The contributions of host population size and maternal transmission rate to fluctuating *Wolbachia* frequencies

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ABSTRACT

Animals form diverse relationships with microorganisms. Many insects host maternally transmitted endosymbionts such as *Wolbachia* that alter host fitness and reproduction. Roughly half of all insect species carry *Wolbachia*, but population frequencies of the endosymbiont can fluctuate across time and geography for reasons that are poorly understood. For instance, the frequencies of facultative *w*Mel *Wolbachia* in host *Drosophila melanogaster*, as well as related "*w*Mel-like" strains in *D. yakuba*-clade hosts, fluctuate over timescales as short as a month at a given location. Recent work suggests that *Wolbachia* frequencies may fluctuate under certain conditions due to temperature effects that disrupt maternal transmission. We simulated temporal *Wolbachia* dynamics to test whether fluctuating frequencies can be explained solely by the stochasticity of a finite host population or, alternatively, due to perturbations of the maternal transmission rate over time. We implemented a discrete generation model in the newly developed R package *wlbcmodeler* to evaluate how host population size, maternal transmission rates, and *Wolbachia* effects on host fitness (fecundity) and reproduction (cytoplasmic incompatibility; CI) contribute to *Wolbachia* fluctuations over time. Broadly, we find that strains like *w*Mel that do not cause strong CI tend to generate larger stochastic fluctuations over time, as compared to *Wolbachia* like *w*Ri in *D. simulans* that cause strong CI. Within a biologically plausible set of parameter values, we find that the maternal transmission rate has the strongest effect on the variance and mean of temporal *Wolbachia* frequencies, relative to host fitness effects and CI. Large frequency fluctuations can arise under certain specific conditions due to either stochastic or deterministic dynamics, for example, due to a decline in the maternal transmission rate of all hosts or a subpopulation of "low transmitters." While these simulated dynamics may help explain empirical examples of *w*Mel-like *Wolbachia* fluctuations over years-long periods, other factors (e.g., insect demography) likely contribute to fluctuations observed over short month-long periods.

INTRODUCTION

Animal life is characterized by interactions with microbes that have diverse host effects spanning a continuum from beneficial to antagonistic. Many insects and other animals carry heritable endosymbionts that reside within their own cells. These microorganisms can alter basic aspects of host fitness, including reproduction, immune function, and nutrient acquisition (McFall-Ngai et al. 2013; Shropshire et al. 2020; Perreau and Moran 2022; Bennett et al. 2024; Hoffmann and Cooper 2024). Heritable endosymbionts often form close associations with female ovaries and the germline to ensure that microbes are maternally transmitted from one host generation to the next (Russell et al. 2019; Hague et al. 2024). This process of vertical-maternal transmission is a key determinant of the spread, persistence, and the long-term evolutionary dynamics of endosymbiosis. Strict vertical maternal transmission aligns the fitness interests of both hosts and endosymbionts, which may lead to long-term, stable relationships that can approach the status of organelles (Wade 2007; McCutcheon and Moran 2012; Fisher et al. 2017; McCutcheon et al. 2019; Perreau and Moran 2022).

Ultimately, the ecological and evolutionary consequences of endosymbioses are determined by whether or not endosymbionts are prevalent in host populations. Many endosymbionts are facultative from the host perspective, such that a proportion of host individuals in the population do not carry endosymbionts due to imperfect maternal transmission (Mateos et al. 2006; Carrington et al. 2011; Hamm et al. 2014; Corbin et al. 2017; Hague, Mavengere, et al. 2020). The frequency of facultative endosymbionts in host populations can vary widely across host systems, geography, and time (Hamm et al. 2014; Kriesner et al. 2016; Cooper et al. 2017; Hague, Mavengere, et al. 2020), suggesting that variable maternal transmission rates contribute to variation in endosymbiont prevalence. For instance, if maternal transmission is perturbed by environmental conditions (e.g., temperature), high rates of imperfect maternal transmission may cause endosymbiont frequencies to decline in certain host populations (Hague, Mavengere, et al. 2020; Ross et al. 2020; Hague et al. 2022). Nonetheless, the relative contribution of imperfect maternal transmission to variation in endosymbiont prevalence is poorly understood, especially in the context of other important contributing factors, such as endosymbiont effects on host fitness and stochastic dynamics associated with host population size (Caspari and Watson 1959; Hoffmann et al. 1990).

Maternally transmitted *Wolbachia* are the most common endosymbionts on earth, infecting roughly half of all insect species, as well as other arthropods and nematodes (Ferri et al. 2011; Zug and Hammerstein 2012; Weinert et al. 2015). *Wolbachia* are vertically maternally transmitted through the female germline (Russell et al. 2019; Porter and Sullivan 2023; Radousky et al. 2023; Hague et al. 2024); although, phylogenetic evidence indicates that horizontal and introgressive transfers occur frequently on evolutionary timescales (O'Neill et al. 1992; Raychoudhury et al. 2009; Conner et al. 2017; Gerth and Bleidorn 2017; Turelli et al. 2018; Cooper et al. 2019; Vancaester and Blaxter 2023; Shropshire et al. 2024). *Wolbachia* generally form facultative relationships with insects (from the host perspective), as the endosymbionts are generally found in only a proportion of host individuals due to imperfect maternal transmission. *Wolbachia* frequencies have been shown to vary considerably among and within host systems (Hoffmann et al. 1990; Kriesner et al. 2013; Hamm et al. 2014; Kriesner et al. 2016; Cooper et al. 2017; Hague, Mavengere, et al. 2020; Hague et al. 2022; Turelli et al. 2022); however, the relative contribution of imperfect maternal transmission to this frequency variation is unclear.

Population frequencies and equilibria dynamics of *Wolbachia* can be approximated by a discrete generation, deterministic model that incorporates three parameters: (1) the proportion of uninfected ova produced by *Wolbachia*-positive females (μ; i.e., imperfect maternal transmission), (2) the fitness of *Wolbachia*-positive females relative to *Wolbachia*-negative females (F; i.e., components of host fitness like fecundity), and (3) the relative egg hatch of uninfected eggs fertilized by *Wolbachia*-positive males (s_h) due to *Wolbachia*-induced cytoplasmic incompatibility (CI) (Hoffmann et al. 1990). The initial spread and persistence of *Wolbachia* in natural host populations is generally considered to be driven by beneficial effects on host fitness ($F > 1$), which are balanced by imperfect maternal transmission ($\mu > 0$) (Hoffmann et al. 1990; Turelli and Hoffmann 1991; Kriesner et al. 2013; Kriesner and Hoffmann 2018; Ross, Turelli, et al. 2019; Turelli et al. 2022). Although beneficial *Wolbachia* effects on host fitness are poorly understood in nature, recent work suggests *Wolbachia* block viruses in their native *Drosophila* hosts (Hedges et al. 2008; Teixeira et al. 2008; Osborne et al. 2009; Martinez et al. 2014; Cogni et al. 2021; Bruner-Montero and Jiggins 2023), in addition to a potential role of nutrient provisioning (Brownlie et al. 2009; Nikoh et al. 2014; Newton and Rice 2020). Some *Wolbachia* strains also cause CI ($s_h > 0$), a crossing incompatibility that generates a

frequency dependent benefit for *Wolbachia*-positive females (Shropshire et al. 2020; Turelli et al. 2022). Once a CI-causing strain exceeds a critical frequency threshold, *Wolbachia* will rapidly spread to high frequencies, which are balanced by imperfect maternal transmission (Hoffmann and Turelli 1988; Hoffmann et al. 1990; Turelli and Hoffmann 1995; Carrington et al. 2011; Kriesner et al. 2013; Turelli et al. 2022).

In host systems where *Wolbachia* do not cause strong CI, infection frequencies tend to fluctuate across time and geographic space (e.g., Kriesner et al. 2013; Hamm et al. 2014; Kriesner et al. 2016; Cooper et al. 2017; Hague, Mavengere, et al. 2020). For instance, *w*Mel in *Drosophila melanogaster* and the closely related ("*w*Mel-like") *w*San and *w*Yak strains in *D. yakuba*-clade hosts (diverged from *w*Mel ~30,000 years ago) generally cause no or weak CI. The frequencies of these *w*Mel-like *Wolbachia* can fluctuate dramatically over time periods as short as a month at a given location (Figure 1) (Hoffmann et al. 1998; Reynolds and Hoffmann 2002; Cooper et al. 2017; Cooper et al. 2019). For example, at Gold Coast in subtropical eastern Australia, the frequency (p) of *w*Mel in *D. melanogaster* dropped from $p = 0.919$ (binomial confidence interval: 0.787, 0.972) in March 1995 down to $p = 0.25$ (0.12, 0.449) just a month later in April and then rebounded back up to $p = 0.615$ (0.355, 0.823) in September (Hoffmann et al. 1998). Similarly, on the island of São Tomé off the coast of west Africa, the frequency of *w*San in host *D. santomea* dropped from $p = 0.772$ (0.648, 0.862) in 2009 to $p = 0.368$ (0.234, 0.527) in 2015 and then back up to $p = 0.795$ (0.692, 0.87) in 2018 (Cooper et al. 2017; Hague, Mavengere, et al. 2020). The frequencies of *w*Mel and *D. yakuba*-clade *Wolbachia* have also been shown to vary geographically among host populations by latitude and altitude (Kriesner et al. 2016; Cooper et al. 2017; Hague, Mavengere, et al. 2020). These variable patterns contrast those of strong CI-causing *Wolbachia* strains (e.g., *w*Ri in *D. simulans*), which tend to persist at high, stable frequencies due to the frequency dependent advantage of CI (Hoffmann et al. 1990; Turelli and Hoffmann 1991; Rousset and Solignac 1995; Turelli and Hoffmann 1995; Bourtzis et al. 1996; James and Ballard 2000; Ballard 2004; Carrington et al. 2011; Choi et al. 2015; Turelli et al. 2018).

The temporal fluctuations of *w*Mel-like *Wolbachia* could be strictly due to stochasticity associated with a finite host population. Alternatively, changes in the maternal transmission rate, host fitness effects, and/or CI between host generations could generate deterministic changes to *Wolbachia* equilibria over time. We first explore the hypothesis that *Wolbachia* frequencies

fluctuate over time as a result of finite-population stochasticity (Jansen et al. 2008; Kriesner and Hoffmann 2018; Turelli et al. 2022; Turelli and Barton 2022). The deterministic model of *Wolbachia* equilibria can be adapted to approximate the stochasticity induced by finite population size using a stochastic transition matrix analogous to a haploid Wright-Fisher model of genetic drift (Crow and Kimura 1970; Turelli et al. 2022; Turelli and Barton 2022). The model uses an effective population size of N female hosts (because *Wolbachia* are maternally transmitted) and binomial sampling to model the stochastic effect of finite population size and their interactions with maternal transmission (μ), host fitness effects (F), and CI (s_h). Following Turelli et al. (2022) and Turelli and Barton (2022), we evaluate how host population size influences the stochasticity of *Wolbachia* frequencies over time, measured as the standard deviation of *Wolbachia* frequencies (p_{SD}), in simulated host populations across a wide range of biologically plausible values for *w*Mel-like *Wolbachia*.

