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1 **Insect population dynamics under *Wolbachia*-**  
2 **induced cytoplasmic incompatibility:**  
3 **puzzle more than buzz in *Drosophila suzukii***

4

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13

## 14 Abstract

15 In theory, the introduction of individuals infected with an incompatible strain of *Wolbachia pipientis* into a  
16 recipient host population should result in the symbiont invasion and reproductive failures caused by cytoplasmic  
17 incompatibility (CI). Modelling studies combining *Wolbachia* invasion and host population dynamics show that  
18 these two processes could interact to cause a transient population decline and, in some conditions, extinction.  
19 However, these effects could be sensitive to density dependence, with the Allee effect increasing the probability  
20 of extinction, and competition reducing the demographic impact of CI. We tested these predictions with laboratory  
21 experiments in the fruit fly *Drosophila suzukii* and the transinfected *Wolbachia* strain *wTei*. Surprisingly, the  
22 introduction of *wTei* into *D. suzukii* populations at carrying capacity did not result in the expected *wTei* invasion  
23 and transient population decline. In parallel, we found no Allee effect but strong negative density dependence.  
24 From these results, we propose that competition interacts in an antagonistic way with *Wolbachia*-induced  
25 cytoplasmic incompatibility on insect population dynamics. If future models and data support this hypothesis, pest  
26 management strategies using *Wolbachia*-induced CI should target populations with negligible competition but a  
27 potential Allee effect, for instance at the beginning of the reproductive season.

28 Keywords: Density dependence, Allee effect, intraspecific competition, *Drosophila suzukii*, *Wolbachia*, cytoplasmic  
29 incompatibility, eco-evolutionary dynamics.

## 30 Introduction

31 *Wolbachia pipientis* is an endosymbiotic bacterium found in a large number of nematode and arthropod species,  
32 including insects [1,2]. The overall record of interactions between *Wolbachia* and their hosts is highly variable,  
33 ranging from parasitism to mutualism. The ability of *Wolbachia* to manipulate the reproduction of their hosts at  
34 the advantage of its own transmission is the most widespread facet of these interactions [3]. Cytoplasmic  
35 incompatibility (CI) is one such manipulation that occurs, in its simplest form, when infected males cross with  
36 uninfected females. *Wolbachia*-induced sperm modification in males cannot be restored by females. In diploid  
37 species, these crosses result in embryo death while others produce viable offspring [4]. Hence, infected females

38 have more offspring on average than uninfected females because they can produce offspring with all types of  
39 males, be they infected or not. Since *Wolbachia* is transmitted maternally and vertically, these females are more  
40 likely to transmit the incompatible strain, thus promoting its spread [5].

41 The high invasion success of *Wolbachia* combined with its ability to reduce the transmission of viruses or other  
42 pathogens [6,7] has aroused the interest of epidemiologists. Disease-vector control strategies have been  
43 developed based on the introduction of individuals artificially infected with a virus-blocking incompatible strain of  
44 *Wolbachia* into a target population. The reproductive advantage of infected females favors the invasion of the  
45 virus-blocking strain, which increases in frequency until it becomes the majority or fixed strain. In the event of a  
46 resident strain not inducing CI is present in the population, it would be replaced by the introduced incompatible  
47 strain, which results in a host population infected with a *Wolbachia* strain with traits of interest for pest control.  
48 For instance, such a replacement strategy has been shown to be effective in suppressing dengue transmission by  
49 the mosquito *Aedes aegypti* [8].

50 In order to optimize the success of such *Wolbachia* replacement programs, it is necessary to identify the factors  
51 that influence *Wolbachia* invasion dynamics. Historically, most theoretical models of CI dynamics are analogous  
52 to population genetic models and have focused on the evolution of the frequencies of an incompatible strain  
53 within the host population, neglecting the specificities of host population dynamics [9–13]. Only a limited number  
54 of host demographic parameters directly affecting changes in frequencies were considered and assumed to be  
55 constant [8,13,14]. These models predict the existence of a critical infection frequency, referred to as the invasion  
56 threshold, above which an incompatible strain will deterministically invade the host population. The threshold  
57 depends on three parameters: the intensity of infection-induced CI, the success of maternal transmission and the  
58 costs resulting from infection, such as reduced fertility. More recently, Hancock *et al.* [15–17] added density  
59 dependence on host demographic parameters in order to study *Wolbachia* invasion dynamics with more realistic  
60 assumptions. They concluded from different models that the intensity of competition, by varying the reproductive  
61 success of individuals, significantly reduces the ability of *Wolbachia* to invade a population [16,17].

62 The ability of *Wolbachia* to invade may serve another pest management strategy, alternative to the drive of  
63 interesting characters. In a landmark publication, Dobson *et al.* [18] have proposed a novel CI-based insect pest  
64 suppression strategy based on *Wolbachia* invasion. They extended classical CI models by assuming realistic host

65 population dynamics with density dependent survival of immature stages and parameter values taken from the  
66 insect literature. They simulated the introduction of an incompatible *Wolbachia* strain into a host population that  
67 had reached carrying capacity. While the incompatible strain spreads through the population, the frequency of  
68 incompatible crosses increased, resulting in a transient reduction in host population size. Population size declined  
69 to a minimum when the proportions of infected and uninfected individuals were similar and then increased again  
70 when the incompatible strain became the majority. One of their predictions is that the minimum population size  
71 reached during invasion is influenced not only by *Wolbachia*'s own invasion parameters, but also by demographic  
72 parameters intrinsic to host populations. For instance, under conditions of high host reproductive rate and  
73 intraspecific competition, the demographic consequences of CI may go unnoticed.

74 The transient population decline caused by *Wolbachia*-induced cytoplasmic incompatibility, as predicted by  
75 Dobson *et al.* [18], is a form of demographic destabilization that, in conjunction with other processes, could  
76 cascade towards population extinction. As other process, the Allee effect is an excellent candidate. It is  
77 characterized by population growth rate decreasing with the decrease of the population size, which triggers an  
78 extinction vortex if the growth rate becomes negative below a critical number of individuals (the Allee threshold)  
79 or if the growth rate remains positive but combines with stochasticity. The Allee effect has thus been envisioned  
80 as a catalyst for extinction upon which novel tactics could be developed to eradicate pest populations [19–23].  
81 Building on these ideas, Blackwood *et al.* [24] assumed an Allee effect to investigate the conditions under which  
82 CI expression would serve pest eradication. To this end, they simulated *Wolbachia* introductions into populations  
83 affected by a more or less intense Allee effect. When the Allee effect was strong and the population reproduction  
84 rate low, the model predicted that the minimum population size reached during *Wolbachia* invasion would fall  
85 below the Allee threshold, leading to population extinction. On the other hand, at high reproduction rates, the  
86 transient reduction induced by CI was not large enough to bring the population size below the Allee threshold.

87 The theoretical models of Dobson *et al.* [18] and Blackwood *et al.* [24] are of major interest for insect pest  
88 management but, to our knowledge, they have not been tested yet. We are unaware of any empirical evidence of  
89 either transient population declines caused by the invasion of an incompatible *Wolbachia* strain and subsequent  
90 increase in CI or population extinctions if CI combines with an Allee effect. We therefore initiated a research  
91 program using these models as a general theoretical framework and *Drosophila suzukii* as a model organism.

