegypti* population of Nogotirto, Indonesia, *w*Mel frequency was raised to

106   more than 80% at the end of field release period, but then oscillated strongly between 50%

107   and 100% (Tantowijoyo et al. 2020). Collapse of *w*Mel introduction has also been observed

108   in Westcourt, Australia (Schmidt et al. 2017) and Rio de Janeiro, Brazil (Garcia et al. 2019).

109   Following the releases of *w*AlbB-carrying *A. aegypti* in Malaysia, the *Wolbachia* frequency

110   once exceeded 95% but subsequently fluctuated and even decreased to 20% at two of four

111   primary intervention sites (Nazni et al. 2019). Temperature susceptibility of *Wolbachia*

112   strain or lack of insecticide resistance in released mosquitoes may account for some

113   unavailing releases (Ant et al. 2018; Garcia et al. 2019), but was proved to have minor

114   effect in some other cases (e.g. Nazni et al. 2019).

115   An alternative and probably more general factor that underlies large fluctuations and

116   thereby collapse of *Wolbachia* introduction is stochastic processes (Engelstadter and

117 Telschow 2009). Previous drift models showed weak impacts of stochasticity on *Wolbachia*

118 dynamics in large host populations, leading to a conclusion that employment of *Wolbachia*

119 as a driving element in pest control normally does not need to consider stochastic effects

120 (Egas et al. 2002; Jansen et al. 2008). However, those models invoked the population

121 genetic parameter 'effective population size', i.e. the size of an idealized panmictic

122 population that experiences the same strength of genetic drift as the actual population

123 (Fisher 1930; Wright 1931). This parameter facilitates modelling but can hardly assist

124 *Wolbachia*-based epidemic management, because effective population size is usually

125 unknown for most vector populations and is difficult to estimate accurately (Wang 2016).

126 In contrast, more variation in fecundity than expected by the Poisson distribution (termed

127 'overdispersion'), the latter assumed in the Wright–Fisher idealized population (Kimura

128 and Crow 1963), has been observed in many insect species including mosquitoes

129 (Blackmore and Lord 2000; Ferguson et al. 2003; Vezilier et al. 2012). It should be

130 expected that such overdispersion generates more stochasticity to the invasion dynamics

131 of *Wolbachia*, and importantly, that the degree of the overdispersion can be easily estimated

132 in the laboratory or in the field. Overdispersion may also provide an explanation for the

133 paradox between the model prediction of poor performance of *Wolbachia* in a novel host

134 from few infections and the reality that *Wolbachia* have successfully infected millions of

135 arthropod species (Fenton et al. 2011). Nevertheless, fecundity overdispersion has rarely

136 been evaluated empirically in insect populations, nor is it understood how fecundity

137 overdispersion impacts the fates of *Wolbachia* in a local host population.

138   Here we present evidence of fecundity overdispersion in insects through reanalyzing

139   empirical datasets, and construct a general *Wolbachia* frequency-dynamic model explicitly

140   considering host fecundity variation. Based on the empirical estimates of overdispersion,

141   we performed a general analysis using the model. We then further used the model to

142   estimate the number of infected vectors required for successful population replacement, to

143   offer some operational suggestions for optimum release strategies in *Wolbachia*-based

144   biocontrol. Additionally, we also explore how and how often *Wolbachia* succeed to invade

145   a novel host population starting from a single infection, which may provide mechanistic

146   insights into the ubiquity of those endosymbionts.

147

148   **Materials and methods**

149   **Literature search**

150   We conducted a literature search using "TI=(fecundity) AND TS=(insect*)" on the ISI

151   Web of Knowledge database (http://apps.webofknowledge.com) in April 2020 to identify

152   experimental data of insect fecundity, yielding a total of 1914 records. We also searched

153   for the fecundity of *A. aegypti* using "TS=(*Aedes aegypti* AND fecundity)", yielding 672

154   records. We then screened these records according to the following criteria: (i) realized

155   fecundity (not potential fecundity) was measured, (ii) insect fecundity was counted at the

156   individual level, and (iii) there was at least one group of studied insects free of the

157   experimental treatments that are unlikely to occur in natural conditions (e.g., pesticides and

158   RNA interference). After screening, we retained 56 studies for estimation of fecundity

159   dispersion (Appendix S1, Supporting Information).

160

161   **Estimation of fecundity dispersion**

162   For each species examined in each retained study, we calculated the sample size (n),

163   the mean ($\bar{x}$) and variance (S^2) of fecundity from the deposited data. If original data were

164   not available, these statistics were extracted from main texts or graphs only concerning the

165   insect individuals that met the third criterion. When multiple groups were needed to be

166   combined together (the conditions could occur in the same population, e.g. treatments with

167   different hosts) but the statistics were reported separately for those groups, the total

168   statistics were calculated as $\bar{x} = \sum n_i \bar{x}_i / \sum n_i$ and $S^2 = \sum n_i (S_i^2 + \bar{x}_i^2) / \sum n_i -$

169    $(\sum n_i \bar{x}_i / \sum n_i)^2$ (see Appendix S2). In the cases where only the ranges of fecundity were

170   reported for each group, we randomly sampled n_i values from the corresponding uniform

171   distribution. We then pooled all groups together and calculated the summary statistics

172   based on 10,000 simulations for each species. The Kolmogorov–Smirnov test was used to

173   examine whether the fecundity data were Poisson distributed in each species. If not,

174   overdispersion parameter θ was estimated by $\theta = \bar{x}^2 / (S^2 - \bar{x})$. Zero inflation is a source

175   of overdispersion (Linden and Mantyniemi 2011), but zeros might have been eliminated in

176   data analyses, leading to an underestimation of overdispersion. Hence, we recorded

177   whether zero values were included in each dataset.