Second, we investigate the hypothesis that *Wolbachia* frequencies may increase/decrease deterministically over time as a result of temporal changes in the maternal transmission rate, host fitness effects, or CI strength between host generations (Turelli and Hoffmann 1995; Carrington et al. 2011; Hague, Mavengere, et al. 2020). Recent work on *w*Mel and *w*Yak showed that rearing hosts at a relatively cool temperature (20ºC) reduces maternal transmission rates compared to standard conditions (25ºC) (Hague, Mavengere, et al. 2020; Hague et al. 2022). Maternal transmission estimates from field-collected females in these systems also indicate that a proportion of *Wolbachia*-positive females may be "low transmitters" with especially high rates of imperfect maternal transmission ($\mu > 0.7$) (Hoffmann et al. 1998; Carrington et al. 2011; Hamm et al. 2014; Hague, Mavengere, et al. 2020). This phenomenon has been observed in wild-caught females transmitting *w*Mel (Hoffmann et al. 1998), *w*San, and *w*Yak (Hague, Mavengere, et al. 2020), as well as other *Wolbachia* strains (Carrington et al. 2011; Hamm et al. 2014). Thus, it is conceivable that rapid changes in environmental conditions (e.g., a bout of cold weather) could perturb maternal transmission for all hosts or a subpopulation of low transmitters, causing deterministic changes to *Wolbachia* frequencies in subsequent host generations. Changes in the strength of host fitness effects or CI could similarly generate deterministic *Wolbachia* fluctuations, because these factors can also be context-dependent, for example, depending on environmental conditions, host age, and the *Wolbachia* and host genomes (Hoffmann et al. 1990; Clancy and Hoffmann 1998; Olsen et al. 2001; Reynolds and Hoffmann 2002; Bordenstein and

151 Bordenstein 2011; Versace et al. 2014; Kriesner et al. 2016; Cooper et al. 2017; Cao et al. 2019;
152 Shropshire et al. 2021). We used the stochastic model to evaluate how biologically plausible
153 values of μ, F, and s_h influence *Wolbachia* equilibria, measured as the mean *Wolbachia*
154 frequency ($\bar{p}$) from each simulated host population.
155 We implemented the simulations in a new publicly available R package *wlbcmodeler*.
156 The package simulates temporal *Wolbachia* dynamics within a host population with discrete
157 generations and a given effective population size. We used the R package to run 491,400
158 simulations across 3,276 unique parameter combinations to explore how biologically plausible
159 values of host population size ($10^3 \leq N \leq 10^6$), imperfect maternal transmission ($0.001 \leq \mu \leq 0.5$),
160 host fitness effects ($1 \leq F \leq 1.5$), and CI ($s_h = 0$ or 0.1) influence the temporal dynamics of
161 *w*Mel-like *Wolbachia*. The package treats μ as a random binomial variable and allows for the
162 inclusion of a subpopulation of low transmitter females (e.g., 10% of *Wolbachia*-positive
163 females with $\mu = 0.8$). We also allow for fluctuating host fitness effects by treating F as a log-
164 normal random variable (Turelli et al. 2022). We began each simulation at an arbitrary
165 intermediate *Wolbachia* frequency of $p_0 = 0.4$ and ran the population for 10,000 host generations.
166 After removing the initial 500 host generations as burn-in, we calculated the standard deviation
167 (p_{SD}) and mean ($\bar{p}$) *Wolbachia* frequency from generations 501-10,000 for each simulation.
168 Broadly, our results demonstrate that strains that weak or no CI, like *w*Mel-like *Wolbachia*, tend
169 to generate larger stochastic fluctuations over time compared to strong-CI causing strains like
170 *w*Ri in *D. simulans*. While the simulated stochastic and/or deterministic dynamics in our model
171 may explain the observed fluctuations of *w*San on São Tomé over years-long periods, other
172 factors likely contribute to the fluctuations of *w*Mel at Gold Coast over short month-long
173 periods.
174
175 **THEORETICAL FRAMEWORK**
176 *Deterministic analysis of Wolbachia frequencies*
177 The deterministic model of *Wolbachia* population frequencies and equilibria dynamics
178 incorporates imperfect maternal transmission (μ), host fitness effects modeled as differential
179 fecundity (F), and CI (s_h) (Hoffmann et al. 1990). We assume that uninfected ova produced by
180 *Wolbachia*-positive females (due to imperfect maternal transmission) are susceptible to CI, just
181 as uninfected ova from *Wolbachia*-negative females (Carrington et al. 2011). Embryos produced

182 by fertilizations of *Wolbachia*-negative ova with sperm from *Wolbachia*-positive males hatch
183 with frequency $H = 1 - s_h$ relative to the other three possible fertilizations, which are all
184 considered equally compatible (Cooper et al. 2017). Thus, s_h represents the severity of CI, or the
185 frequency of unhatched eggs in pairings between *Wolbachia*-negative ova and *Wolbachia*-
186 positive sperm.

187 We first ignore CI and consider a stable equilibrium ($\hat{p}$) balanced by positive host fitness
188 effects ($F > 1$) and imperfect maternal transmission ($\mu > 0$). When initially rare, *Wolbachia* must
189 generate $F(1 - \mu) > 1$ to spread deterministically from low frequency, regardless of whether they
190 cause CI. Here, we assume $F(1 - \mu) > 1$ given the spread and persistence of non-CI causing
191 *Wolbachia* strains in nature (Hoffmann and Turelli 1997; Kriesner et al. 2013; Hamm et al. 2014;
192 Kriesner et al. 2016; Kriesner and Hoffmann 2018; Meany et al. 2019). In contrast, when $F(1 -$
193 $\mu) < 1$, rare infections will be deterministically lost (Hoffmann et al. 1990). A value of $F(1 - \mu) >$
194 1 also provides an explanation for the low frequency spread of strong CI-causing strains,
195 particularly *w*Ri (Turelli and Hoffmann 1991; Turelli and Hoffmann 1995; Bakovic et al. 2018).
196 When $F(1 - \mu) > 1$, the stable equilibrium frequency is

$$\hat{p} = 1 - \frac{\mu F}{F-1}. \quad [1]$$

197 In this case, $\hat{p}$ increases from 0 to $1 - \mu$ as F increases from $1/(1 - \mu)$.

198 We explored a range of plausible μ values based on previous estimates of maternal
199 transmission in the field, as well as under varying conditions in the lab. Empirical estimates of
200 mean μ values ($\pm BC_a$ confidence intervals) for *w*Mel generated from field-collected *D.*
201 *melanogaster* have ranged from low ($\mu = 0.026$ [0.008, 0.059]; Hoffmann et al. 1998) up to
202 moderate levels of imperfect transmission ($\mu = 0.11$ [0.07, 0.17]; Olsen et al. 2001). Estimates
203 generated in the lab range as high as $\mu = 0.415$ (0.332, 0.493) when females are reared in the
204 cold (20ºC) relative to a standard temperature (25ºC) (Hague et al. 2022; Hague et al. 2024).
205 Estimates of mean μ values for *w*San from field collected *D. santomea* on the island of São
206 Tomé were moderate ($\mu = 0.068$ [0.027, 0.154]), whereas *w*Yak in *D. yakuba* ranged from low at
207 low altitude ($\mu = 0.038$ [0.003, 0.184]) to moderate at high altitude ($\mu = 0.20$ [0.087, 0.364])
208 (Hague, Mavengere, et al. 2020). Estimates of μ generated under lab conditions for *w*Yak in *D.*
209 *yakuba* ranged has high as $\mu = 0.15$ (0.109 0.196) when females were reared in the cold (Hague,
210 Mavengere, et al. 2020). We thus decided to explore a broad range of μ values from $0.001 \leq \mu \leq$
211 0.5.

We know far less about *Wolbachia* effects on host fitness (F) in natural populations due to the challenges of estimating fitness of *Wolbachia*-positive and -negative individuals in the wild (Ross, Turelli, et al. 2019). *Wolbachia* cells are found throughout host somatic tissue (Pietri et al. 2016), which is likely to have complex effects on components of host fitness in nature (e.g., Hoffmann et al. 1998; Weeks et al. 2007; Kriesner et al. 2016; Hague, Caldwell, et al. 2020; Cogni et al. 2021; Hague et al. 2021; Bruner-Montero and Jiggins 2023). Prior work found no evidence for fecundity effects of *w*Mel in *D. melanogaster* (Hoffmann et al. 1994) or *Wolbachia* in *D. yakuba*-clade hosts (Cooper et al. 2017); however, fecundity represents only one aspect of host fitness. *Wolbachia* effects on other measures of host fitness in the lab have ranged from negative (Hoffmann and Turelli 1988; Hoffmann et al. 1990; Kriesner et al. 2016) to positive (Hedges et al. 2008; Teixeira et al. 2008; Brownlie et al. 2009; Russell et al. 2023) to context-dependent (Olsen et al. 2001; Fry et al. 2004). Beneficial *Wolbachia* effects of $F > \sim 1.2$ (roughly a 20% relative fitness advantage for *Wolbachia*-positive females) have not been documented in any system (Weeks et al. 2007; Hamm et al. 2014; Meany et al. 2019; Hague, Mavengere, et al. 2020). We generally expect $F > 1$ based of the spread and persistence of *Wolbachia* strains in natural host populations (described above); however, we expect values of $F > 1.5$ to be biologically unrealistic (Weeks et al. 2007; Hamm et al. 2014; Cooper et al. 2017; Meany et al. 2019; Hague, Mavengere, et al. 2020; Hague et al. 2022). Therefore, we chose to explore F values ranging from $1 \leq F \leq 1.5$.

Next, we considered equilibria that incorporate CI ($s_h > 0$; Figure 2B). Like non-CI causing strains, CI-causing strains must still increase host fitness to spread deterministically from low frequency such that $F(1-\mu) > 1$. CI-causing infections that generate $F(1-\mu) > 1$ and $F\mu < 1$ produce a single stable equilibrium between 0 and 1 given by

$$\hat{p} = \frac{s_h + 1 - F + \sqrt{(s_h + 1 - F)^2 + 4s_h[F(1-\mu) - 1](1 - F\mu)}}{2s_h(1 - F\mu)}. \quad [2]$$

Prior estimates of CI using field-collected *D. melanogaster* males from central and northern Australia suggest that *w*Mel causes weak CI on the order of $s_h = 0.05$ (Hoffmann et al. 1998; Kriesner et al. 2016). CI strength declines rapidly with male age (Shropshire et al. 2021) and Reynolds and Hoffmann (2002) found that one-day-old males derived from wild-collected larvae and pupae produce hatch rates of 0.39 in incompatible crosses, with large confidence intervals (0.15, 0.64). Given this information, Kriesner et al. (2016) conjectured that s_h values for *w*Mel of 0 to 0.1 are plausible, but $s_h > 0.1$ is unlikely. We are not aware of CI estimates for *D. yakuba*-

242 clade *Wolbachia* using field-collected males; however, estimates in the laboratory indicate that
243 these strains reduce egg-to-adult viability in CI crosses by about 10-20% (*w*San: s_h = 0.15 [0.12,
244 0.18]; *w*Yak: s_h = 0.16 [0.13, 0.20]; *w*Tei: s_h = 0.2 [0.17, 0.23]), although the presence and
245 intensity of CI can also vary depending on host and *Wolbachia* genotype (Cooper et al. 2017).
246 Based on these previous data, we decided to explore the effects of weak CI (s_h = 0.1) on
247 *Wolbachia* equilibria and temporal dynamics. We also considered strong CI (s_h = 0.45) that is
248 characteristic of field-collected male *D. simulans* carrying *w*Ri (Turelli and Hoffmann 1995;
249 Carrington et al. 2011); although, this level of CI has not been observed for the *w*Mel-like
250 *Wolbachia* described here.