92 There are several reasons for choosing *Drosophila suzukii* as a model organism. First, *D. suzukii* is a major invasive  
93 pest of berry and stone fruit crops [25]. Native from Southeast Asia, *D. suzukii* was first reported in the USA and  
94 Europe in the late 2000s and continues to spread worldwide (e.g. Africa; [26–28]). Unlike other *Drosophila* species,  
95 the serrated ovipositor of *D. suzukii* allows females to lay eggs under the skin of ripening or mature fruits, causing  
96 significant damage to cultivated fruits and consequently, huge economic losses [25]. This calls for research to  
97 develop environmentally friendly methods to control this major agricultural pest. Second, *D. suzukii* is naturally  
98 infected with *Wolbachia*. Contrary to the common observation of “most-or-few” infection frequencies, the  
99 prevalence of the resident *wSuz* strain in European populations is highly variable, averaging 46% [29]. This  
100 infection polymorphism could be a consequence to the fact that *wSuz* induced no or weak CI [29,30] as well as the  
101 variable benefits and costs of *wSuz* protection from viruses [31]. Third, we capitalized on a strong body of  
102 experimental work based on the transinfection of *D. suzukii* with various *Wolbachia* strains, including *wHa* and  
103 *wTei*, which cause cytoplasmic incompatibility [32–34]. Finally, the expression of negative density dependence on  
104 individual performance has already been observed in *D. suzukii* [35]. Under experimental conditions with high  
105 larval densities, some individuals were observed to leave the breeding site, which was interpreted as a response  
106 to intraspecific competition for resources [36]. While the Allee effect has not yet been demonstrated in *D. suzukii*,  
107 it has been documented in other closely related *Drosophila* species [37].

108 The aim of this study is to estimate the strength of positive and negative density dependence in *D. suzukii* and to  
109 test the effect of *Wolbachia*-induced CI on the dynamics of experimental populations. In this article, we report the  
110 absence of an Allee effect under our laboratory conditions. In contrast, we found strong negative density  
111 dependence resulting in logistic population growth. Finally, we found no effect of introducing an incompatible  
112 *Wolbachia* strain on *D. suzukii* population dynamics. We discuss these results in the light of the models developed  
113 by Dobson *et al.* [18] and Blackwood *et al.* [24] and propose that density dependent competition may have much  
114 stronger influences than expected on *Wolbachia* invasion and the demographic consequences of cytoplasmic  
115 incompatibilities.

## 116 **Materials and methods**

### 117 **Insect rearing**

118 Two lines of *D. sukukii* have been maintained in the laboratory since 2018, *i.e.*, for around 24 generations before  
119 experiments. These lines were infected with either *wSuz*, the strain naturally present in *D. sukukii* [29,30,38] or  
120 the transinfected *Wolbachia* strain *wTei*, the natural symbiont of *D. teissieri*. The latter was selected because of  
121 the incompatibility induced by transinfected *D. sukukii* males when crossed with *wSuz*-infected females [32,34].  
122 The two lines were reared on cornmeal-based food medium [39] containing, per 1 L of water: 10 g agar, 80 g corn  
123 flour, 80 g brewer's yeast and 60 ml moldex (100 g methyl hydroxybenzoate in 1 L 70% ethanol). Rearing, as well  
124 as all experiments, occurred in a built-in climatic chamber at 23-24°C, 50-60% humidity and a photoperiod of 12  
125 h-12 h.

### 126 **Experiment 1. Estimation of population size**

127 In order to estimate accurately the number of flies in population cages, we developed a rapid photo-based census  
128 method. Prior to experiments, we assessed the relevance of this method. For this, between 4 and 396 individuals  
129 were introduced into 59 cubic experimental cages (21 cm x 21 cm x 21 cm) made of fine-mesh fabric and a  
130 transparent plastic front panel. The cages were then photographed using a Nikon N750D camera and a Nikon lens  
131 (AF-S Micro Nikkor 60 mm f/2.8G ED). Visible Individuals were counted on a computer using the software ImageJ  
132 1.52 [40] on three predefined areas (S1 Fig): (i) individuals appearing on the front and on the rear panels (area 1),  
133 (ii) individuals appearing on the whole surface except the left side and the cage floor (area 2), or (iii) only individuals  
134 appearing on the front panel (area 3). Areas 1 and 2 were used for the experiments 2 and 3, respectively. Area 3  
135 was not used in the present study but it will be used in follow-up articles. The reasons for this choice are given in  
136 the Results section.

137 For each area, linear regressions between the numbers of individuals on pictures and the total numbers in the  
138 cage were fitted, and the coefficients of determination  $R^2$  estimated. Kolmogorov-Smirnoff tests, from the

139 DHARMA package in R, were used to test normality of the residual distribution. These and the following statistical  
140 analyses were carried out using R 4.2.2 software [41].

## 141 **Experiment 2. Early dynamics of small populations**

### 142 **Founding populations**

143 This experiment aimed at quantifying a demographic Allee effect, *i.e.*, positive density dependence at low  
144 population size. To this end, we founded *D. sukukii* populations with different numbers of male-female pairs and  
145 estimated subsequent growth rates. Founding pairs were formed with 1 male and 1 female, all virgins and less  
146 than 24 hours old. Populations were founded with either 1, 3, 10, 30 or 100 pairs, and each of these initial  
147 population size was replicated between 8 and 13 times (13 populations with 1 pair, 13 with 3 pairs, 10 with 10  
148 pairs, 8 with 30 pairs, and 8 with 100 pairs, for a total of 52 populations). Replicated populations were distributed  
149 across four blocks spaced one week apart. For each population, the pairs were introduced into a cage as described  
150 above. Weekly, flies were provided with a new oviposition patch consisting of 100 ml (75 mm diameter) rearing  
151 medium supplemented with a fresh raspberry. As the generation time was around two weeks in our rearing  
152 conditions, each patch was exposed three weeks to ascertain that eggs laid in the patch yielded new adults  
153 emerging into the cages. Immature drosophila developed by feeding on the rearing medium whereas adult flies  
154 were supplied with water and honey three times a week. To estimate population size, each cage was photographed  
155 once a week. Populations were followed nine weeks post foundation; generations were completely overlapping,  
156 but if they were discrete, nine weeks would represent 4 to 5 generations of *D. sukukii*.

### 157 **Fitting an exponential growth model**

158 The early dynamics of populations founded with a small number of individuals is expected to depend on initial  
159 population size and the strength of an Allee effect. In the absence of an Allee effect, exponential population growth  
160 is expected. With a weak Allee effect, exponential growth is also expected, but the growth rate should be lower  
161 for initially small populations (*e.g.*, 1 pair, 3 pairs, etc.) than for larger ones (*e.g.*, 100 pairs). With a strong Allee  
162 effect, populations of initial size smaller than the Allee threshold should go extinct whereas populations initially



163 above the Allee threshold should increase exponentially, with a growth rate again depending on population size.

164 We tested these predictions by counting populations that went extinct and estimating the growth rate of those

165 that persisted. The growth rate ( $r$ ) was estimated by fitting a simple exponential growth model such as:

$$166 \quad N_t = N_0 e^{r t} \quad (1)$$

167 to each population time series, excluding the last week at which the largest populations had stopped increasing.