178

179   **The model of *Wolbachia* dynamics**

180   We construct a general dynamic model of infection frequency to describe how host

181   fecundity variation together with CI and infection cost impact the fate of *Wolbachia* in a

182   host population. We focus on CI because it is the most frequently found *Wolbachia*-induced

183   reproductive effect (Werren et al. 2008) and most relevant to *Wolbachia* invasion for pest

184   management (Ritchie et al. 2018).

185   We denote the fecundity of the *i*th infected female and that of the *j*th uninfected female

186   using $F_{I(i)}$ and $F_{U(j)}$ (both can be zero), which have expected values of λ_1 and λ_2, respectively.

187   The fecundity cost due to *Wolbachia* parasitism is thus given by $s_f = 1 - \lambda_1/\lambda_2$. We let $H <$

188   1 represent the hatch rate from a CI cross relative to other crosses, and let $\mu \leq 1$ denote the

189   probability for an infected female to transmit *Wolbachia* to its eggs. Thus, $s_h = 1 - H$

190   quantifies the intensity of CI, and $1 - \mu$ quantifies imperfect maternal transmission. We

191   assume discrete generations of the host population, but generation overlap and age structure

192   may be incorporated into our model like Turelli (2010) and Hancock et al. (2011). Panmixia

193   is assumed for the host population, and more realistic nonrandom mating may be reconciled

194   by partitioning the population into several panmictic subpopulations linked via gene flow.

195   The population dynamics of *Wolbachia* can be modelled by tracking changes in the

196   frequency of infected hosts across generations. If the frequency of hosts being infected at

197   generation t is p_t, and the total number of reproductive females within the population is N,

198   the numbers of crosses are then expected to be Np_t^2, $Np_t(1-p_t)$, $Np_t(1-p_t)$, and $N(1-p_t)^2$, for

199   IF $\times$ IM, IF $\times$ UM, UF $\times$ IM, and UF $\times$ UM, respectively (I: infected, U: uninfected; F:

200   female, M: male). Multiplying them by the corresponding fecundity and hatch rate, we

201   obtain the recursion equation

$$p_{t+1} = \frac{\mu \sum_{i=1}^{Np_t} F_{I(i)}}{\sum_{i=1}^{Np_t} F_{I(i)} + \sum_{j=1}^{N[1-(1+s_h)p_t+s_h p_t^2]} F_{U(j)}} \qquad \text{(eqn 1)}$$

203   Stochastic deviation of the numbers of crosses from the expectations may exist due to finite

204   population size, but we argue that the variation can be regarded as a source of dispersion

205   in fecundity (F_I and F_U). Therefore, equation (1) holds in such cases. For investigating the

206   role of host fecundity variation in the dynamics of *Wolbachia*, we characterize F_I and F_U

207   in the following three cases.

208   *Case 1: No variation of fecundity*

209   We first assume constant fecundity, that is, $F_I = \lambda_1$ and $F_U = \lambda_2$. Then, equation (1)

210   reduces to

$$p_{t+1} = \frac{(1-s_f)\mu}{1 + s_h p_t^2 - (s_f + s_h)p_t} \cdot p_t \qquad \text{(eqn 2)}$$

212   and yields three equilibria by setting $p_{t+1} = p_t$:

213    $p = 0, \; p = \dfrac{s_f + s_h - \sqrt{(s_f - s_h)^2 - 4s_h(1-\mu)(1-s_f)}}{2s_h}, \;$ and $\; p = \dfrac{s_f + s_h + \sqrt{(s_f - s_h)^2 - 4s_h(1-\mu)(1-s_f)}}{2s_h}.$

214   For a simple example, if we assume perfect maternal transmission (i.e., $\mu = 1$), there

215   will be two stable equilibria ($p = 0$ and $p = 1$) and one unstable equilibrium ($\hat{p} = s_f/s_h$)

216   for the infection frequency. When the initial frequency (p_0) is above the establishment

217   threshold $\hat{p}$, *Wolbachia* will spread throughout the whole population, but when $p_0 < \hat{p}$,

218   *Wolbachia* will ultimately be lost in this population. Thus, this case corresponds to those

219   deterministic models that predict bistable dynamics (Barton and Turelli 2011; Caspari and

220   Watson 1959; Turelli and Barton 2017; Turelli and Hoffmann 1991).

11

221 *Case 2: Poisson distribution for dispersed fecundity*

222 We use the Poisson distribution to model moderate variation of host fecundity: $F_I \sim$

223 Poisson (λ_1) and $F_U \sim$ Poisson (λ_2), where the expected variances of F_I and F_U equal to their

224 means (λ_1 and λ_2), respectively. This case corresponds to stochastic models that test for the

225 effects of drift (Jansen et al. 2008; Rigaud and Rousset 1996).