251 Assuming discrete host generations, the total population fraction of offspring in the next
252 generation can be divided with respect to whether mated males and females are carrying
253 *Wolbachia*:

254 1. *Wolbachia-positive male and female*. In this case, there is a population of $F(1-\mu)p_t^2$
255 and also a population of $F\mu(1-s_h)p_t^2$ when the ova become uninfected due to imperfect
256 maternal transmission, resulting in a population fraction of $F(1-\mu s_h)p_t^2$.
257 2. *Wolbachia-negative male and female*. The population fraction is simply $(1-p_t)^2$.
258 3. *Wolbachia-negative male, Wolbachia-positive female*. Accounting for fecundity in
259 *Wolbachia*-positive females, the population fraction is then $Fp_t(1-p_t)$.
260 4. *Wolbachia-positive male, Wolbachia-negative female*. The population from generation
261 *t* is $p_t(1-p_t)$. A fraction $1-s_h$ of this population survives from this incompatible
262 pairing, so the population fraction for generation *t* + 1 is $p_t(1-p_t)(1-s_h)$.

263 Assuming equal infection frequencies in males and females, the adult infection frequency in
264 generation *t*, denoted p_t, changes between generations as follows:

$$p_{t+1} = \frac{p_t F(1-\mu)}{1 + p_t(F-1-s_h) + p_t^2 s_h(1-\mu F)} \quad [3]$$

265 (Hoffmann et al. 1990). In the absence of CI (s_h = 0), this model becomes

$$p_{t+1} = \frac{p_t F(1-\mu)}{1 + p_t(F-1)}. \quad [4]$$

266 For a stability analysis of [4], see Appendix 1.

267

268

Simulations to approximate temporal infection dynamics under finite population size

Following Turelli and Barton (2022) and Turelli et al. (2022), we approximated the stochasticity produced by finite population size using a stochastic transition matrix analogous to a haploid Wright-Fisher model of genetic drift, which assumes that generations are discrete and the new generation is a random sample of constant population size drawn from the total number of offspring produced by the previous generation (Crow and Kimura 1970). The model uses an effective population size of *N* females. The finite-population stochasticity, modeled as binomial sampling, is superimposed on the deterministic equilibria dynamics described by [3]. We explored a range of biologically plausible host population sizes ranging from 10^3 to 10^6 (McKenzie 1980; McInnis et al. 1982; Powell 1997; Gravot et al. 2004; Bergland et al. 2014). Estimates of local population sizes of *Drosophila* made from mark-release-recapture methods report local census sizes on the order of 10^4 to 10^5, with considerable variation among seasons, years, and localities (McKenzie 1980; McInnis et al. 1982; Powell 1997; Gravot et al. 2004; Bergland et al. 2014).

We now derive a model for modeling infection frequency p_t of *Wolbachia*-positive hosts which includes randomness effects from a finite female population of size N, $I_t = Np_t$ of which are infected. We assume females produce the same number of eggs with each mating and that N does not change over generations. Starting with the current adult (female) infection frequency p_t, the infection frequency among viable gametes in the next generation is determined by [3]. Then, the infection frequency in the next generation of *N* adult females is obtained from binomial sampling of this deterministic projection.

First, we compute the total number of *Wolbachia*-positive adult offspring in generation $t + 1$ in the absence of CI ($s_h = 0$). For *Wolbachia*-positive females, we note that there are FI_t ova produced. Each ova has a probability $1 - \mu$ of being infected, so the total number of infected offspring can be represented by a binomial random variable

$$X_t \sim \mathrm{Bin}\big(FI_t, (1 - \mu)\big). \qquad [5]$$

For the total number of offspring produced, we observe that all *Wolbachia*-negative females produce *Wolbachia*-negative ova. This gives a total of $N - I$ *Wolbachia*-negative offspring for the next generation. For *Wolbachia*-positive females, we noted that FI_t offspring are produced from matings. This gives a total population of $N + (F - 1)I_t$ offspring. The proportion of *Wolbachia*-positive adults for generation $t + 1$ is then

$$p_{t+1} = \frac{X_t}{FI_t + N - I_t} = \frac{X_t/N}{p_t(F-1)+1}. \quad [6]$$

From [6], the expected value and variance of p_{t+1}, given knowledge of p_t, are readily computed as

$$\mathbb{E}[p_{t+1}|p_t] = \frac{F(1-\mu)p_t}{p_t(F-1)+1}, \quad \mathrm{Var}(p_{t+1}|p_t) = \frac{F\mu(1-\mu)p_t}{N(p_t(F-1)+1)^2}. \quad [7]$$

From the law of large numbers, as the population $N \to \infty$, the variance in [7] tends to 0, and the random recursion [6] converges to the deterministic model [4].

Now, allowing for CI ($s_h > 0$), we can derive a random recursion for p_t. For each of the stages of mating, ova production, and the generation of adult offspring, we record total populations in Table S1. The total *Wolbachia*-positive adult offspring resulting from *Wolbachia*-positive male and female matings is given by $X_1 \sim \mathrm{Bin}\big(FpI, (1-\mu)\big)$. For *Wolbachia*-negative males and -positive females, total *Wolbachia*-positive offspring is $X_2 \sim \mathrm{Bin}\big(F(1-p)I, (1-\mu)\big)$. The total number of offspring is found by summing the four terms in the row labeled "adult offspring after CI" in Table S1. Denoting the normalized fractions $\tilde{X}_i = X_i/N$, the fraction of *Wolbachia*-positive offspring over the total population is then

$$p_{t+1} = \frac{\tilde{X}_1 + \tilde{X}_2}{s_h(1-F)p_t^2 + (F - s_h - 1)p_t + s_h\tilde{X}_1 + 1}. \quad [8]$$

As $N \to \infty$, from the law of large numbers [8] approaches the deterministic model [3].

Let us also consider the case where a subpopulation of "low transmitter" females with high rates of imperfect maternal transmission are included in the host population. Here, the host population consists of M groups with group fractions $(r_1, \dots, r_M)$. Rather than a single μ value, the ith subpopulation assumes a value μ_i for $i = 1, \dots, M$. To compute *Wolbachia*-positive offspring, we are now summing M separate binomial distributions. However, most computations from above carry over. For each group i, we compute $X_i \sim \mathrm{Bin}(FIr_i, 1-\mu_i)$. The number of *Wolbachia*-positive female eggs then becomes $S^M = \sum_{i=1}^{M} X_i$. For no CI, the total population is still $FI + N - I$, and

$$p_{t+1} = \frac{S_{t+1}^M}{FI_t + N - I_t} = \frac{S_{t+1}^M/N}{p_t(F-1)+1}. \quad [9]$$

When $\mu_i \equiv \mu$ for all $i = 1, \dots, M$, then $S^M \sim \mathrm{Bin}(1-\mu, FI)$ and we recover [6]. Expected values are also straightforward to compute, with

$$\mathbb{E}[p_{t+1}|p_t] = \frac{F(1-\sum_{i=1}^{M}\mu_i r_i)p_t}{p_t(F-1)+1}. \quad [10]$$

Each of the *Wolbachia*-positive populations X_i for $i \ldots, M$ terms are independent, so by additivity of variance,

$$\mathrm{Var}(p_{t+1}|p_t) = \frac{F(\sum_{i=1}^{M}\mu_i(1-\mu_i)r_i)p_t}{N(p_t(F-1)+1)^2}. \quad [11]$$

The stable equilibrium, where $\hat{p} = \mathbb{E}[p_{t+1}|p_t = \hat{p}]$, is very similar to [1]. Accounting for multiple μ_i values, we have

$$\hat{p} = 1 - \frac{F}{F-1}\sum_i \mu_i\, r_i. \quad [12]$$

Finally, to generalize [8], we replace X_1 and X_2 with sums of independent binomials $\{Y_i\}_{1\le i\le M}$ and $\{Z_i\}_{1\le i\le M}$, with

$$X_1 = \sum_{i=1}^{M} Y_i\,, \qquad Y_i \sim \mathrm{Bin}(Fpr_iI, 1-\mu_i), \quad [13]$$

$$X_2 = \sum_{i=1}^{M} Z_i\,, \qquad Z_i \sim \mathrm{Bin}(F(1-p)r_iI, 1-\mu_i). \quad [14]$$

Previous estimates of μ from a large sample of field-collected female *D. santomea* (*N* = 62) and *D. yakuba* (*N* = 71) on São Tomé in 2018 suggest the majority of females have perfect or near perfect transmission rates, but a small proportion of low transmitters have high values of μ. For *w*San, the total sample of *Wolbachia*-positive females had a mean value of μ = 0.068 (0.027, 0.154), but 8% of these females had a μ value greater than 0.5 with a mean of μ = 0.728. Similarly, for *w*Yak, the total sample of *Wolbachia*-positive females had a mean value of μ = 0.126 (0.062, 0.238), but approximately 11% of the females had a μ value greater than 0.5 with a mean of μ = 0.803 (Hague, Mavengere, et al. 2020; see Figure 3). Previous work using field-collected females found similar evidence of low transmitters for *w*Mel in *D. melanogaster* (Hoffmann et al. 1998), *w*Suz in *D. suzukii* (Hamm et al. 2014; see Figure 3), *w*Inn in *D. innubila* (Unckless et al. 2009), and *w*Ri in *D. simulans* (Carrington et al. 2011; see Figure 6). Hamm et al. (2014) termed females with high rates of imperfect *w*Suz transmission as "low transmitters" and we follow the same terminology here. Using the 2018 *D. yakuba*-clade *Wolbachia* data as a guide, we explored the effect of treating a 10% subpopulation of *Wolbachia*-positive females as low transmitters with a random binomial μ variable set to μ = 0.8. The rest of the *Wolbachia*-positive females had μ values set as described above ($0.001 \le \mu \le 0.5$).

While we initial assumed a fixed F value, we also considered the case where host fitness effects fluctuate from one host generation to the next as independent, identically distributed log-normal random variables (Turelli et al. 2022). We assume that each generation F is chosen independently from a log-normal distribution such that $F = e^X$, where X is a normal random variable with mean μ_X and variance σ^2 (Turelli et al. 2022). This implies that F has median $m = e^{\mu_x}$ and a squared coefficient of variation $CV^2 = \mathrm{Var}(F)/[\mathrm{E}(F)^2] = e^{\sigma^2} - 1$. Thus, to produce a particular median, m, and CV for F, we set $\mu_x = \ln(m)$ and $\sigma^2 = \ln(CV^2 + 1)$. While it is generally unknown whether host fitness effects fluctuate in natural host populations (Turelli et al. 2022), there is evidence that the fitness effects of *w*Mel on *D. melanogaster* fecundity (Kriesner et al. 2016) and virus-blocking (Chrostek et al. 2021) can depend on the environmental context. We explored scenarios with weakly ($CV = 0.01$) and strongly ($CV = 0.1$) fluctuating host fitness effects. The illustrate how these values correspond to fluctuating F values in our simulations, when median(F) = 1.05, a $CV = 0.01$ value corresponds to 2.5 and 97.5 percentiles of 1.03 and 1.071, respectively, and a $CV = 0.1$ value corresponds to 0.863 and 1.271, respectively.