168 In practice, we fitted weekly counts with a generalized linear model based on a Poisson distribution and a log link

169 function, *i.e.*:

$$170 \quad \log (N_t) = \log (N_0) + r t \quad (2)$$

171 The initial number of individuals observed just after the populations had been founded was defined as an offset

172 ( $\log (N_0)$ ), that is, a parameter included in the model but not estimated. The number of individuals estimated

173 immediately after release (week 1) was missing for all populations from the first block. It was impossible to add

174 the initial number as an offset to the model. Therefore, the five populations of the first block were discarded due

175 to missing values. Growth curves and estimated growth rates  $r$  were obtained for each population. A linear

176 regression was then used to analyze the effect of the number of founders on growth rate.

## 177 **Testing density dependence**

178 A complementary method to test for positive density dependence is to assess the relation between the proportion

179  $p$  of populations growing between  $t$  and  $t + 1$  and the population size at time  $t$ . Analyzing  $p$  rather than individual

180 counts circumvents the high sampling variance at low numbers and the consequent spurious estimations of growth

181 rates [42]. With an Allee effect, we expect  $p$  to increase with population size at low population size, with  $p = 0.5$

182 being the Allee threshold. Independently of the experimental manipulation of initial population size, we associated

183 all possible pairs of  $N_t$  and  $N_{t+1}$  for each of the 47 populations at each weekly interval. A population was considered

184 stable or growing for  $N_{t+1} \geq N_t$  (further referred to as a growing population given the low number of pairs, 14 of

185 410 pairs, with  $N_{t+1} = N_t$ ) or decreasing otherwise. We then calculated the proportion  $p$  of growing populations

186 within 18 classes of population sizes (1-49, 50-99, etc.). This proportion was fitted with a logistic regression to test

187 three alternative hypotheses: (i) a first null model suggests no relation between population size and growth

188 
$$p = \beta_0, \tag{3}$$

189 (ii) a second standard model suggesting a linear relation

190 
$$p = \beta_0 + \beta_1 N_t, \tag{4}$$

191 and (iii) a third polynomial model allowing a non-linear relationship

192 
$$p = \beta_0 + \beta_1 N_t + \beta_2 N_t^2 \tag{5}$$

193 The Eq (5) is well suited to describe bell-shaped curves that characterize populations with both an Allee effect and  
194 negative density dependence. The Akaike Information Criterion (AIC) was used to select the best model.

### 195 **Experiment 3. *Wolbachia* invasion and consequent population** 196 **dynamics**

#### 197 **Experimental populations**

198 The aim of this experiment was to assess changes in *D. sukukii* population dynamics caused by the introduction of  
199 individuals infected with an incompatible *Wolbachia* strain. To this end, we founded eight populations with 100  
200 *wSuz*-infected individuals (sex ratio 1:1) aged 3 to 5 days. Each population was studied continuously across three  
201 distinct periods, for a total of 23 weeks: a first period of population growth (5 to 8 weeks); a seven-week period of  
202 dynamic equilibrium around the carrying capacity, starting upon observation of the first decrease in number; a  
203 post-introduction period (8 to 11 weeks) starting upon the introduction of infected flies. Populations were  
204 maintained as in the previous experiment, with a new reproduction patch provided weekly and exposed during  
205 three weeks, and water and honey to feed adults three times a week. Cages were again photographed every week  
206 for estimation of population sizes.

#### 207 **Introducing *Wolbachia* and characterizing infection status**

208 Individuals introduced after the seven-week period of dynamic equilibrium were infected by either *wSuz* for the  
209 four "control" populations (without cytoplasmic incompatibility) or *wTei* for the four "CI" populations (with  
210 cytoplasmic incompatibility). Theoretically, *Wolbachia* invasion depends on the intensity of CI, transmission  
211 efficiency, and infection cost [9–13]. Assuming perfect transmission, an incompatible strain should invade if it is  
212 introduced above a threshold equal to the ratio of infection cost (parameter  $sf$  in the models referenced above)  
213 to incompatibility (parameter  $sh$ ). In our system of *D. sukukii* and *wTei*, incompatibility is incomplete, with an egg  
214 hatch rate of about 33% after incompatible crosses (*i.e.*,  $sh = 0.67$ ; [34]). In parallel, the analyses of traits such as  
215 fecundity, longevity, egg hatch rate and developmental time all suggest an absence of cost of *wTei* infection on *D.*  
216 *sukukii* [32,34]. This apparent absence of infection costs ( $sf = 0$ ), even with incomplete incompatibility, suggests a  
217 theoretical invasion threshold of 0%. In practice, this would mean just enough individuals to compensate for the  
218 stochastic events that are not considered in the theoretical models. A number of individuals close to 10% of the  
219 mean number of individuals over equilibrium period should be sufficient to trigger invasion. Before introduction,  
220 we verified the fixation of *wSuz* for all populations by genotyping 20 individuals per population (methods below).  
221 After introduction, we monitored the evolution of *wTei* frequencies over time in the four CI populations. For this,  
222 we randomly sampled 20 individuals per week and stored them in 99% ethanol at - 20°C until analysis. Individual  
223 infection status was assessed *via* HRM (High Resolution Melting), a genotyping method that allows the detection  
224 of changes in the melting profiles of double-stranded DNA (S2 Fig, [43]). For each individual, DNA was extracted  
225 using Macherey Nagel's NucleoSpin kit. *Wolbachia*-specific primers 81f (5'-TGGTCCAATAAGTGATGAAGAAAC-3';  
226 [44]) and 2R (5'-CAGCAATTCAGGATTAG-3'; [43]) were used to amplify the *wsp* ("*Wolbachia Surface Protein*")  
227 gene. PCRs were performed in a 10  $\mu$ L volume containing 500 nM primers, 1X Precision Melt Supermix (Biorad),  
228 containing dNTPs, iTaq™ DNA polymerase, MgCl<sub>2</sub>, EvaGreen buffer, stabilizers, and 2  $\mu$ L of 10-fold diluted DNA  
229 [43]. Cycle conditions were 95°C for 120 s, then 30 s at 95°C, 30 s at 54°C and 30 s at 72°C for 40 cycles, followed  
230 by 30 s at 95°C and 60 s at 60°C (Biorad CFX96). The temperature ramp rate was 1.6°C/s.

## 231 **Estimating carrying capacity**

232 The eight populations were studied over enough time to estimate the carrying capacity  $K$  in our environmental  
233 conditions. For this, we fitted non-linear regressions to the number of adults observed each week, from foundation  
234 to the introduction of infected flies (first and second time periods). Three candidate population growth models

235 were fitted: Gompertz, logistic, and Weibull. SSlogis, SSgompertz and SSweibull functions in R were used to obtain  
236 the initial parameter values for each candidate model (so-called "selfStart" models [41,45]). All these models share  
237 one parameter, the asymptotic population size defined as the carrying capacity. The AIC and root-mean-square  
238 error (RMSE) were then used to select the best of the three models fitted to each population.

## 239 **Testing the consequences of CI on population dynamics**

240 Evolution of the frequency of *wTei*-infected individuals was analyzed using a generalized linear mixed model based  
241 on a negative binomial distribution and a logit link function, with time as a fixed effect and population as a random  
242 effect. We excluded the first period of population growth to focus on the influence of introducing incompatible  
243 *Wolbachia* on the dynamics of populations that had reached carrying capacity. The main prediction tested here is  
244 that the introduction of individuals infected by the incompatible *wTei Wolbachia* strain should lead to a decline in  
245 population size, co-occurring with the invasion of *wTei* and subsequent increase in the proportion of incompatible  
246 crosses. This decline should not be observed in control populations with introductions of individuals infected by  
247 *wSuz*. We thus fitted the following model:

$$248 \quad N_{ijt} = \beta_0 + \beta_1 t + \beta_2 \text{intro} + \beta_3 t \text{intro} + \beta_4 t \text{intro strain} + \varepsilon_{ijt} \quad (6)$$

249 where  $N_{ijt}$  is the number of individuals in populations of introduction status  $i$  (before vs after introduction; variable  
250 *intro* in the model) having undergone treatment  $j$  (introduction of *wTei* or *wSuz*; variable *strain* in the model) at  
251 time  $t$  relative to the introduction (rescaled so that  $t = 0$  upon introduction). This model allowed to test four  
252 predictions (S3 Fig).