226 *Case 3: Negative binomial distribution for overdispersed fecundity*

227 We take the negative binomial distribution to model F_I and F_U to include the

228 overdispersion of host fecundity, because this distribution has a good performance in

229 characterizing overdispersion of biological count data (Linden and Mantyniemi 2011;

230 Warton et al. 2016). Specifically, the probability functions of F_I and F_U are defined by

$$f(F_I = k) = \frac{\Gamma(\theta+k)}{k!\Gamma(\theta)} \left(\frac{\lambda_1}{\theta+\lambda_1}\right)^k \left(\frac{\theta}{\theta+\lambda_1}\right)^\theta \qquad \text{(eqn 3)}$$

232 and

$$f(F_U = k) = \frac{\Gamma(\theta+k)}{k!\Gamma(\theta)} \left(\frac{\lambda_2}{\theta+\lambda_2}\right)^k \left(\frac{\theta}{\theta+\lambda_2}\right)^\theta \qquad \text{(eqn 4)}$$

234 with the variances $\lambda_1 + \lambda_1^2/\theta$ and $\lambda_2 + \lambda_2^2/\theta$, respectively, where $\theta > 0$ is the dispersion

235 parameter with smaller values representing stronger overdispersion. When θ approaches

236 infinity, the distribution converges to the Poisson distribution.

237 To illustrate how variation of host fecundity affects the fates of *Wolbachia*, we perform

238 numerical simulations for a total of 60 scenarios over the three cases, setting four levels of

239 fecundity cost ($s_f = 0$, 0.01, 0.1, and 0.25), three levels of host population size ($N = 10^2$,

240 10^3, and 10^4), and six distributions of fecundity (constant, Poisson, and four negative

241 binomials with $\theta = 0.01$, 0.1, 1 and 10 according to the empirical results). Perfect maternal

242 transmission (i.e., $\mu = 1$) and complete CI (i.e., $s_h = 1$) are assumed in all scenarios, which

243 is reasonable for a variety of *Wolbachia* strains in *A. aegypti* (Ant et al. 2018; Hoffmann et

244 al. 2014). For each scenario, we set the initial infection frequency (p_0) from 0.01 to 0.99

245 with an increase of 0.01 each time and then calculated $p_1, p_2, \ldots$ using equation (1) until p_t

246 = 0 or 1. In the scenarios with fecundity variation, values of $F_{I(i)}$ and $F_{U(j)}$ are sampled from

247 the Poisson distribution or using equations (3) and (4). One thousand simulations are run

248 for each p_0 of each scenario to determine the probability of *Wolbachia* fixation.

249

250 **Applications of the model**

251   We used the model to address two specific issues. First, we estimated the number of

252 infected *A. aegypti* mosquitoes that need to be released in order to ensure a high probability

253 (say, ≥ 0.95) for successful *Wolbachia* invasion, when there is overdispersion in host

254 fecundity. The fecundity cost on *Wolbachia*-infected *A. aegypti* was estimated at *c*. 20%

255 for *w*Mel infection (Hoffmann et al. 2011) and 10%–15% for *w*AlbB infection (Axford et

256 al. 2016; Xi et al. 2005). We thus assumed fecundity cost at $s_f = 0.15$. The dispersion

257 parameter θ was set to vary over the range 0.01–10, according to the empirical estimates.

258 The number of reproductive female mosquitoes N was set at 10^2, 10^3 and 10^4. For

259 comparison, simulations with the same settings except for θ were also performed in cases

260 where host fecundity is fixed or Poisson distributed.

261   Second, the model was used to estimate the probability of *Wolbachia*'s fixation

262 following the introduction of a single infected female into a population (i.e. $p_0 = 1/N$)

263   through interspecific horizontal transfers. For comparison, we used the same settings of

264   fecundity cost as Jansen et al. (2008). Given the empirical results of fecundity

265   overdispersion in insects, we considered four plausible scenarios of overdispersion, i.e. θ

266   = 10, 1, 0.1, and 0.01, and use Poisson-distributed fecundity as the control. We ran the

267   model one million times for each combination of parameters to get the probability of

268   fixation of *Wolbachia*.

269

270   **Results**

271   **Fecundity overdispersion**

272   We obtained a total of 56 articles that met the three criteria, involving 47 species and

273   79 datasets. Only one of them (grain aphid *Sitobion avenae*) showed a Poisson-type

274   distribution of fecundity, with the variance close to the mean (23.74 vs. 26.09, $P = 0.58$).

275   Overdispersion was found in all other cases, with estimates of overdispersion parameter θ

276   0.01−20.29 (median: 2.6). The largest overdispersion ($\theta = 0.01$) was found in the eusocial

277   red ant *Myrmica rubra*. Besides *S. avenae*, 57 datasets did not include zero values, which

278   produced significantly larger θ (i.e., less overdispersion) than the 21 datasets with zeros

279   ($5.04 \pm$ SE 0.58 vs. $0.80 \pm$ SE 0.11, Mann–Whitney $U = 1121$, $P < 0.001$; Fig. 1). For the

280   same species, parameter θ was overestimated by approximately one order of magnitude

281   (median: 4.08-fold) when zero values were overlooked (Fig. 1). Summary statistics of all

282   studies are provided in Table S1.

283   Among the datasets involving *A. aegypti* ($n = 14$), six studies reported zero values and

284 displayed substantial fecundity overdispersion, with θ ranging 0.55–1.57 (median 1.05).

285 The remaining eight datasets that did not include zero values showed much weaker

286 overdispersion, where θ varied between 1.97 and 7.39 (median 4.50). When zero values

287 were deliberately excluded from the with-zero datasets, the increases in the estimate of θ

288 were large in two of three cases (Fig. 1).