We ran 25 simulations for every unique set of parameter combinations. Each individual simulation began with the arbitrary intermediate *Wolbachia* frequency of $p_0 = 0.4$ and ran for 10,000 host generations (similar p_0 values do not qualitatively alter the results). We removed an initial burn-in of 500 host generations as *Wolbachia* spread from $p_0 = 0.4$ to a stable equilibrium, and then calculated the standard deviation (p_{SD}) and mean ($\bar{p}$) *Wolbachia* frequency from generations 501-10,000 for each simulation. To quantify the influence of the different parameter values on the outcome of simulations, we modeled the p_{SD} and $\bar{p}$ values with linear effects for N, μ, the presence/absence of low transmitters, F, the presence/absence of fluctuating F values (i.e., treating F as fixed or a random variable), and s_h. Since the frequency is always between zero and one, we modeled the distribution of the p_{SD} and $\bar{p}$ values as beta random variables. Models were implemented in R version 4.4.2 using the gamlss package version 5.4. We found that a small handful of simulations had infections that persisted at $p > 0$ for the full 10,000 host generations for combinations of parameter values that do not predict a stable equilibrium of $\hat{p} > 0$ (e.g., $F = 1.05$, $\mu = 0.05$, $s_h = 0$). We removed these simulations from our regression analysis, because they are predicted to be deterministically lost over time and we did not want the p_{SD} and $\bar{p}$ values to bias our analyses of stable equilibria.

375 **RESULTS**

376 We ran 491,400 simulations to examine how different parameter combinations of host
377 population size, imperfect maternal transmission, host fitness effects, and CI contribute to
378 stochastic fluctuations (measured as p_{SD}) and *Wolbachia* equilibria (measured as $\bar{p}$) over time in
379 simulated host populations (Figures 3, 4). Multiple regression was used to summarize how N, μ,
380 F, and s_h impacted p_{SD} and $\bar{p}$ in our simulations (Tables 1, 2). We first explore how different
381 parameters values influence the size of stochastic fluctuations over time and then examine how
382 temporal changes to parameter values, particularly the maternal transmission rate, between host
383 generations alter deterministic *Wolbachia* equilibria. For both scenarios, we consider whether the
384 dynamics in our simulations can explain empirical examples of temporally fluctuating *w*Mel-like
385 *Wolbachia* in Australia and São Tomé.

386 All the variables in our regression model had a significant effect on stochastic
387 fluctuations measured as p_{SD} (Figure 3); however, some parameters were more influential than
388 others (Table 1). Notably, host population size had only a small effect ($\beta = -4.14 \times 10^{-7}$, $P <$
389 0.001), although p_{SD} generally declined as population size increased and *Wolbachia* frequencies
390 hovered close to predicted equilibria. Given that host population size had only a minor effect on
391 p_{SD}, we focus here on our results from a single biologically plausible population size of $N = 10^4$
392 as a simple case to illustrate how other parameters contribute to p_{SD} (Figures 5, S1). Broadly, our
393 results illustrate an intuitive pattern where the parameter values that yield high mean infection
394 frequencies—high rates of maternal transmission, strong fitness benefits, and CI—tend to
395 generate lower p_{SD} values and smaller fluctuations. In contrast, the parameters that yield low
396 infection frequencies ($\bar{p} < 0.2$) typically generate the largest *Wolbachia* fluctuations over time.
397 Within the biologically plausible range of μ, F, and s_h values that we explored, μ had the
398 strongest effect on p_{SD} ($\beta = 8.51$, $P < 0.001$), as lower maternal transmission rates (i.e., larger μ
399 values) are associated with lower infection frequencies and larger fluctuations. To a lesser extent,
400 the inclusion of a subpopulation (10%) of low transmitter females ($\mu = 0.8$) also generally
401 increased p_{SD} values ($\beta = 1.12$, $P < 0.001$). Here, we also found an interaction effect between the
402 value of μ and the presence of low transmitters ($\beta = -4.42$, $P < 0.001$), such that the rate of
403 increase in p_{SD} as a function of μ is smaller when low transmitters are present. The value of F
404 had a smaller relative effect on p_{SD} than μ ($\beta = -2.04$, $P < 0.001$), wherein stronger host fitness
405 benefits are associated with higher infection frequencies and smaller fluctuations. If we instead

treat F as a log-normal random variable and assume fluctuating host effects, weak fluctuations ($CV = 0.01$) only had a minor effect on increasing p_{SD} values ($\beta = 0.423$, $P < 0.001$), whereas strong fluctuations predictably had a larger effect ($\beta = 1.78$, $P < 0.001$). Finally, the addition of weak CI at a biologically plausible level for *w*Mel-like *Wolbachia* ($s_h = 0.1$) had a relatively small effect on p_{SD} values ($\beta = -9.85 \times 10^{-2}$, $P < 0.001$). CI generally causes higher infection frequencies and smaller fluctuations. Strong CI characteristic of strains like *w*Ri ($s_h = 0.45$) had a larger effect on p_{SD} ($\beta = -0.783$, $P < 0.001$), as strong CI is associated with high, stable infection frequencies (Figure S1).

Can these stochastic dynamics explain empirical observations of temporally fluctuating *w*Mel-like *Wolbachia*? At Gold Coast in Australia, *w*Mel frequencies dropped from $p = 0.919$ (0.787, 0.972) in March 1995 down to $p = 0.25$ (0.12, 0.449) in April and then back up to $p = 0.615$ (0.355, 0.823) in September, representing a maximum absolute change in frequencies ($p_{max} - p_{min}$) of 0.669 occurring over just a month-long period (Figure 1). On São Tomé, *w*San frequencies dropped from $p = 0.772$ (0.648, 0.862) in 2009 to $p = 0.368$ (0.234, 0.527) in 2015 and then back up to $p = 0.795$ (0.692, 0.87) in 2018, representing a maximum frequency change of 0.427 over the course of a six-year period (Figure 1). Generally, the simulations assuming fixed or weakly fluctuating host effects ($CV = 0.01$) produced small frequency fluctuations that cannot explain *w*Mel or *w*San (Figures 6, S2). Assuming a host population size of $N = 10^4$, we found that the parameter combination of $\mu = 0.1$ (without low transmitters), a fixed value of $F = 1.125$, and $s_h = 0.1$ produced the largest frequency fluctuations, with an absolute difference between the maximum and minimum frequency of 0.07 in an individual simulation spanning 10,000 host generations. Adding weakly fluctuating host fitness effects ($CV = 0.01$) produced only a slightly larger maximum absolute difference of 0.12 from the parameters $\mu = 0.05$ (without low transmitters), $F = 1.05$, and $s_h = 0.1$. Only when we assume strongly fluctuating fitness effects ($CV = 0.1$) do we find large fluctuations that can potentially account for empirical observations of *w*Mel and *w*San. The parameters $\mu = 0.01$ (without low transmitters), $F = 1.025$, and $s_h = 0$ produced the largest absolute frequency difference of 0.855 between p_{max} and p_{min} over the course of a single simulation (Figure S3).

These results highlight a pattern where the specific combination of parameters that generate the largest p_{SD} values does not necessarily align strictly with the general trends outlined above in the multiple regression. In the absence of strong fluctuating host effects, simulations

with CI (s_h = 0.1) and intermediate values of μ ($0.05 \leq \mu \leq 0.15$) generated the highest p_{SD} values (Figure 5A). With the addition of strong fluctuating host effects, simulations with no CI (s_h = 0) and small μ values ($0.005 \leq \mu \leq 0.01$) combined with small F values (F = 1.025) generated the highest p_{SD} values (Figure 5B). In this region of parameter space, no CI and the presence of strongly fluctuating F values with a median slightly greater than one cause *Wolbachia* to fluctuate between strongly favored ($F[1 - \mu] > 1$) and disfavored ($F[1 - \mu] < 1$) in the host population (Figure S3). With a median of F = 1.025 and CV = 0.1, the 2.5 and 97.5 percentiles are 0.843 and 1.251, respectively. Only the simulations with no CI and strong fluctuating host effects (CV = 0.1) produced absolute frequency differences ≥ 0.669 in individual simulations that were on the scale of *w*Mel fluctuations observed at Gold Coast. Because the *w*San fluctuations at São Tomé were not quite as large, both the no CI and weak CI simulations with strongly fluctuating host effects produced maximum absolute frequency differences ≥ 0.427 that are comparable to *w*San. Without CI, the presence of a low transmitter subpopulation generally reduced maximum observed frequency differences (Figure 6) and low transmitters cannot be present in order to generate maximum frequency differences ≥ 0.669 comparable to *w*Mel. In fact, the values of $0.005 \leq \mu \leq 0.01$ (without low transmitters), and F = 1.025 (CV = 0.1), and s_h = 0 were the only combinations of parameters that generated absolute frequency differences ≥ 0.669 as observed for *w*Mel at Gold Coast.

We also examine how the values of μ, F, and s_h influenced *Wolbachia* equilibria as measured by $\bar{p}$ (Figure 4) to explore the alternative possibility that changes to these parameter values between host generations could generate deterministic increases/decreases in *Wolbachia* frequencies over time that explain *w*Mel-like *Wolbachia* dynamics. Again, we used multiple regression to summarize how different values of N, μ, F, and s_h impacted $\bar{p}$ in our simulations (Table 2). Host population size had a very small relative effect on $\bar{p}$ values ($\beta = -1.58 \times 10^{-8}$, P = 0.009). As expected, our results otherwise align tightly with equilibria predictions from the strictly deterministic model (Figure 2), which shows that high rates of transmission (smaller μ values), strong beneficial host effects (larger F values), and strong CI (larger s_h values) yield high equilibrium frequencies. Within the biologically plausible range of parameters we explored, the value of μ had the strongest effect on $\bar{p}$ (β = -22.4, $P < 0.001$). To a lesser extent, the inclusion of a subpopulation of low transmitter females (μ = 0.8) was also associated with lower $\bar{p}$ values (β = -2.43, $P < 0.001$). We also found an interaction effect between the μ value and the

468 presence of low transmitters (β = 8.26, P < 0.001), such that the rate of decrease in $\bar{p}$ as a
469 function of μ is slightly less when low transmitters are present. The value of F had a smaller
470 relative effect on $\bar{p}$ compared to μ (β = 3.77, P < 0.001) and the addition of fluctuating host
471 fitness effects had only a minor effect on $\bar{p}$ (CV = 0.01: β = 1.30 x 10^{-4}, P = 0.961; CV = 0.1: β =
472 9.68 x 10^{-3}, P < 0.001). Finally, the addition of weak CI had a small effect on $\bar{p}$ (β = 0.427, P <
473 0.001), whereas strong CI had a much stronger effect (β = 1.92, P < 0.001), as the frequency
474 dependent benefit of CI drives *Wolbachia* to high equilibrium frequencies.

475 While including a subpopulation of low transmitters (μ = 0.8) generally reduced $\bar{p}$ values,
476 we note that this effect was strongest for *Wolbachia* strains that do not cause CI (Figure 4).
477 Again, we focus on the simple case of $N = 10^4$ to illustrate how different parameter values
478 contribute to $\bar{p}$ (Figures 5, S1). Here, with no CI (s_h = 0), the median $\bar{p}$ value dropped from 0.807
479 for all our simulations with a strictly binomial μ value to 0.472 for the simulations that included
480 a low transmitter subpopulation. In contrast, the median $\bar{p}$ value dropped from 0.875 to 0.591
481 with low transmitters for weak CI (s_h = 0.1) and from 0.945 to 0.858 for strong CI (s_h = 0.45).
482 The impact of low transmitters on $\bar{p}$ is especially strong for relatively small values of μ ($\leq$ 0.01)
483 and F ($\leq$ 1.15), which are otherwise predicted to generate high equilibrium frequencies (Figure
484 2). For instance, the values of μ = 0.001, F = 1.1, and s_h = 0 generated a pooled mean of $\bar{\bar{p}}$ =
485 0.989 across the set of 25 simulations, but the addition of low transmitters dropped the mean
486 down to $\bar{\bar{p}}$ = 0.11.