- 253 • H1: Control and CI populations were at equilibrium before introduction so that there should be no effect of  
254 time during the seven weeks prior to the introduction ( $\beta_1 = 0$ ).
- 255 • H2: Introductions should result in an immediate but small increase in population size ( $\beta_2 \neq 0$ ).
- 256 • H3: Introductions may destabilize populations from equilibrium and hence, induce a change in the effect of  
257 time on population size ( $\beta_3 \neq 0$ ).
- 258 • H4: The latter change is expected to be affected by the strain, with *wTei*, contrary to *wSuz*, inducing a  
259 decrease in population size ( $\beta_4 \neq 0$ ).

260 These four hypotheses were tested with likelihood ratio tests between models with and without the variable or  
261 interaction concerned.

## 262 **Results**

### 263 **Estimation of population size (experiment 1)**

264 In order to estimate population size, we counted adult flies on pictures of three different areas of the experimental  
265 cages. Whatever the area considered, we found a clear relationship between the number counted on pictures and  
266 the total number of flies in the cages (Fig 1). Linear regressions were characterized by high coefficients of  
267 determination:  $R^2 = 0.99$  for areas 1 and 2 and  $R^2 = 0.86$  for the last area. Kolmogorov-Smirnov tests confirmed the  
268 normal distribution of errors, and hence, an absence of sampling bias (Fig 1; KS test:  $p = 0.83, 0.85$  and  $0.34$  for  
269 areas 1, 2 and 3 respectively). Given the high coefficients of determination obtained for areas 1 and 2 and the  
270 small number of populations involved, these two areas were chosen to estimate the population size in experiments  
271 2 and 3 respectively. However, should the number of experimental populations increase, we would recommend  
272 the use of area 3, because counting higher numbers of insects in the other two areas would be too time-consuming  
273 to characterize many populations on a weekly basis. Therefore, area 3 was not used in the present study, but it  
274 will be used in the future.

### 275 **Early dynamics of small populations (experiment 2)**

276 None of the newly founded populations went extinct, even introducing a single pair. Population growth during the  
277 first eight weeks was well described by an exponential model, regardless of the number of founding pairs (Fig 2).  
278 The estimated growth rate  $r$  was influenced by initial population size (Anova,  $F_{4,42} = 14.6, p < 0.001$ ). Contrary to  
279 expectation with an Allee effect, the growth rate  $r$  was highest at smallest population sizes, from  $1.73 \pm 0.03$  for  
280 single pairs to  $1.4 \pm 0.04$  for 100 pairs.

281 The detailed analysis of week-to-week variations in population sizes confirmed the absence of Allee effect. Among  
282 the three alternative models, the second order polynomial logistic regression best fitted the proportion of  
283 increasing populations (AIC = 61.3, *versus* 82.9 for the null model and 63.5 for linear regression). The resulting  
284 model ( $\text{logit } p = 1.814 + 2.014 \cdot 10^{-3} N_t - 1.056 \cdot 10^{-5} N_t^2$ ) suggests that the proportion of populations growing between  
285 consecutive weeks ( $p$ ) peaks at a population size of about  $N_t = 95$  individuals. This is the population size at which  
286 the derivative of the above equation equals zero. However, the predicted value of  $p$  for this "optimal" population  
287 size ( $p = 0.87$ ) is not different from that predicted for  $N_t = 0$ , *i.e.* the intercept:  $p = 0.86$  [0.77-0.92]. Thus, despite  
288 a second-order logistic regression best fitting the data, there is no evidence of an Allee effect. This also appears  
289 clearly in Fig 3.

290 Altogether, these results suggest an absence of Allee effect in our conditions. Rather, the decrease in population  
291 growth with increasing population size reflects negative density dependence.

## 292 **Reaching carrying capacity (Experiment 3)**

293 The eight experimental populations studied over a longer time period were well described by Gompertz, Logistic  
294 or Weibull population growth models (Table 1). Notwithstanding slightly different shapes, these three models are  
295 characterized by a plateau, the carrying capacity  $K$  (Fig 4). Although the estimated values of  $K$  varied between  
296 populations, they were of similar order of magnitude, between 455 and 593 (Table 1). This carrying capacity was  
297 reached after five to eight weeks.

298

## 299 **Consequences of cytoplasmic incompatibilities at carrying** 300 **capacity (Experiment 3)**

301 To test the effect of introducing CI into a recipient population of *D. sukukii*, we fitted a generalized linear model to  
302 population size, with a negative binomial distribution of errors and a log link function (Fig 5). The variance in

303 numbers within replicate was low ( $5.3 \cdot 10^{-12}$ ) so we added no random effect. Hypotheses H1 to H4 were tested *via*  
304 likelihood ratio tests for comparisons of models with and without parameters under scrutiny.

- 305 • H1: as expected from the design of the experiment, prior to the introductions of infected flies, the  
306 numbers of individuals varied at random around the carrying capacity ( $\beta_0 = 6.21$ ; test for parameter  $\beta_1$   
307  $= -0.02$ ;  $LR_{1,135} = 0.98$ ,  $p = 0.32$ ).
- 308 • H2: as expected, introducing infected flies had an immediate, positive influence on population size (test  
309 for parameter  $\beta_2 = 0.24$ ;  $LR_{1,135} = 6.13$ ,  $p = 0.01$ ).
- 310 • H3: following the immediate increase in numbers, the introduction of infected individuals had no  
311 general influence on the variation over time (test for parameter  $\beta_3 = -0.009$ ;  $LR_{1,135} = 0.18$ ,  $p = 0.67$ ).
- 312 • H4: most importantly, this absence of effect of introduction on population dynamics was consistent for  
313 the two strains of *Wolbachia*, *i.e.*, the interaction *time*  $\times$  *strain* after introduction was not significant  
314 (test for parameter  $\beta_4 = -0.02$ ;  $LR_{1,135} = 3.11$ ,  $p = 0.08$ ). A trend in the expected direction can be observed  
315 and could be responsible for this marginally significant test, but this trend seems to be partly driven by  
316 only few data points (populations 6 and 8; Fig 5).

317 Given the importance of this latter test and the low  $p$ -value, we complemented the parametric analysis with a chi-  
318 square test to compare the proportion of weekly observations where population sizes were below and above the  
319 carrying capacity. Before introduction, 53% (15/28) and 64% (18/28) observations were below  $K$  for control and CI  
320 populations respectively, and these proportions did not differ ( $\chi^2 = 0.30$ ,  $p = 0.59$ ). After introduction 38% (14/37)  
321 observations were below  $K$  for CI populations, a proportion that, contrary to theoretical predictions, was not  
322 higher than that observed for control populations (47%, 18/38;  $\chi^2 = 0.36$ ,  $p = 0.55$ ).