289

290 ***Wolbachia* fixation from different initial frequencies**

291   The fixation probability displays a step function of initial infection frequency (p_0) if

292 assuming no variation for host fecundity (case 1), but turns to a sigmoid function when

293 fecundity is Poisson-distributed (case 2) or overdispersed (case 3). Compared to Poisson-

294 distributed fecundity, overdispersed fecundity produces smoother curves for the fixation

295 probability function (Fig. 2). More generally, when the overdispersion in fecundity become

296 larger (with a smaller θ), the model provides smoother curves for the fixation probability

297 function at all levels of fecundity cost (Fig. 2).

298   When p_0 is smaller than the threshold ($\hat{p}$) predicted by the deterministic model, the

299 model predicts non-zero fixation probabilities when there are some variations in fecundity,

300 and these probabilities generally increase with larger extent of fecundity dispersion (Fig.

301 2). For example, a *Wolbachia* strain causing fecundity cost of 25% is always unable to fix

302 itself in a host population of 1000 reproductive females, when the initial infection

303 frequency is 0.17 and host fecundity is Poisson distributed (Fig. 2f). In contrast, the strain

304 has a 9.2% chance to achieve fixation if host fecundity shows a negative binomial

15

305　distribution with $\theta = 0.1$. When $p_0 > \hat{p}$, however, overdispersion can introduce larger

306　variation to *Wolbachia* fixation, and the uncertainty of fixation increases with stronger

307　overdispersion (e.g., $p_0 > 0.25$ in Fig. 2f). This trend is extremely strong when population

308　size is relatively small (e.g., comparing Fig. 2c and f).

309

310　**Application 1: replacement releases of *Wolbachia*-infected vectors**

311　　In the vector population replacement with *Wolbachia*, fecundity variation significantly

312　elevates the number of infected vectors that should be released to ensure the successful

313　invasion of *Wolbachia* (Fig. 3). For a vector population of 1000 reproductive females, 27%

314　more *Wolbachia*-carrying vectors are required than predicted by the deterministic model

315　when accounting for Poisson-distributed fecundity. This proportion increases to 33%, 40%,

316　80% and 220% when fecundity is overdispersed with θ of 10, 1, 0.1 and 0.01, respectively.

317　The strength of such effect of fecundity variation depends negatively on population size.

318　The required initial infection frequency increases at an astonishing rate in a population of

319　100 reproductive females when fecundity variation becomes larger, whereas the impacts of

320　overdispersion are much weaker in a very large population (e.g. 10000 reproductive

321　females) (Fig. 3). Nevertheless, there is still a substantial effect that need to be considered

322　in such large populations if fecundity overdispersion is of the order of $\theta = 0.1$ (Fig. 3).

323　　When regarding the primary vector of Zika and dengue fever, *A. aegypti*, based on the

324　empirical estimates of fecundity overdispersion of $\theta = 0.55–1.57$ (Fig. 1), our model

325　predicts that the numbers of released mosquitoes into a population of 100, 1000 and 10000

326   reproductive females need to exceed the expectations from the deterministic model by

327   107%–130%, 33%–40%, and 13%, and exceed those from the stochastic model by 11%–

328   23%, 5%–11%, and 0%, respectively (Fig. 3).

329

330   **Application 2: spread of *Wolbachia* from a single infection**

331   When assuming Poisson-distributed host fecundity, the fixation probability declines

332   with population size in an approximately linear manner on the log-log plot if the *Wolbachia*

333   strains are neutral to their hosts (Fig. 4a). When population size is small, the fixation

334   probability of a weakly deleterious *Wolbachia* strain is close to the neutral one, but the

335   discrepancy enlarges rapidly as the deleterious effect of *Wolbachia* and/or population size

336   increases (Fig. 3a). Note that a strain with cost $s_f = 0.125$ is still unable to establish itself

337   in any population of $N > 600$ following one million events of lateral introduction of a single

338   infection.

339   Overdispersion of host fecundity increases the probability of *Wolbachia* spreading to

340   fixation from a single infection in a host population, and this effect becomes stronger when

341   population size, the deleterious effect of *Wolbachia*, and the magnitude of overdispersion

342   increase (Fig. 3 b–d). In contrast to the Poisson scenario, a strain with cost $s_f = 0.125$

343   achieves fixation in a host population of $N = 631$ every 550 events of introducing a single

344   infection if host fecundity is overdispersed with $\theta = 0.1$.

345

346   **Discussion**

347   This study provides to our knowledge the first assessment of overdispersion in insect

348 fecundity and its effect on the invasion dynamics of *Wolbachia*. Empirical data present

349 compelling evidence for overdispersion of fecundity in most studied insect species.

350 Furthermore, our model demonstrates that the overdispersion may help CI-inducing

351 *Wolbachia* spread in novel hosts from few infections, but can also undermine vector

352 population replacement with *Wolbachia* in epidemic management.

353   Our analysis may provide an underestimate for fecundity overdispersion in many insect

354 species due to several reasons. First, the conspecific insects used here to estimate fecundity

355 variation were reared under almost the same condition in each dataset. However,

356 environmental heterogeneity, variation in the availability and quality of food and mates,

357 and other biotic factors may augment the overdispersion of fecundity in field populations

358 (Awmack and Leather 2002; Borer et al. 2009; Reigada et al. 2018; Zanchi et al. 2012).