487 Can changes to the maternal transmission rate or the presence of low transmitters
488 between host generations generate large deterministic drops in *Wolbachia* frequencies over time
489 on the scale observed for *w*Mel-like *Wolbachia*? We further examined the $\bar{\bar{p}}$ values from our
490 simulations (Figure 5C) to infer how changes to μ, F, or s_h between host generation could alter
491 equilibrium frequencies over time. We found that a range of parameter values could plausibly
492 explain the drop in *w*Mel frequencies from p = 0.919 in March down to p = 0.25 in April of 1995
493 at Gold Coast if we assume that changes to μ values or the presence of low transmitters occur
494 between host generations (Figures S4). For instance, the values of μ = 0.005, F = 1.075, and s_h =
495 0 produced a value of $\bar{\bar{p}}$ = 0.928, which falls within the confidence interval of p for *w*Mel in
496 March. Then, assuming a drop in the transmission rate to μ = 0.05 in subsequent generations
497 (while all else remains equal), *Wolbachia* frequencies drop to $\bar{\bar{p}}$ = 0.283, which falls within the
498 confidence interval of p in April (Figure S5). Or, assuming the values of μ = 0.005, F = 1.125,

and $s_h = 0$ in March ($\bar{\bar{p}} = 0.955$), and then adding a subpopulation of low transmitters in April ($\bar{\bar{p}} = 0.239$) could also explain the drop. Of course, subtle changes in multiple parameters, including F and s_h, could also explain the temporal drop in frequencies. For example, the values of $\mu = 0.01$, $F = 1.025$, and $s_h = 0.1$ could explain March ($\bar{\bar{p}} = 0.920$) and then a slight decrease in the maternal transmission rate, increase in host fitness effects, and the loss of CI ($\mu = 0.05$, $F = 1.075$, and $s_h = 0$) could explain April ($\bar{\bar{p}} = 0.283$).

We found that a similar range of parameter values could plausibly explain the drop in *w*San from $p = 0.772$ in 2009 to $p = 0.368$ in 2015 on São Tomé (Figure S6). For instance, the values of $\mu = 0.01$, $F = 1.075$, and $s_h = 0$ produced a value of $\bar{\bar{p}} = 0.857$, which falls within the confidence interval of p for *w*San in 2009. Then, assuming a drop in the transmission rate to $\mu = 0.05$ (while all else remains equal), *Wolbachia* frequencies drop to $\bar{\bar{p}} = 0.283$, falling within the 2015 p estimate (Figure S7). Assuming the addition of low transmitters in 2015 could also plausibly explain the drop in *Wolbachia* frequencies. For example, the values of $\mu = 0.05$, $F = 1.175$, and $s_h = 0.1$ produced $\bar{\bar{p}} = 0.782$, and could explain 2009, and then adding a subpopulation of low transmitters drops *Wolbachia* frequencies to $\bar{\bar{p}} = 0.284$, falling within the 2015 p estimate. Subtle changes in multiple parameters could also plausibly explain the drop in frequencies. For instance, the values of $\mu = 0.005$, $F = 1.025$, and $s_h = 0$ produced $\bar{\bar{p}} = 0.795$ and could explain 2009, whereas a slight drop in the maternal transmission rate and increase in host fitness effects ($\mu = 0.05$, $F = 1.075$, and $s_h = 0$) produced $\bar{\bar{p}} = 0.283$ and could explain 2015.

DISCUSSION

We explored how finite host population size in conjunction with maternal transmission rates, host fitness effects, and CI contribute to the temporal dynamics of *w*Mel-like *Wolbachia*. Broadly, we find that strains like *w*Mel and *w*San that cause no or weak CI are more likely to generate large stochastic fluctuations over time than strains like *w*Ri that cause strong CI. The combination of no CI, host effects that fluctuate between mutualistic and deleterious, and a modest maternal transmission rate generate the largest stochastic fluctuations over time in our model, regardless of population size. Changes between host generations to the maternal transmission rate or the presence/absence of low transmitters can also generate large deterministic changes to *Wolbachia* frequencies, as can between-generation changes to host fitness effects and CI. The results suggest that stochastic dynamics and/or between-generation

530 changes to parameter values can provide plausible explanations for the temporal fluctuations of
531 *w*San on São Tomé over years-long periods, but the fluctuations of *w*Mel at Gold Coast are more
532 difficult to explain in the context of our model.

533 We first evaluated whether finite-population stochasticity alone can explain the temporal
534 dynamics of *w*Mel-like *Wolbachia*. Assuming the values of μ, F, and s_h remain stable over time,
535 we found that stochasticity in our model generates only minor fluctuations over time, regardless
536 of host population size. Even small host population sizes (10^3) were generally outweighed by
537 deterministic dynamics, as *Wolbachia* frequencies tend fluctuate closely around predicted
538 equilibria. Notably, across all the biologically plausible parameter values that we explored, poor
539 rates of maternal transmission (i.e., increasing values of μ) had the strongest relative effect on
540 increasing *Wolbachia* fluctuations in our simulations (Table 1). However, very specific
541 conditions were required to generate the absolute largest frequency fluctuations. Assuming fixed
542 host fitness effects, weak-CI causing *Wolbachia* ($s_h = 0.1$) with moderate fitness benefits (1.125
543 $\leq F \leq 1.2$) and relatively poor rates of maternal transmission ($0.1 \leq \mu \leq 0.2$) generated that
544 largest p_{SD} values (Figure 5A). Adding a subpopulation of low transmitters generally increased
545 the size of fluctuations; however, low transmitters were not present in the simulations the
546 produced the largest p_{SD} values. Only the addition of strongly fluctuating host fitness effects (CV
547 $= 0.1$) under a limited set of conditions can replicate fluctuations on the scale of *w*Mel-like
548 *Wolbachia*, particularly for *w*Mel at Gold Coast in Australia. Here, non-CI causing *Wolbachia*
549 with median F values close to one ($1.025 \leq F \leq 1.075$) combined with a high fidelity of
550 transmission ($0.005 \leq \mu \leq 0.05$) generated the largest p_{SD} values (Figure 5B), as *Wolbachia*
551 fluctuate between mutualistic ($F[1 - \mu] > 1$) and deleterious ($F[1 - \mu] < 1$) (Figure S3). In
552 contrast, conditions that yield equilibrium frequencies near one—strong CI, near perfect
553 maternal transmission, and large host fitness benefits—generate smaller stochastic fluctuations
554 over time.

555 These results are generally consistent with empirical observations of *Wolbachia*
556 frequency fluctuations in natural populations. *w*Mel-like *Wolbachia* that cause no or weak CI and
557 are imperfectly transmitted in nature tend to show evidence of temporally and/or spatially
558 fluctuating *Wolbachia* frequencies (Hoffmann et al. 1994; Kriesner et al. 2016; Cooper et al.
559 2017). Estimates of CI for *w*Mel using crosses between field-collected male *D. melanogaster*
560 from Australia and *Wolbachia*-negative laboratory females are consistent with $s_h \approx 0$ (unless

561 males are very young) (Hoffmann et al. 1998; Reynolds and Hoffmann 2002; Kriesner et al.
562 2016; Shropshire et al. 2021). Estimates of *w*Mel maternal transmission rates from field-
563 collected females have been recorded as high μ = 0.11 (0.07, 0.17) (Olsen et al. 2001). Similar
564 levels of imperfect maternal transmission have also been recorded for *w*San from field-collected
565 *D. santomea* (μ = 0.120, [0.032, 0.346]) and for *w*Yak from *D. yakuba* (μ = 0.20 [0.087, 0.364])
566 on São Tomé (Hague, Mavengere, et al. 2020). These patterns are not strictly characteristic of all
567 "*w*Mel-like" *Wolbachia*, as some *w*Mel-like strains cause strong CI in their native hosts (e.g.,
568 *w*Seg in *D. seguyi*; Shropshire et al. 2024) and other *Wolbachia* such as the *w*Ri-like strain *w*Suz
569 in *D. suzukii* do not cause CI, show imperfect transmission in the field (μ = 0.14 [0.04, 0.27]),
570 and fluctuate across geographic space (Hamm et al. 2014; Turelli et al. 2018). The patterns for
571 non-CI causing *Wolbachia* contrast empirical observations of the *w*Ri strain in *D. simulans*,
572 which causes strong CI ($s_h \approx 0.45$) and tends to persist at high, stable infection frequencies. *w*Ri
573 also seems to have relatively high rates of maternal transmission in the field (e.g., μ = 0.026
574 [0.004, 0.057]) and in the lab at different temperatures (Hoffmann et al. 1990; Turelli and
575 Hoffmann 1995; Carrington et al. 2011; Kriesner et al. 2013; Hague et al. 2024). Similarly, the
576 CI-causing strains *w*Ha (in *D. simulans*) and *w*Ana (in *D. ananassae*) persist at high, stable
577 infection frequencies (Rousset and Solignac 1995; Bourtzis et al. 1996; James and Ballard 2000;
578 Ballard 2004; Choi et al. 2015) and have thermally stable maternal transmission rates in the lab
579 (Hague et al. 2024).

580 It is important to note that strongly fluctuating host fitness effects are required as a
581 condition to generate stochastic fluctuations on the scale of *w*Mel at Gold Coast or *w*San at São
582 Tomé (Figure 6). Unfortunately, we have a poor understanding of *Wolbachia* effects on host
583 fitness in nature, although the endosymbionts are generally considered to be beneficial based on
584 the spread and persistence of *Wolbachia* in nature (Weeks et al. 2007; Hamm et al. 2014; Cooper
585 et al. 2017; Meany et al. 2019; Ross, Turelli, et al. 2019; Hague, Mavengere, et al. 2020; Hague
586 et al. 2022; Hoffmann and Cooper 2024). We do not know if host fitness effects fluctuate in
587 nature (Turelli et al. 2022), but there is evidence that the effects of *w*Mel on *D. melanogaster*
588 fecundity (Kriesner et al. 2016) and virus-blocking (Chrostek et al. 2021) can depend on the
589 environmental context. The non-CI causing strain *w*Au in *D. simulans* has also been shown to
590 have context-dependent host effects depending on where host breeding occurs (Cao et al. 2019).
591 Given that temperature has pervasive effects on *Wolbachia* densities in host tissues (Hague,