323 Altogether, these results suggest that introducing the incompatible *Wolbachia* strain *wTei* into a population of *D.*  
324 *suzukii* at carrying capacity produced an immediate and limited burst in population size but no consistent decrease  
325 in population size over time.

## 326 **Invasion dynamics of the incompatible strain (Experiment 3)**

327 After introducing flies infected by *wTei* at an initial frequency of 10% of the estimated population size, the  
328 prevalence of *wTei* did not change consistently over time ( $\chi^2 = 3.55$ ,  $df = 1$ ,  $p = 0.06$ ). The prevalence of *wTei*  
329 reached up to 25% in two populations, which probably produced the marginally significant test, but it was below  
330 the initial frequency introduced in the other two (Fig 6). For populations with weak evidence for invasion of *wTei*,  
331 weekly variations in the frequency of infected individuals were high, which could reflect random effects such as  
332 sampling variance, drift, or the combination of both processes.

## 333 Discussion

334 The endosymbiotic bacterium *Wolbachia pipientis*, widespread in insects, often causes cytoplasmic  
335 incompatibility, a reproductive failure that occurs when males and females do not have the same infection status  
336 [3]. This has led to novel and interesting ideas for insect pest management based on the introduction of an  
337 incompatible *Wolbachia* strain into a target population, with the assumption that the resulting incompatibilities  
338 will affect insect population dynamics. The reasoning is that if the incompatible strain is introduced at an initial  
339 frequency above its invasion threshold, it will invade the population, which should then lead to an increase in the  
340 proportion of incompatible crosses and, in turn, a decrease in the population growth rate. This basic idea has been  
341 formalized in the theoretical models of Dobson *et al.* [18] and Blackwood *et al.* [24] which predict that the  
342 introduction of one or more incompatible *Wolbachia* strains into a target population could lead to a transient  
343 decrease in population size [18]. Furthermore, assuming that a pre-existing Allee effect is enhanced by  
344 complementary tactics such as mating disruption, the unwanted population could be driven to extinction [24].  
345 Although these models are of great interest for their efforts to link *Wolbachia* invasion to insect population  
346 dynamics, their hypotheses and predictions have not been tested with appropriate experiments. Using *Drosophila*  
347 *suzukii* as a model organism, we present here a first step in this direction.

348 Our main result is that introducing the incompatible *Wolbachia* strain *wTei* into a recipient *Drosophila suzukii*  
349 population at carrying capacity does not result in an observable decrease in population size. This finding, with this  
350 specific *Wolbachia* strain and insect host, contradicts theoretical predictions [18,24], and moderates the



351 perspective on using cytoplasmic incompatibility as a lever for insect pest management. In the remainder of the  
352 discussion, we explore the various hypotheses that could explain this result.

353 The most trivial hypothesis to explain the lack of influence of *Wolbachia*-induced CI on population dynamics is that  
354 contrary to expectations, the introduced incompatible *Wolbachia* did not invade the recipient populations and  
355 therefore, did not produce enough incompatible crosses to produce an effect. This is a hypothesis that we cannot  
356 firmly reject. Although the prevalence of individuals infected with *wTei* reached up to 25% in some populations,  
357 weekly estimates revealed important variations, with frequencies that were sometimes lower than the 10% fixed  
358 at introduction. This result contrasts with the published data, showing that incompatible *Wolbachia* strains  
359 introduced above their invasion threshold readily invade recipient populations. In the mosquito *Aedes aegypti*,  
360 the introduction of 20% of *Wolbachia*-infected females (with strain *wAlbB*) into an experimental population of  
361 uninfected individuals resulted in the fixation of the introduced strain in seven generations [46]. In another study  
362 on the same species, 65% of *wMel*-infected mosquitoes were introduced into two populations of uninfected  
363 individuals, and again, the introduced strain became fixed 3 to 8 generations after introduction (30 to 80 days  
364 respectively [47]). In the fruit fly *Drosophila simulans*, caged populations were established with a mixture of  
365 uninfected individuals and either 35% of *wAu*-infected individuals or 4% of *wRi*-infected individuals. A significant  
366 increase in *wAu* and *wRi* frequencies was observed in all populations from the 10<sup>th</sup> generation onwards, except in  
367 two populations where *wRi* was either lost or at low frequency [48]. With a similar invasion dynamic, our 4-5  
368 generations experiment (seven to ten weeks) should have revealed the invasion of *wTei* more clearly than it did.  
369 In this respect, our results are surprising.

370 Invasion may have failed if the number of infected drosophila introduced was too low to trigger invasion. We  
371 showed in the methods section that the absence of infection costs ( $sf = 0$ ) suggests a theoretical invasion threshold  
372 close to 0% [9-13]. This means that in practice the introduction of very few individuals infected with *wTei* should  
373 result in the invasion of this strain. Under these conditions, the number of flies we introduced experimentally -  
374 between 76 and 100 individuals in populations that were an order of magnitude larger - should have been high  
375 enough to induce invasion.

376 The observed frequencies of *wTei* in the experimental populations with CI can alternatively be interpreted as a  
377 successful but slow invasion. In this context, the generational overlap inherent in our biological model could have

378 allowed older males, inducing lower CI, to participate in reproduction. Indeed, CI intensity decreases rapidly with  
379 male age in several *Drosophila* species [49–51]. In *D. suzukii*, CI intensity is reduced to 27% in mass crosses between  
380 *w*Suz-infected females and *w*Tei-infected males aged 9-10 days, compared to 53% when males are 2-3 days old  
381 [32,34]. If older males are as competitive for access to females as younger ones, the negative relationship between  
382 CI intensity and male age could reduce the demographic consequences of CI, particularly in species such as *D.*  
383 *suzukii*, where individuals can survive for over 58 days at 23-24°C [34].

384 A final hypothesis, not exclusive to the previous arguments, is that the influence of negative density dependence  
385 on host population dynamics overwhelms that of *Wolbachia*-induced CI. In our experiment, after an initial phase  
386 of exponential growth, the populations were well described by logistic-type models characterized by a carrying  
387 capacity estimated between 450 and 600 adults. This upper limit to population growth is probably a consequence  
388 of competition among larvae for limited food resources. Indeed, intraspecific competition certainly occurs in  
389 natural populations of *D. suzukii*. Our data suggest that the number of larvae developing in small fruits can be high  
390 (for instance, we could find up to 21 individuals at emergence from raspberry or blackberry exposed 48 h in the  
391 field; S1 Table). This suggests that larval intraspecific competition may be severe *in natura*, in particular when fruit  
392 abundance is weak, *i.e.*, at the beginning or at the end of the season. Laboratory experiments are consistent.  
393 According to Bezerra Da Silva *et al.* [36], the recurrent observation of *D. suzukii* pupae outside oviposition patches  
394 is a sign of high sensitivity to larval competition. The number of eggs and larvae developing in damaged fruits  
395 affects development time and further reduces adult size and fecundity [52,53]. In adults, increasing density results  
396 in females laying in fewer eggs laid per fruit, a behavior interpreted as an adaptation to alleviate competition  
397 among immature stages [54].