359 Second, zero-inflation is an important source of overdispersion (Linden and Mantyniemi

360 2011), as shown by the large overdispersion of the eusocial species *Myrmica rubra* ($\theta =$

361 0.01). Most studies reviewed here were not designed to test the variation of fecundity, and

362 therefore some oviposition failures might be treated as noise and excluded artificially.

363 However, null fecundity has a close relevance to the invasion of *Wolbachia*, because it may

364 directly lead to the loss of *Wolbachia*. Additionally, copious oviposition sites were supplied

365 for each adult in those studies, whereas competition for oviposition sites may occur in field

366 populations of some species, resulting in lower or even null fecundity of some adults. This

367 is likely the case in two populations of *A. aegypti* with *w*AlbB-releases in Malaysia, where

368 the population sizes decreased considerably following the cessation of releases (Nazni et

369 al. 2019).

370 　　Consistent with the results of previous stochastic models (Egas et al. 2002; Jansen et

371 al. 2008), our model suggests it is usually difficult for a deleterious *Wolbachia* strain to

372 spread from few initial infections if host fecundity is Poisson distributed. Repeated

373 *Wolbachia* transfers can compensate for the extremely low fixation probability of a single

374 transfer event (Jansen et al. 2008), but in this situation it is unclear whether the waiting

375 time for fixation is biologically relevant. In our study, the estimated probabilities of

376 *Wolbachia* reaching fixation from a single infection in host populations of overdispersed

377 fecundity appear to relax the assumptions of Jansen et al. (2008) on transfer rate and

378 waiting time, and may reconcile the contradictions among occasional and probably rare

379 interspecific transfer events (Turelli et al. 2018), bistable population dynamics (Barton and

380 Turelli 2011), and the remarkable prevalence of *Wolbachia* (Werren et al. 2008).

381 　　The uncertainty in the fixation of *Wolbachia* caused by overdispersion of host fecundity

382 (see Figs. 2 and 3) may to some extent explain the large fluctuations of *Wolbachia*

383 frequency in some *A. aegypti* populations after replacement releases (Nazni et al. 2019;

384 Schmidt et al. 2017; Tantowijoyo et al. 2020). Especially for the releases of *A. aegypti* in

385 Malaysia, lack of *w*AlbB invasion was found in low-density sites where oviposition sites

386 might be rare (Nazni et al. 2019). This phenomenon is consistent with the expectations of

387 large overdispersion of fecundity. Although we illustrate a special case of $s_f = 0.15$, the

388 need for increasing released individuals is prevalent across diverse fecundity costs of

389   *Wolbachia* infection and becomes even more critical when the cost is smaller (see Fig. 2).

390   In light of great efforts being made to search for *Wolbachia* strains of low fecundity costs

391   (Ant et al. 2018; Pance 2018), the significance of considering fecundity overdispersion in

392   successful vector control is likely to increase further.

393   Nevertheless, the effects of fecundity overdispersion may be counterbalanced by other

394   factors that have not been included in the model. An important factor particularly for *A.*

395   *aegypti* is larval density-dependent competition (Hancock et al. 2016a). This density effect

396   is likely to weaken the overdispersion effects, due to the tradeoff between adult fecundity

397   and larval fitness. Yet, the outcomes of density dependence for *Wolbachia*-based biocontrol

398   strategies are very similar to those of overdispersion, i.e. greater numbers of released

399   mosquitoes and longer time of *Wolbachia* establishment following releases (Hancock et al.

400   2016a; Hancock et al. 2016b). While increased mortality due to *Wolbachia* infection,

401   imperfect maternal transmission (e.g. at high temperatures), or occurrence of adverse

402   conditions, may augment the effects of overdispersion (Jansen et al. 2008).

403   Our model focuses on CI-inducing *Wolbachia* strains especially *w*Mel and *w*AlbB.

404   However, the *w*Au strain which does not induce CI shows more efficient virus blocking

405   and greater temperature stability than the two strains (Ant et al. 2018). Hence, a

406   superinfection *w*Au-*w*AlbB was created to combine strong viral inhibition and CI, and has

407   been proposed as a novel biocontrol agent (Ant et al. 2018). Intriguingly, our model can

408   also apply for the superinfection, but if *w*Au and *w*AlbB decoupled over time in the field,

409   only the dynamics of *w*AlbB would be described by our model.

410   Our model assumes a panmictic population, but mating is often nonrandom in the field

411   populations, especially when it routinely takes place at breeding sites before individuals

412   disperse (Macke et al. 2011). After an initial phase of local establishment, *Wolbachia* may

413   spread to other areas in a wave of colonization (Barton and Turelli 2011; Schmidt et al.

414   2017) or via stepping stone subpopulations (Engelstadter and Telschow 2009). In both

415   cases, spatial spread can be slowed or even stopped if the host density is much higher in

416   surrounding uninfected areas, because there are insufficient migrants transporting

417   *Wolbachia*. These areas are analogous to the 'tension zones' where transitions occur

418   between alternative genetic equilibria. Local fluctuations in population density and

419   dispersal rate can drive development and movement of these tension zones (Barton 1979;

420   Barton and Hewitt 1989). Nonetheless, the increased probabilities of fixation caused by

421   overdispersion in host fecundity should help *Wolbachia* to pass through such tension zones

422   via few migrants.