592 Caldwell, et al. 2020; Hague et al. 2024), temperature-induced changes to *Wolbachia* densities
593 could in turn alter *Wolbachia* effects on various host fitness components. The strongly
594 fluctuating host effects we modeled here may also help explain altitudinal variation in *w*Yak
595 frequencies in *D. yakuba* observed on São Tomé in 2018, which otherwise generally requires
596 invoking unrealistic values of F and s_h (Figure S8; see Hague et al. 2020 for further discussion).
597 Our simulations build on previous work focusing on the role of finite-population
598 stochasticity in the initial establishment of *Wolbachia* from low frequencies in host populations
599 (Jansen et al. 2008). At low initial frequencies, *Wolbachia* that generate $F(1 - \mu) > 1$ may be
600 stochastically lost and, conversely, CI-causing strains that generate $F(1 - \mu) < 1$ may become
601 established; however, these probabilities decreases as host population size increases (Rigaud and
602 Rousset 1996; Egas et al. 2002; Jansen et al. 2008). *Wolbachia* that generate $F(1 - \mu) < 1$ are
603 disfavored by selection; however, strong-CI causing *Wolbachia* may still become established if
604 stochastic fluctuations carry *Wolbachia* above the critical threshold frequency at which CI favors
605 infection spread. This is especially relevant for biocontrol programs using transinfected *Aedes*
606 *aegypti* mosquitoes, because *w*Mel is deleterious but also generates strong CI in the mosquito
607 background (Ross, Turelli, et al. 2019). Stochastic dynamics can also cause deleterious infections
608 to be lost if *Wolbachia* fluctuate back below the threshold frequency and they are no longer
609 favored by CI (Jansen et al. 2008). Our simulations here reflect the fact that *Wolbachia*
610 infections in natural hosts are now generally thought to generate $F(1 - \mu) > 1$ (Hoffmann and
611 Turelli 1997; Kriesner et al. 2013; Hamm et al. 2014; Kriesner et al. 2016; Kriesner and
612 Hoffmann 2018; Meany et al. 2019).
613 We also considered the hypothesis that changes to maternal transmission rates, host
614 fitness effects, and/or CI between host generations could cause deterministic changes to
615 *Wolbachia* frequencies over time that resemble *w*Mel-like *Wolbachia* in nature. Predictably, the
616 $\bar{p}$ values from our simulations (Figures 5C) closely align with equilibria from the deterministic
617 model [2] (Hoffmann et al. 1990); although, we also considered the addition of low transmitters
618 to the population. Generally, moderate changes to maternal transmission rates (e.g., $\mu = 0.005$ to
619 0.05) and/or the addition of low transmitters between host generations can plausibly explain
620 drops in the frequencies of *w*Mel-like *Wolbachia*. Moderate changes to host fitness effects (e.g.,
621 $F = 1.025$ to 1.075) and CI strength (e.g., $s_h = 0.1$ to 0) can also cause similar changes to
622 infection frequencies over time. These parameter values generally fall within a biologically

plausible range based on aforementioned estimates from field-collected *Drosophila* hosts (Hoffmann et al. 1998; Hoffmann et al. 1998; Olsen et al. 2001; Reynolds and Hoffmann 2002; Kriesner et al. 2016; Shropshire et al. 2021), especially considering that maternal transmission rates of *w*Mel and *D. yakuba*-clade *Wolbachia* can change depending on thermal conditions (Hague, Mavengere, et al. 2020; Hague et al. 2022; Hague et al. 2024). Of course, it is important to note that our simplified model treats μ, F, and s_h as discrete values from one host generation to the next, whereas these parameters are much more likely to exhibit quantitative variation on a continuous scale in natural populations with overlapping host generations.

By incorporating a subpopulation of low transmitters into our simulations, we considered the possibility that maternal transmission rates vary among hosts individuals within a given host generation. This phenomenon has been observed for a number of *Wolbachia* strains in natural host populations, including *w*Mel, *w*San, and *w*Yak (Hoffmann et al. 1998; Carrington et al. 2011; Hamm et al. 2014; Hague, Mavengere, et al. 2020). The presence of low transmitters caused the largest drops in *Wolbachia* frequencies for strains that do not cause CI (Figure 4). These results imply that heterogeneity of transmission rates among females and changes to the presence/absence of low transmitters between host generations may provide another plausible explanation for *w*Mel-like *Wolbachia* fluctuations over time.

Heterogeneity in transmission rates could arise within a generation if host individuals experience different thermal conditions that impact maternal transmission rates and *Wolbachia* densities (Ulrich et al. 2016; Ross et al. 2017; Foo et al. 2019; Ross, Ritchie, et al. 2019; Hague, Mavengere, et al. 2020; Hague, Caldwell, et al. 2020; Chrostek et al. 2021; Hague et al. 2022; Hague et al. 2024). Insects are ectotherms that inhabit complex, heterogeneous environments where thermal conditions can vary on the scale of individual fruits or leaves (Roberts and Feder 2000; Potter et al. 2009; Saudreau et al. 2009; Pincebourde and Woods 2012; Woods et al. 2015). Insects thermoregulate behaviorally, but the immobile egg and pupal stages of development leave hosts more vulnerable to local microclimatic conditions that could influence maternal transmission or *Wolbachia* densities (May 1979; Heinrich 1993; Dillon et al. 2009; Rajpurohit and Schmidt 2016). Heterogeneity of *Wolbachia* densities in hosts could also arise for other reasons, for example, due to individual variation in diet (Serbus et al. 2015; Christensen et al. 2019), age (Reynolds and Hoffmann 2002; Shropshire et al. 2021), and the host and *Wolbachia* genomes (Gu et al. 2022; Hague et al. 2022). The density a *w*Inn, a strain related to *w*Mel and

654 *w*San, varied by 20,000 fold among host individuals in natural populations of *D. innubila* in
655 Arizona (Dyer and Jaenike 2004; Unckless et al. 2009; Hill et al. 2019).
656 We must point out that our discrete generation model generally requires a number of host
657 generations to achieve stochastic or deterministic changes to *Wolbachia* frequencies over time on
658 the order ≥ 0.669 for *w*Mel and ≥ 0.427 for *w*San (Figures S3, S5, S7). These timescales may be
659 plausible for *w*San on São Tomé, where frequencies fluctuated on the scale of years; however, it
660 is implausible for *w*Mel at Gold Coast, where frequencies fluctuated over just a month-long
661 period. This period from March to April corresponds to roughly a single host generation in the
662 field (Turelli and Hoffmann 1995; Hoffmann et al. 1998). Conservatively, the maximum
663 stochastic change in *Wolbachia* frequences (p_{max} - p_{min}) we observed over five consecutive host
664 generations in our simulations was 0.209 using the parameters that generated the largest p_{SD}
665 values (μ = 0.01, F = 1.025, CV_F = 0.1, s_h = 0) (see Appendix 2 for a detailed analysis of
666 stochasticity over many host generations). Even if we assume an implausibly small host
667 population of N = 100, the maximum absolute change in *Wolbachia* frequencies that we observe
668 over five consecutive generations is 0.224 in a set of 25 simulations. If we instead assume that μ,
669 F, and/or s_h values change between host generations, it takes roughly 50 host generations for
670 deterministic changes in equilibria to occur (Figures S5, S7). These results suggest that between-
671 generation changes to maternal transmission, host fitness effects, or CI cannot yield large
672 deterministic changes to *Wolbachia* frequencies over a single host generation according to the
673 assumptions of our model.
674 What else could potentially explain large temporal fluctuations in *Wolbachia* frequencies
675 over just one or two host generations? Importantly, our model assumes that hosts have discrete
676 generations and a fixed population size. Accounting for aspects of insect population dynamics
677 and ecology, such as overlapping generations, density-dependent population regulation, or
678 seasonal variation in population size could also contribute to fluctuating *Wolbachia* frequencies
679 over time (Rasgon and Scott 2004; Farkas and Hinow 2010; Turelli 2010; Hancock et al. 2011;
680 Turelli and Barton 2022). Host migration among structured populations with different *Wolbachia*
681 frequencies could also plausibly alter frequencies within a given focal population (Hoffmann et
682 al. 1998; Flor et al. 2007; Jansen et al. 2008; Engelstädter and Telschow 2009; Haygood and
683 Turelli 2009; Hancock et al. 2011). Along similar lines, *Wolbachia* frequencies could vary on
684 small geographic scales due to the effects of microclimatic variation on μ, F, and s_h. At Gold

685 Coast, *D. melanogaster* hosts were sampled from 20-25 banana mash buckets along transects of
686 at least 3 km. Perhaps microclimatic differences along the transect could generate complex
687 variation in *Wolbachia* frequencies (Roberts and Feder 2000; Potter et al. 2009; Saudreau et al.
688 2009; Pincebourde and Woods 2012; Woods et al. 2015). It is also conceivable that
689 environmental conditions could cause *Wolbachia* frequencies to change within a single host
690 generation if there are extreme effects on *Wolbachia* densities (e.g., a heat wave) that effectively
691 clear *Wolbachia* from some hosts (Ross, Ritchie, et al. 2019; Ross et al. 2020).
692 In summary, we find that stochasticity or between-generation changes to the maternal
693 transmission rate (as well as host effects and CI) can provide plausible explanations for the
694 temporal fluctuations of *w*Mel-like *Wolbachia* over years-long periods, but not shorter time
695 scales. Our results highlight the need for future work to explore the scale of temporal and
696 geographic variation in *Wolbachia* frequencies in the nature, in conjunction with insect
697 population dynamics, like census population size and migration. It is unclear, for example,
698 whether *Wolbachia* frequencies vary temporally on a scale of less than a month or
699 geographically on the scale of kilometers. Interestingly, the temporal dynamics of *w*Mel at Gold
700 Coast seem to be quite variable, but on a broader geographic scale, *w*Mel frequencies have
701 persisted as a stable latitudinal cline for decades along the coast of eastern Australia (Hoffmann
702 et al. 1994; Hoffmann et al. 1998; Kriesner et al. 2016). In the future, field estimates of maternal
703 transmission rates, fecundity, and CI using wild-caught flies sampled at different temporal and
704 geographic scales can be used to model *Wolbachia* dynamics using the publicly available R
705 package *wlbcmodeler*. Uncovering sources of temporal and geographic variation in *Wolbachia*
706 prevalence is ultimately critical for the understanding the long-term evolutionary dynamics of
707 these host-endosymbiont relationships.
708
709 **DATA ACCESSIBILITY STATEMENT**
710 The *wlbcmodeler* R package and scripts to recreate figures will be made publicly
711 available upon publication.
712
713 **COMPETING INTERESTS STATEMENT**
714 The authors declare no competing interests.
715

AUTHOR CONTRIBUTIONS

JMG: funding acquisition (equal), methodology (equal), formal analysis (lead), software (lead), visualization (lead), writing – original draft (supporting), writing – review and editing (supporting). JK: methodology (equal), formal analysis (supporting), writing – original draft (supporting), writing – review and editing (supporting). MTJH: funding acquisition (equal), conceptualization (lead), supervision (lead), writing – original draft (lead), writing – review and editing (lead).

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729 **TABLES**

730 **Table 1.** Regression analysis of the effects of model parameters on the standard deviation of
731 *Wolbachia* frequencies (p_{SD}).