398 If larval survival depends on the intensity of competition within the fruit, a reduction in egg hatch rate resulting  
399 from *Wolbachia*-induced CI should, at high densities, alleviate competition and therefore, cause an immediate  
400 increase in larval survival. Reduced competition would offset incompatibility, and if this were true, CI would not  
401 cause the predicted decline in population size. Such a verbal argument echoes a specific section of the theoretical  
402 analysis of Dobson *et al.* ([18], Fig 2B), where the effect of density on immature survival is scaled by the parameter  
403  $\gamma$  [55]. Increasing the influence of density by increasing  $\gamma$  made the effects of introducing incompatible strains  
404 disappear [18]. Other modelling studies have highlighted similar influence of competition among hosts on

405 *Wolbachia* invasion [15–17]. More generally, the strength of competition may alter the benefits of gene drive [56]  
406 and of the sterile insect technique [57] with undesirable consequences such as increasing (rather than decreasing)  
407 the size of pest populations.

408 The extent to which negative density dependent survival may jeopardize attempts to reduce pest reproduction  
409 appears to be a key issue. However, empirical evidence for the critical role of density dependence in *Wolbachia*  
410 invasion and consequences for host population dynamics is completely lacking. In *D. sukukii*, we suggest that  
411 negative density dependence may explain the discrepancy between prior expectations and observed population  
412 dynamics. However, further species-specific models and experiments are needed to better understand the  
413 interplay between density dependence, the invasion of incompatible *Wolbachia* strains and the resulting  
414 population dynamics. Follow-up experiments could manipulate intraspecific competition to understand how  
415 competition interacts with CI. If there is an antagonistic interaction between the two processes, the population  
416 consequences of CI should only appear when flies are released before the populations reach their carrying  
417 capacity.

418 Positive density dependence, *i.e.*, the Allee effect, is also being proposed as an important process in pest  
419 management. The basic reasoning is that eradication can be achieved *via* a combination of treatments that reduce  
420 population size (pesticides, predators, etc.) and tactics that enhance a pre-existing Allee effect (for instance,  
421 mating disruption). Theoretical models, verbal arguments and reviews are numerous [19,20,58,59] and, for insect  
422 pests, well supported by widespread evidence of Allee effects [21,60]. A keystone example is that of the gypsy  
423 moth *Lymantria dispar*, whose spread is slowed by a combination of insecticides, sterile insect technique and  
424 mating disruption [61]. From this perspective, an important finding of our study is the absence of a demographic  
425 Allee effect in *D. sukukii*. Despite founding populations with the smallest possible number of individuals (one male  
426 and one female) we observed no positive relationship between population size and growth rate, and no extinction.  
427 This finding contrasts with observations of Allee effects in closely related species such as *D. melanogaster* [37,62].  
428 In our experimental conditions with population cages, relatively high densities may have prevented an Allee effect.  
429 Alternatively, a component Allee effect could have occurred at the individual level, but was offset by negative  
430 density dependence and thus did not translate to the population level.

## 431 Conclusion

432 In conclusion, many of our results are puzzling with respect to prior expectations. The absence of a transient  
433 decrease in *D. sukukii* population size after the introduction of the incompatible *Wolbachia* strain *wTei* is most  
434 easily explained by the slow invasion of this introduced strain, which, in itself, lacks clear explanations.  
435 Nevertheless, there were some incompatible crosses in populations where the proportion of *wTei* was sometimes  
436 higher than 20%, but these did not influence the host population dynamics. We therefore conclude that the strong  
437 density dependent competition could be a key driver of insect population dynamics, counterbalancing the  
438 predicted effect of cytoplasmic incompatibility, and possibly hampering pest management programs based on  
439 reproductive failure. *Wolbachia*-based methods, just like any other methods that reduce pest realized fecundity,  
440 should be implemented when populations are not under an overwhelming competition, for instance, at the  
441 beginning of the season.

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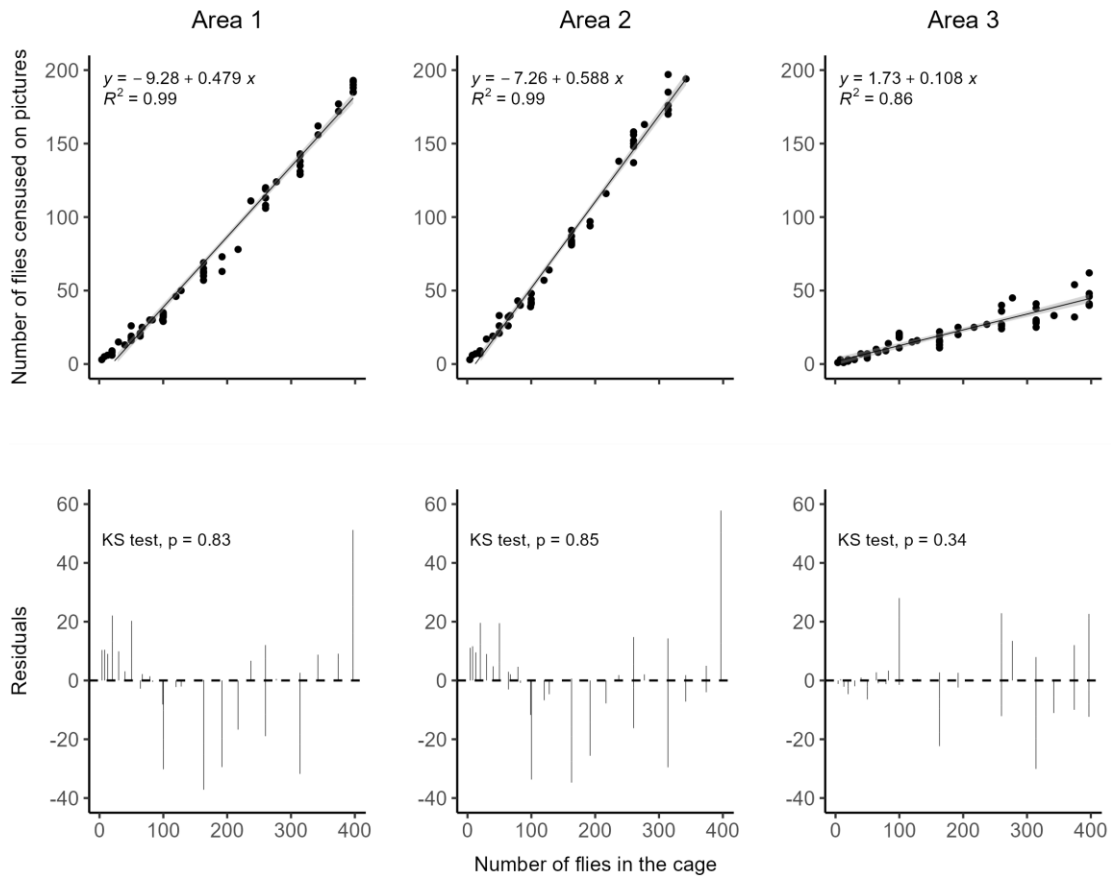
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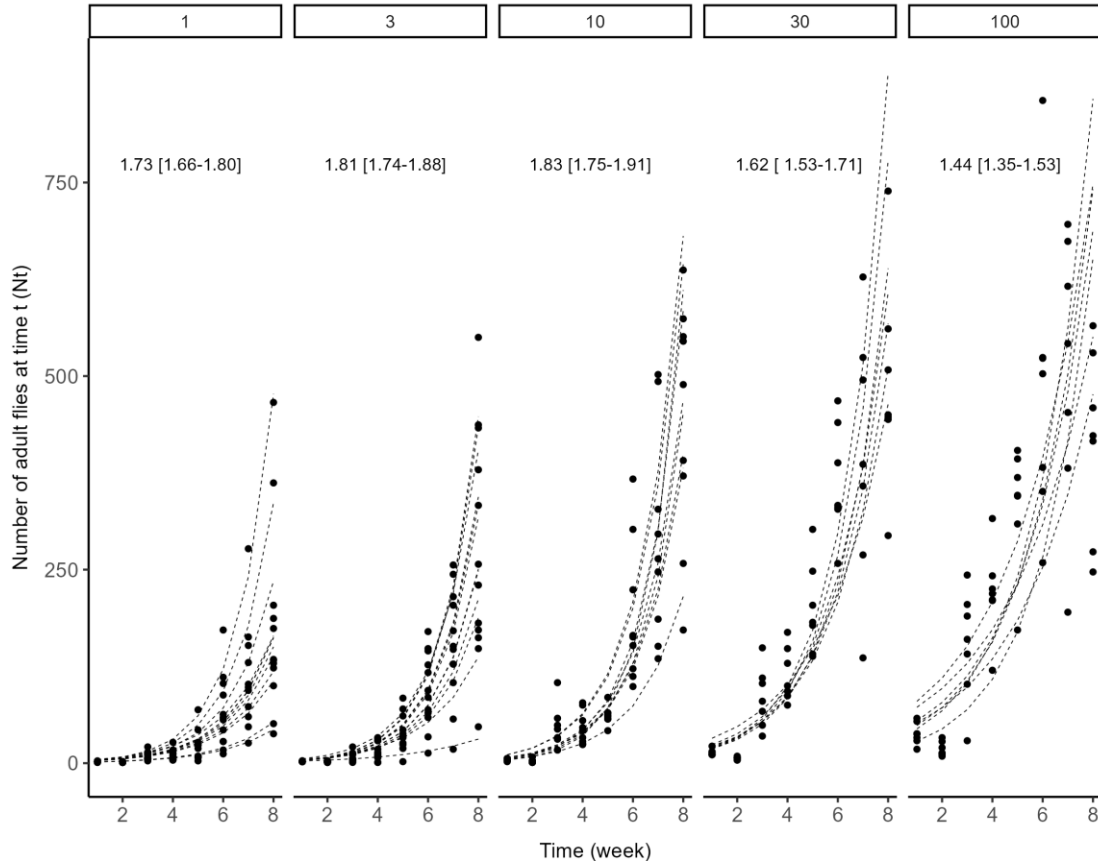