423   In conclusion, fecundity overdispersion is ubiquitous in insect populations, placing

424   large stochasticity on the dynamics of *Wolbachia*. The significance of this stochasticity has

425   been underestimated in both explaining the pandemic distribution of *Wolbachia* and

426   formulating strategies for sustainable biocontrol of insect-borne diseases with *Wolbachia*.

427   Our study address that efforts should be made to understand the dispersion of fecundity in

428   the target vector populations before decision on the nature of the *Wolbachia* releases, and

429   also that the stochasticity brought by fecundity overdispersion greatly contributes to the

430   local establishment and spatial spread of *Wolbachia* after the releases.

434 **Conflict of Interest**: The authors declare that they have no conflict of interest.

435 **Availability of data and material:** Not applicable.

436 **Ethics approval:** Not applicable.

437 **Consent to participate:** Not applicable.

438 **Consent for publication:** Not applicable.

439 **Code availability:** The R code is attached as a supplementary file.

440 **Author Contribution Statement**: XYC and XT conceived and designed research. XT and

441 JJ constructed models. XT, XYC, SGC, YC, YYD, RW and JJ analyzed data. XT wrote the

442 manuscript. All authors read and approved the manuscript.

443

444 **References:**

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Figure captions

Fig. 1 Empirical estimates of overdispersion in realized fecundity for 47 insect species.

Smaller values of θ represent stronger overdispersion. The estimate for *Sitobion*

avenae is not given, because the species showed Poisson-distributed fecundity. Each

estimate is derived from one study except in *Aedes aegypti* and *A. albopictus*, and the

numbers of studies used for the two species are shown. The difference of estimates

between with- and without-zero datasets for the same species is denoted by solid line

when the same study is used in both cases, or by dash line when using different studies.

All estimates from with- and without-zero datasets, respectively, are summarized by

boxplots. Insert shows the estimates for *A. aegypti* from each study.

Fig. 2 The probability of local fixation of *Wolbachia* as a function of initial infection

frequency, with varying levels of parasitism cost (s_f), host population size (N), and

host fecundity variation. The variation of fecundity increases from no dispersion

(black lines), dispersion (green lines), to overdispersion (purple, blue, yellow and red

lines). For each p_0 under each parameter setting, the model is conducted 1000 times

to estimate the fixation probability. Scenarios with $s_f = 0$ are very similar to those with

$s_f = 0.01$, and thus not shown here.

Fig. 3 The initial frequencies for ensuring a probability of 95% for successful establishment

of *Wolbachia* in relation to host fecundity overdispersion. Smaller θ values represent

larger overdispersion. Three population sizes (N) are considered, and we assume $s_f =$

0.15, $s_h = 1$, and $\mu = 1$. The predictions from the deterministic and stochastic models

631   are also shown (dash lines). Grey area denotes the estimation for *Aedes aegypti*.

632   **Fig. 4** The fixation probability of *Wolbachia* following the introduction of a single infected

633   female into a host population of size N with different distributions of fecundity. A

634   range of parasitism costs (s_f) is plotted.