		Coefficient	t value	*P* value
	Host population size	-4.14×10^{-7}	-1.13×10^{2}	< 0.001
Imperfect maternal transmission (μ)	**μ value ($0.001 \leq \mu \leq 0.5$)**	8.51	4.03×10^{2}	< 0.001
	Low transmitters	1.12	2.88×10^{2}	< 0.001
	μ value * low transmitters	-4.42	-1.39×10^{2}	< 0.001
Host fitness effects (F)	**F value ($1 \leq F \leq 1.5$)**	-2.04	-1.68×10^{2}	< 0.001
	Fluctuating F ($CV = 0.01$)	4.23×10^{-1}	1.01×10^{2}	< 0.001
	Fluctuating F ($CV = 0.1$)	1.78	4.49×10^{2}	< 0.001
Cytoplasmic incompatibility (s_h)	**Weak CI ($s_h = 0.1$)**	-9.85×10^{-2}	-3.20×10^{1}	< 0.001
	Strong CI ($s_h = 0.45$)	-7.83×10^{-1}	-2.17×10^{2}	< 0.001

732

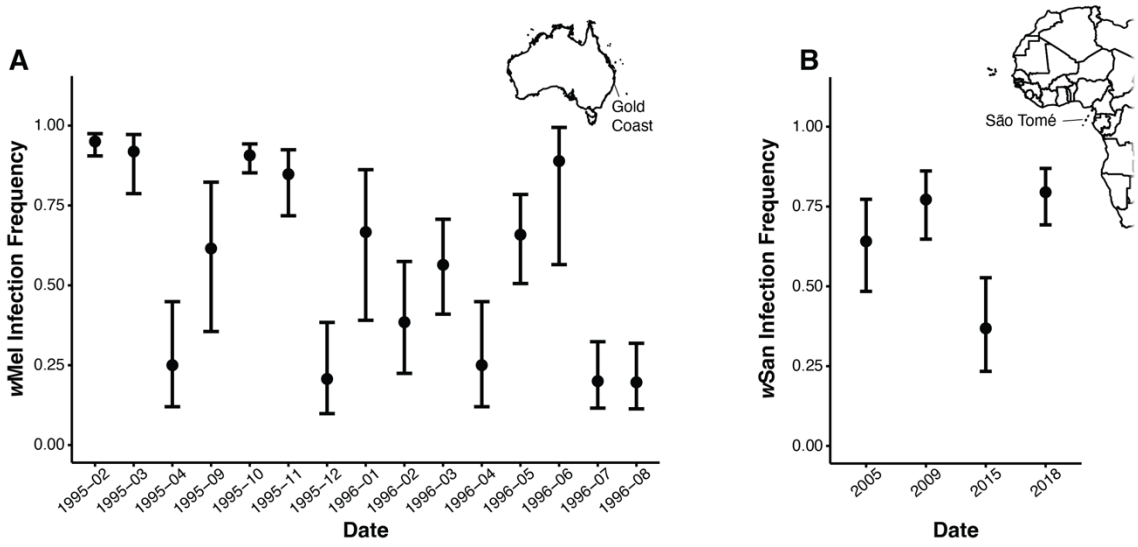
733 **Table 2.** Regression analysis of the effects of model parameters on mean *Wolbachia* frequencies
734 ($\bar{p}$).

		Coefficient	t value	*P* value
	Host population size	-1.58×10^{-8}	-6.09	< 0.001
Imperfect maternal transmission (μ)	**μ value ($0.001 \leq \mu \leq 0.5$)**	-2.24×10^{1}	-1.02×10^{3}	< 0.001
	Low transmitters	-2.43	-7.33×10^{2}	< 0.001
	μ value * low transmitters	8.26	2.76×10^{2}	< 0.001
Host fitness effects (F)	**F value ($1 \leq F \leq 1.5$)**	3.77	4.01×10^{2}	< 0.001
	Fluctuating F ($CV = 0.01$)	1.30×10^{-4}	4.93×10^{-2}	9.61×10^{-1}
	Fluctuating F ($CV = 0.1$)	9.68×10^{-3}	3.62	< 0.001
Cytoplasmic incompatibility (s_h)	**Weak CI ($s_h = 0.1$)**	4.27×10^{-1}	1.63×10^{2}	< 0.001
	Strong CI ($s_h = 0.45$)	1.92	6.82×10^{2}	< 0.001

735

736

737 **FIGURES**



738

739 **Figure 1.** Temporal fluctuations of *Wolbachia* frequencies for **(A)** *w*Mel in *D. melanogaster* and
740 **(B)** *w*San in *D. santomea*. Error bars represent 95% binomial confidence intervals. **(A)** *w*Mel
741 frequencies in *D. melanogaster* at Gold Coast in subtropical eastern Australia from the winter of
742 1995 to the summer of 1996. Data recreated from Hoffmann et al. (1998). The authors reported
743 no obvious seasonal effect on *w*Mel frequencies. **(B)** *w*San frequencies in *D. santomea* in the
744 high-altitude mist forests on the volcanic island of São Tomé estimated from 2005 to 2018. Data
745 recreated from Hague, Mavengere, et al. (2020). *w*Yak frequencies in *D. yakuba* also fluctuate
746 temporally on São Tomé; however, this variation is complicated by altitudinal variation in
747 *Wolbachia* frequencies and transmission rates (see Hague, Mavengere, et al. 2020).

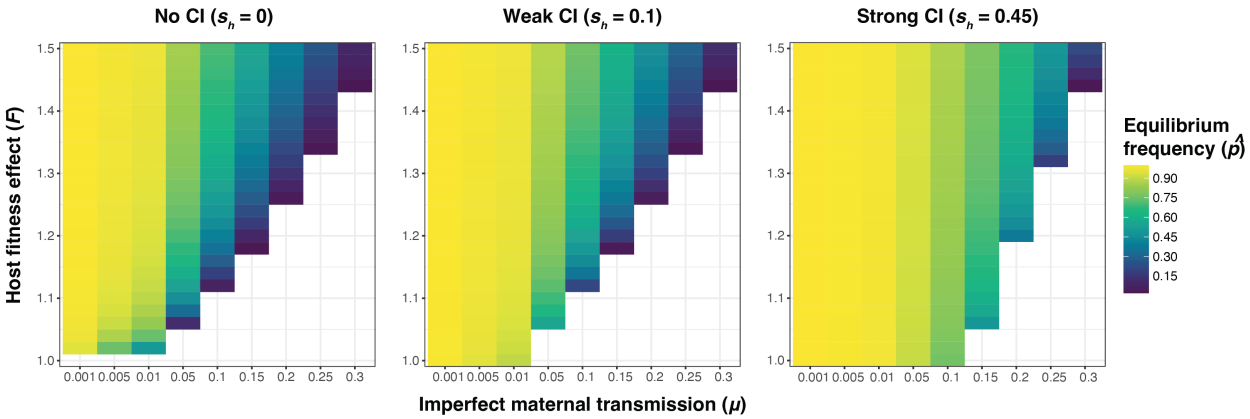


Figure 2. Equilibrium *Wolbachia* frequencies ($\hat{p}$) approximated by a discrete generation deterministic model incorporating imperfect maternal transmission (μ), *Wolbachia* effects on host fitness (F), and cytoplasmic incompatibility (s_h). Equilibrium frequencies are shown across a range of biologically plausible parameter values for no, weak, and strong CI.

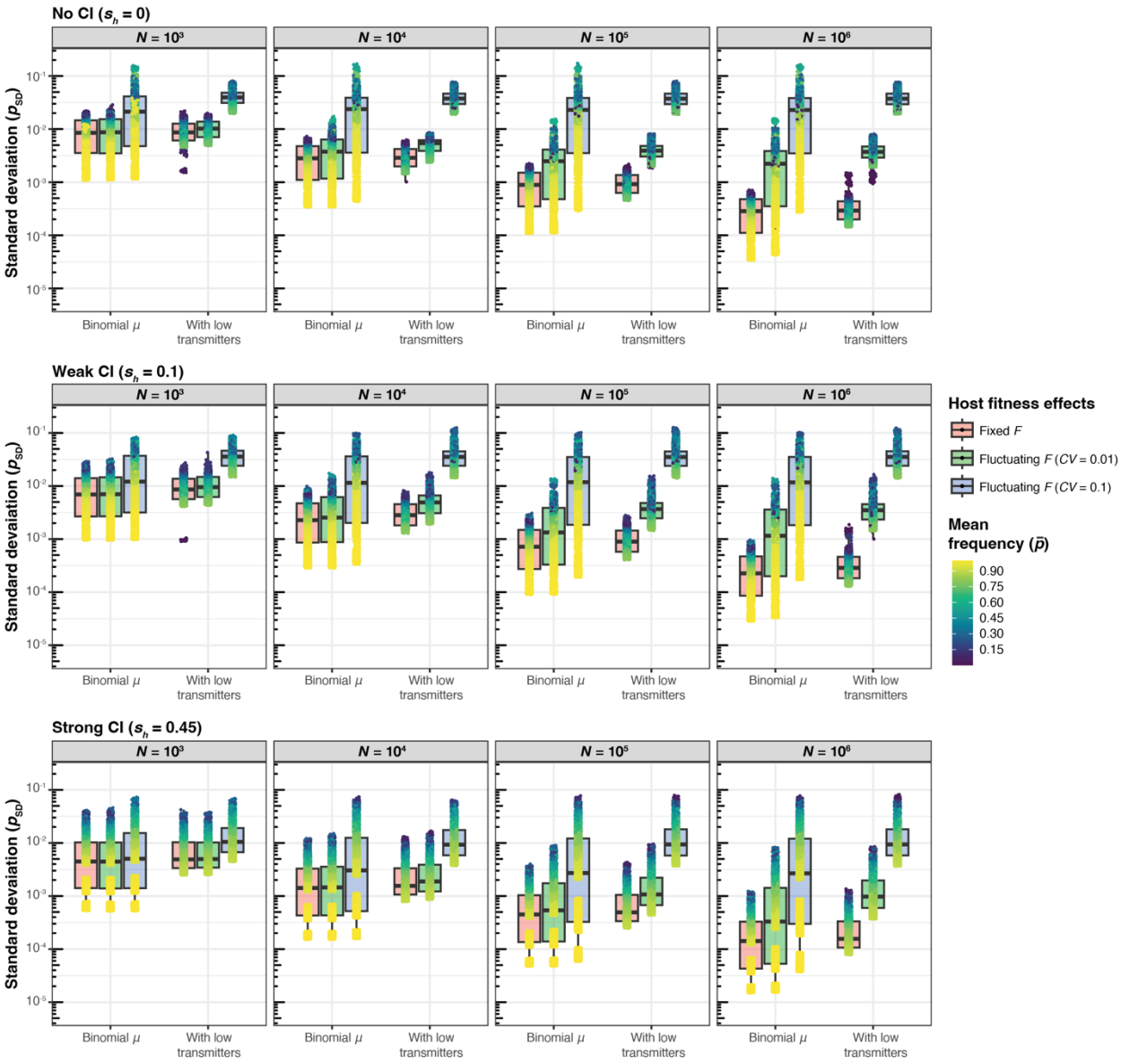


Figure 3. Results from individual simulations exploring the contributions of N, μ, F, and s_h to the standard deviation of *Wolbachia* frequencies (p_{SD}). Each boxplot represents the distribution of p_{SD} values from simulations implemented with different combinations of μ ($0.001 \leq \mu \leq 0.5$) and F ($1 \leq F \leq 1.5$). Datasets are further separated based on whether low transmitters and fluctuating host effects ($CV = 0.01$ or 0.1) are present. Results are shown for different CI strengths (none, weak, or strong) and population sizes (10^3, 10^4, 10^5, 10^6). Individual points represent p_{SD} from a given simulation and are color-coded to indicate the simulation's mean *Wolbachia* frequency ($\bar{p}$). The first 500 generations were removed from each simulation before calculating p_{SD}.