583

584 **Fig 1. Estimation of population size.**

585 Linear regressions between the number of adult *D. sukuzii* censused on pictures of three different areas of the  
586 experimental cages and the total number of individuals in the cage.

587



588

589

590

**Fig 2. Early dynamics of small *D. sukuzii* populations initiated with 1, 3, 10, 30 or 100 male-female pairs.**

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For each replicated population, the eight-week time series of observed number of individuals (circles) was fitted

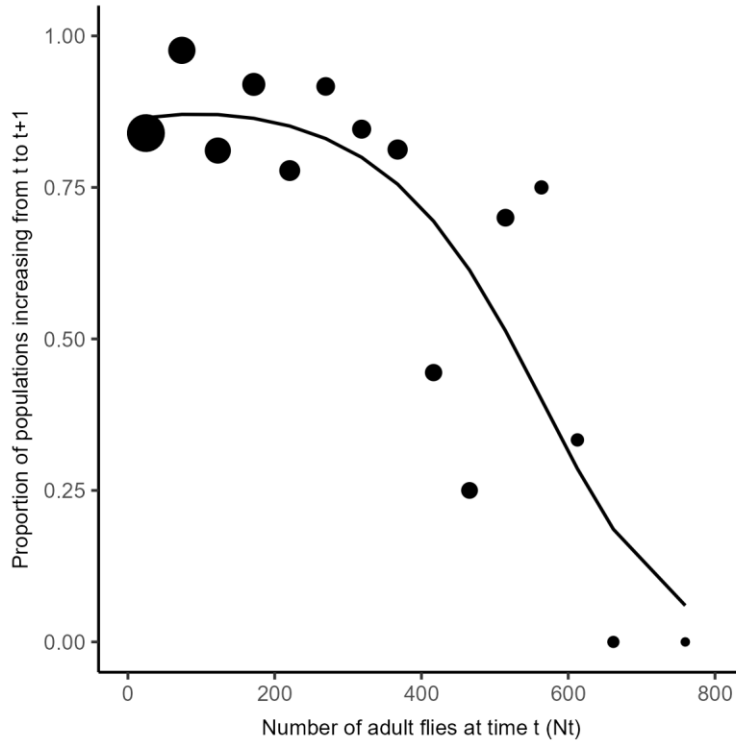
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with an exponential growth model  $N_t = N_0 e^{rt}$  (lines). Estimated marginal means of population growth rate  $r$  and

593

their 95% confidence intervals are shown.

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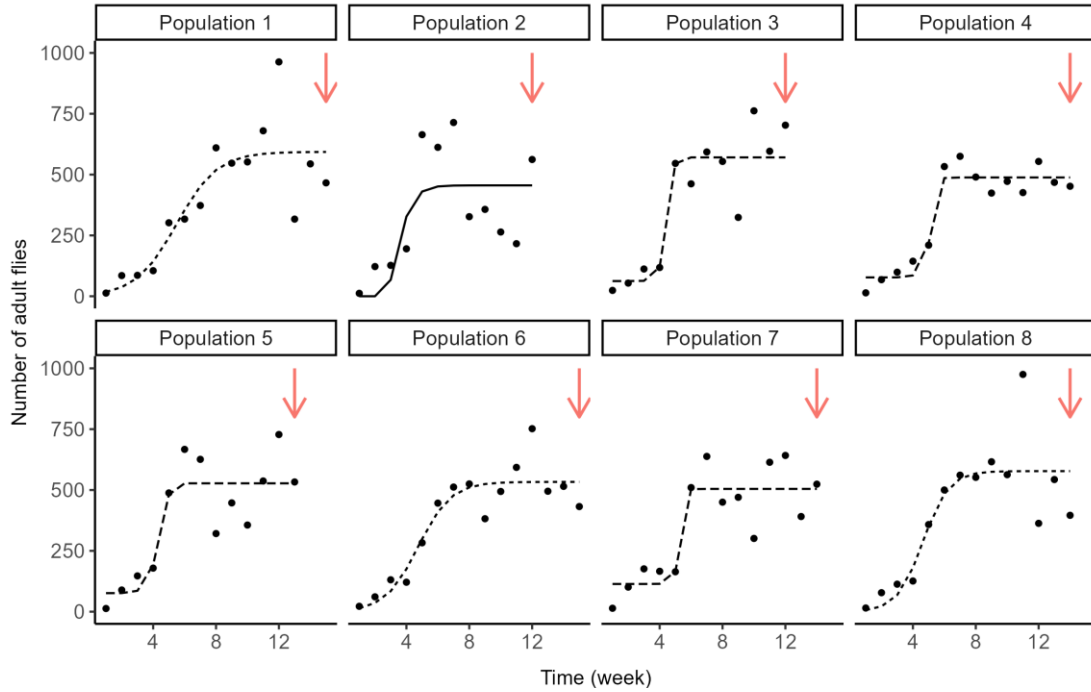


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**Fig 3. Proportion of the populations increasing ( $t$  to  $t + 1$ ).**

598 For each range of population size at time  $t$  ( $N_t$ : 0-49, 50-99, etc.) circles represent the proportion  $p$  of pairs for  
599 which the number of individuals increased ( $N_{t+1} \geq N_t$ ). Circle size represent the number of pairs available to  
600 estimate each proportion (largest circle:  $x$  pairs; smallest circle:  $y$  pairs). The curve represents the second-order  
601 polynomial regression that best fitted the data ( $\text{logit } p = 1.814 + 2.014 \cdot 10^{-3} N_t - 1.056 \cdot 10^{-5} N_t^2$ ).