The figure shows a forest plot of the overdispersion parameter, ϑ, across insect species. Species names (left axis, from top): *Sitobion avenae*, *Musca domestica*, *Aquarius remigis*, *Drosophila mauritiana*, *Callosobruchus maculatus*, *Aphis glycines*, *Nasonia vitripennis*, *Rhyzobius lophanthae*, *Culex pipiens*, *Lygus hesperus*, *Pineus similis*, *Rhynchophorus cruentatus*, *Nilaparvata lugens*, *Drosophila simulans*, *Ostrinia nubilalis*, *Euphydryas editha*, *Phoracantha semipunctata*, *Drosophila melanogaster*, *Epiphyas postvittana*, *Galleria mellonella*, *Operophtera brumata*, *Pineus pinifoliae*, *Lymantria dispar*, *Mepraia spinolai*, *Speyeria mormonia*, *Helicoverpa armigera*, *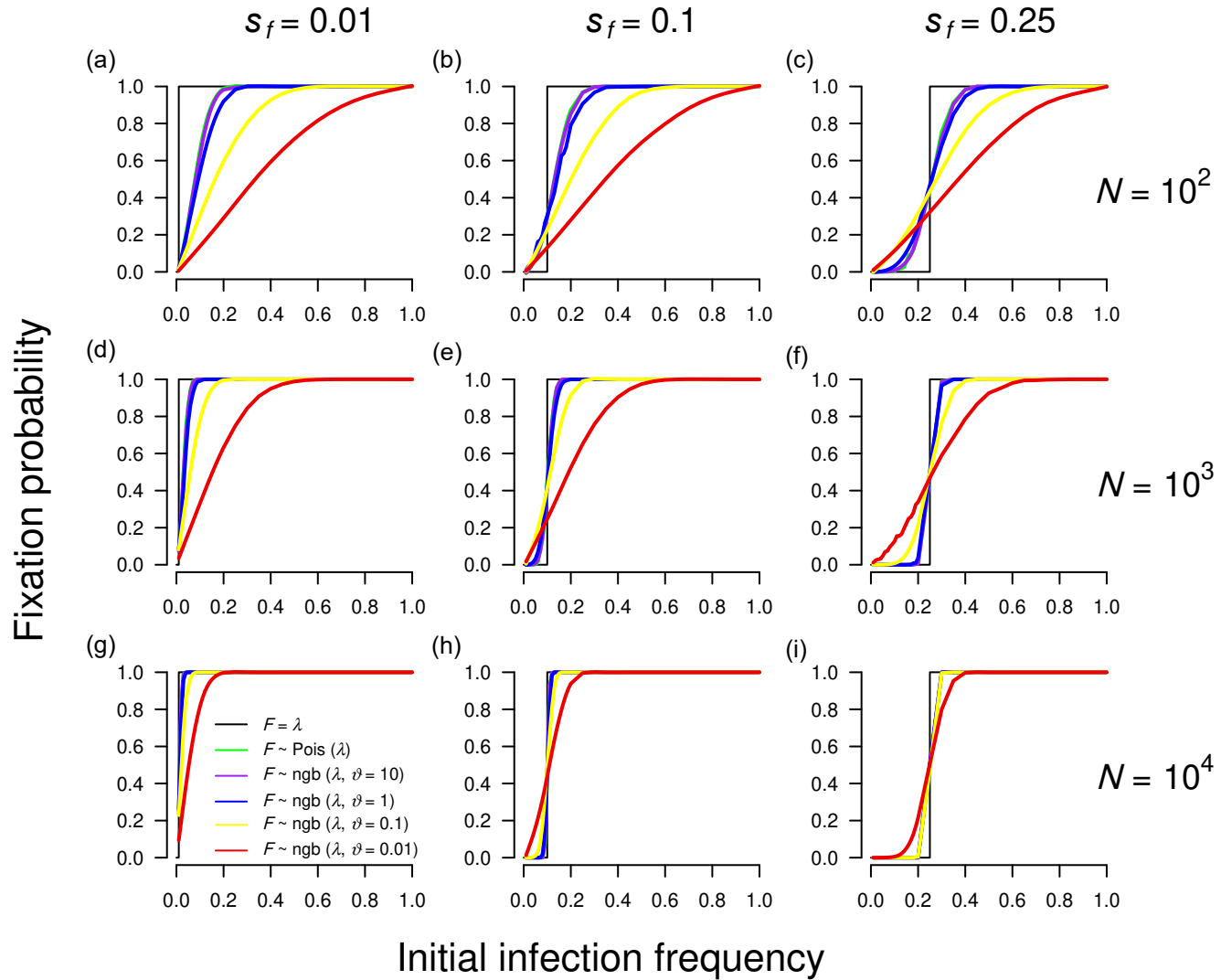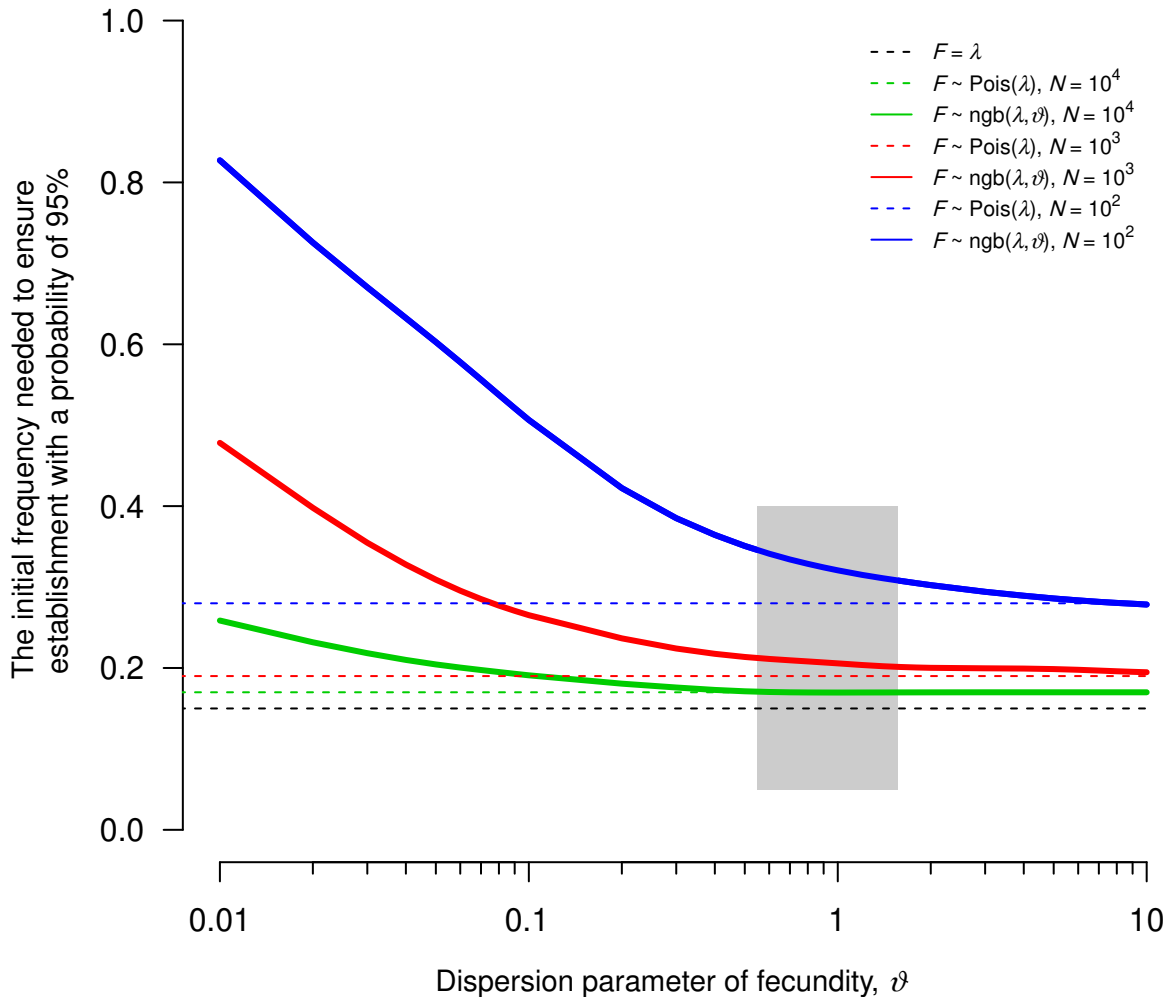Cornuaspis beckii*, *Trichogramma semblidis*, *Paratenodera angustipennis*, *Parlatoria pergandii*, *Acyrthosiphon pisum*, *Amara aenea*, *Orgyia leucostigma*, *Aedes aegypti*, *Aedes albopictus*, *Prokelisia marginata*, *Metamasius callizona*, *Colias philodice*, *Amara similata*, *Diadegma semiclausum*, *Paropsis atomaria*, *Amara familiaris*, *Ceratitis cosyra*, *Tribolium castaneum*, *Rhodnius prolixus*, *Tenebrio molitor*, *Myrmica rubra*.