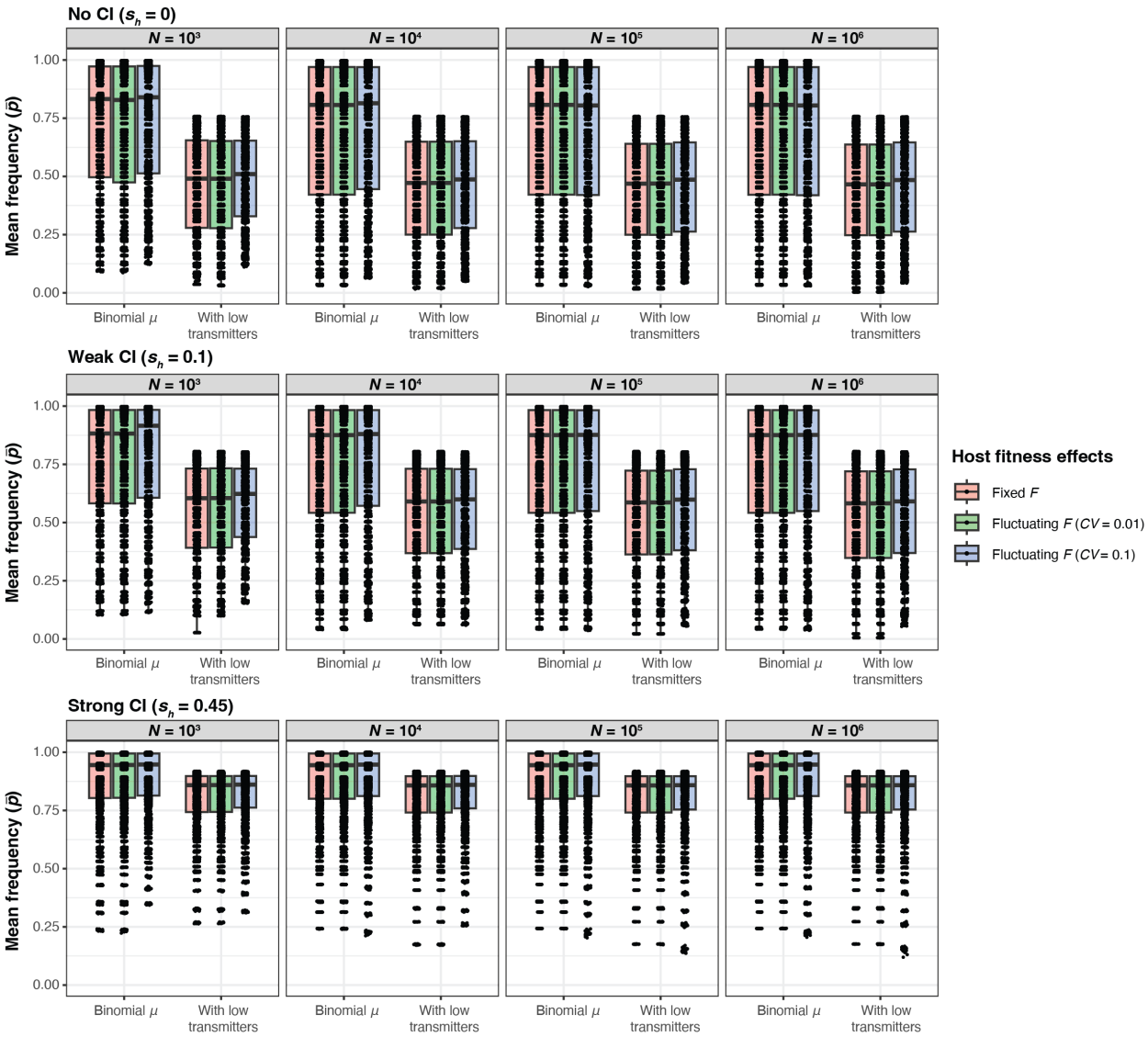


Figure 4. Results from individual simulations exploring the contributions of N, μ, F, and s_h to mean *Wolbachia* frequencies ($\bar{p}$). Each boxplot represents the distribution of $\bar{p}$ values from simulations implemented with different combinations of μ ($0.001 \leq \mu \leq 0.5$) and F ($1 \leq F \leq 1.5$). Datasets are further separated based on whether low transmitters and fluctuating host effects (CV = 0.01 or 0.1) are present. Results are shown for different CI strengths (none, weak, or strong) and population sizes (10^3, 10^4, 10^5, 10^6). Individual points represent $\bar{p}$ from a given simulation. The first 500 generations were removed from each simulation before calculating $\bar{p}$.

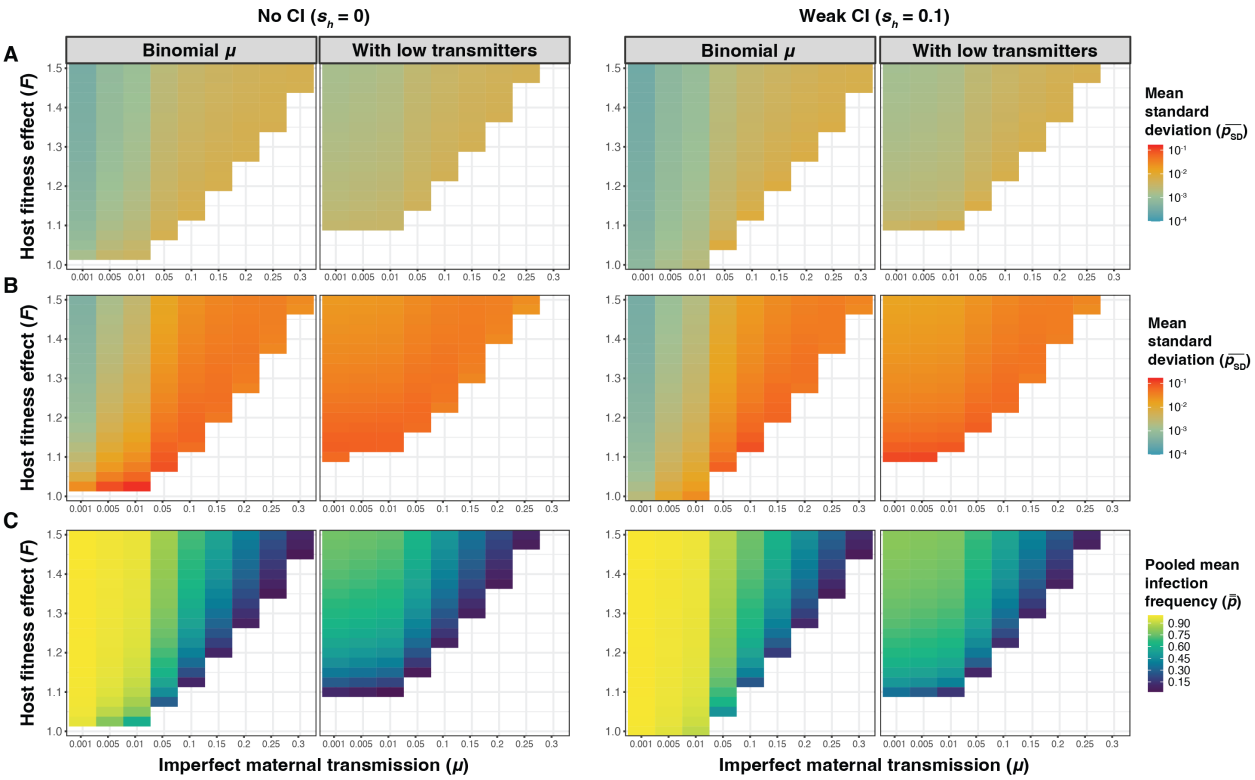


Figure 5. Summary of results from simulations exploring the contributions of μ, F, and s_h to standard deviations (p_{SD}) and mean *Wolbachia* frequencies ($\bar{p}$) with a finite host population size of $N = 10^4$. **(A)** Each cell represents the mean standard deviation ($\overline{p_{SD}}$) of *Wolbachia* frequencies from 25 simulations with a given μ value and a fixed F value. Note that $\overline{p_{SD}}$ values are plotted on a log scale. **(B)** Each cell represents $\overline{p_{SD}}$ from 25 simulations with a given μ value and a strongly fluctuating F value ($CV = 0.1$). **(C)** Each cell represents the pooled mean of mean *Wolbachia* frequencies ($\bar{\bar{p}}$) from a set of 25 simulations with a given μ value and a fixed F value. Similar data are not shown for the simulations with fluctuating F values, because fluctuating host effects had only a small effect on $\bar{p}$. Results for strong CI (considered implausible for *w*Mel and *w*San) are shown in Figure S1.

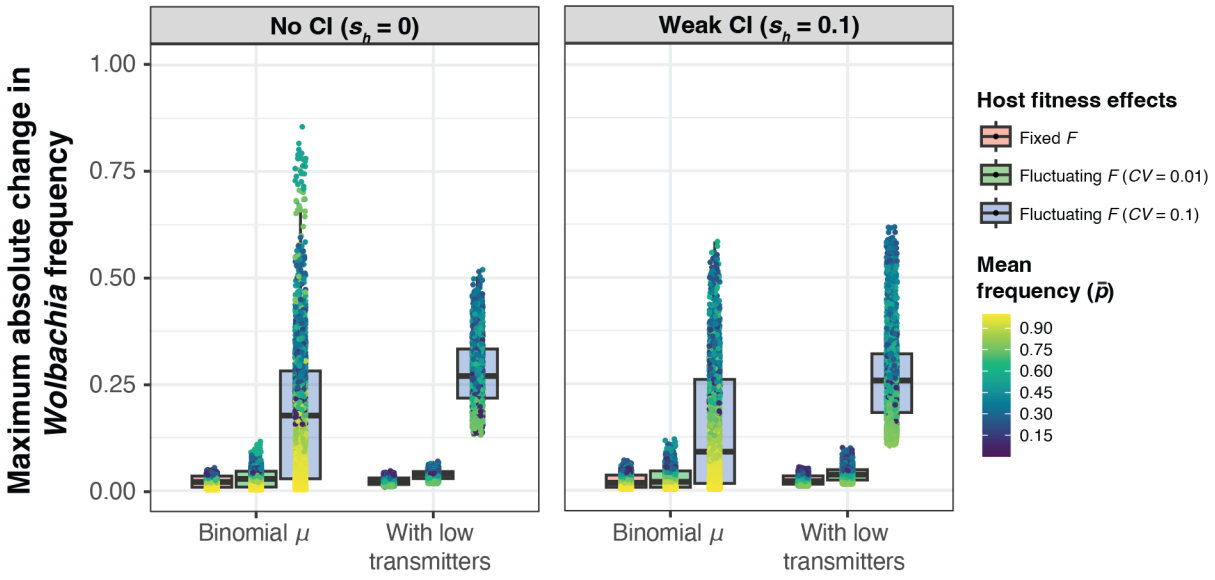


Figure 6. Results from individual simulations exploring the contributions of μ, F, and s_h to the absolute difference between maximum and minimum *Wolbachia* frequencies (p_{max} - p_{min}) observed over 10,000 host generations with a finite population size of $N = 10^4$. Individual points represent the maximum absolute change in *Wolbachia* frequencies observed in a given simulation with various combinations of μ ($0.001 \leq \mu \leq 0.5$) and F ($1 \leq F \leq 1.5$). Datasets are further separated based on whether low transmitters and fluctuating host effects ($CV = 0.01$ or 0.1) are present. Individual points are color-coded to indicate each simulation's mean *Wolbachia* frequency ($\bar{p}$). The full set of results are shown in Figure S2. The first 500 generations were removed from each simulation before calculating p_{max} - p_{min}.

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