Inset for *Aedes aegypti* lists studies: Qureshi et al. (2019), Yadav et al. (2019), G & H (2020), Gleave et al. (2016), Gutiérrez et al. (2020), da Silva et al. (2009), Muttis et al. (2018), Chang et al. (2018), Costanzo et al. (2015), Lee et al. (2018), Bibbs et al. (2018), Briegel (1990), C & P (2017), Harris & Cooke (1969), Summary.

Legend: ● Datasets with zero values; ○ Datasets without zero values.

x-axis: Overdispersion parameter, ϑ

Figure legend (from panels g–i):

$F = \lambda$
$F \sim \text{Pois}(\lambda)$
$F \sim \text{ngb}(\lambda, \vartheta = 10)$
$F \sim \text{ngb}(\lambda, \vartheta = 1)$
$F \sim \text{ngb}(\lambda, \vartheta = 0.1)$
$F \sim \text{ngb}(\lambda, \vartheta = 0.01)$

Column headers: $s_f = 0.01$, $s_f = 0.1$, $s_f = 0.25$

Row labels: $N = 10^2$, $N = 10^3$, $N = 10^4$

Y-axis: Fixation probability

X-axis: Initial infection frequency

The figure shows the initial frequency needed to ensure establishment with a probability of 95% (y-axis) versus the dispersion parameter of fecundity, ϑ (x-axis).

Legend:
- $F = \lambda$
- $F \sim Pois(\lambda), N = 10^4$
- $F \sim ngb(\lambda, \vartheta), N = 10^4$
- $F \sim Pois(\lambda), N = 10^3$
- $F \sim ngb(\lambda, \vartheta), N = 10^3$
- $F \sim Pois(\lambda), N = 10^2$
- $F \sim ngb(\lambda, \vartheta), N = 10^2$
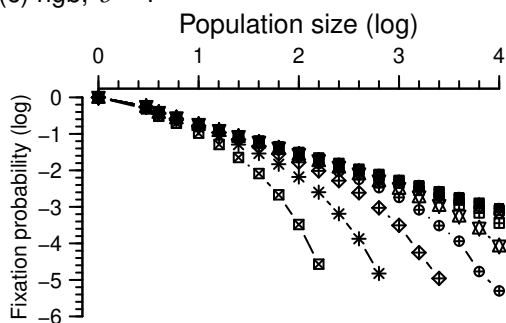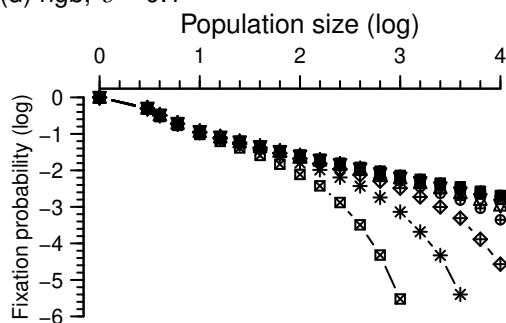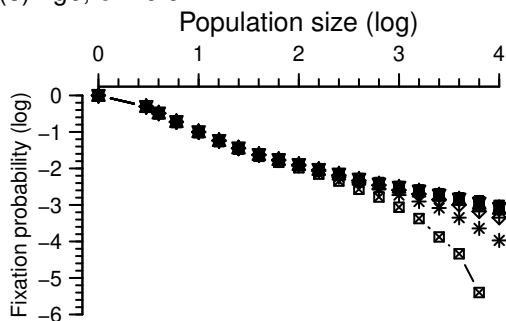
(a) Poisson

(b) ngb, $\vartheta = 10$

(c) ngb, $\vartheta = 1$

(d) ngb, $\vartheta = 0.1$

(e) ngb, $\vartheta = 0.01$

Legends

Population size (log)

Fixation probability (log)

$s_f = 0$	$s_f = 0.016$
$s_f = 0.001$	$s_f = 0.030$
$s_f = 0.002$	$s_f = 0.060$
$s_f = 0.004$	$s_f = 0.125$
$s_f = 0.008$	$s_f = 0.250$