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**Fig 4. Reaching the carrying capacity.**

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Observed and predicted time variations of *D. sukuii* population size for eight replicated populations before the

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introduction of *Wolbachia*-infected individuals. Line types represent the logistic population growth model that

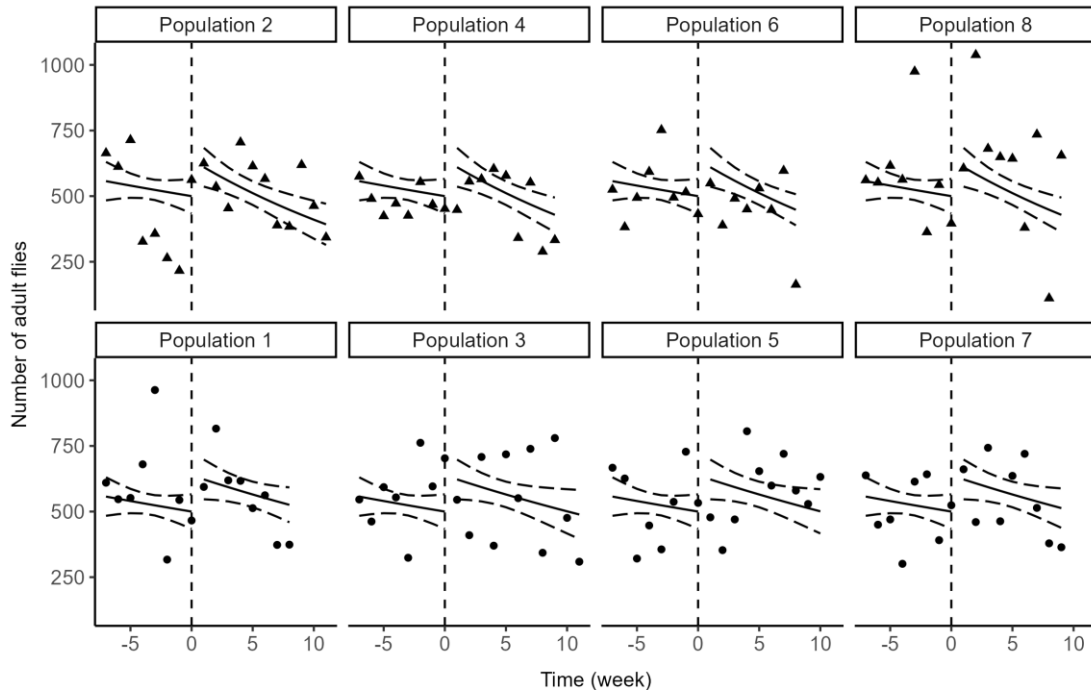
608

best fitted the data (dashed = Weibull, solid = Gompertz, dotted = logistic). Red arrows indicate the time at which

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infected flies were introduced, *i.e.*, seven weeks after the first observed decrease in population size.

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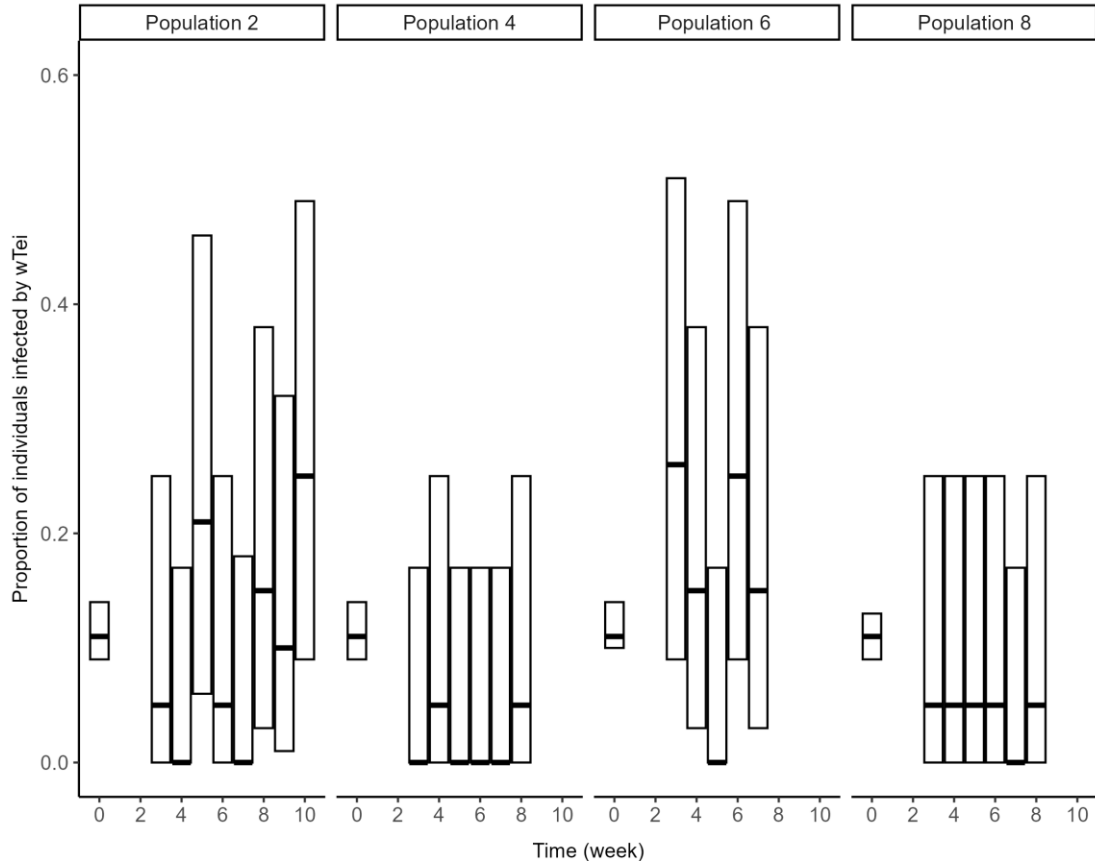
**Fig 5. Impacts of introduced CI-inducing flies.**

614 Observed and predicted number of adult *D. sukikii* in populations with CI (triangles; introduction of individuals

615 infected by *wTei*) and without CI (circle; introduction of individuals infected by *wSuz*). Time is scaled so that

616 introductions occur at  $t = 0$ .

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**Fig 6. Dynamics of *Wolbachia* infection.**

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Evolution of the proportion of *D. sukuzii* infected by the introduced *Wolbachia* strain *wTei*. Each proportion is

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presented with its theoretical 95% confidence interval, based on the binomial distribution. The first bar at  $t = 0$

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corresponds to the proportion of *wTei*-infected flies actually introduced (10%). Subsequent prevalence was

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estimated from the third week post-introduction, after emergence of offspring from the introduced individuals.

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628 **Table 1. Gompertz, logistic and Weibull population growth models fitted to each of the eight experimental**  
 629 **population and values of Akaike Information Criterion (AIC), Root-Mean-Square Error (RMSE) and carrying**  
 630 **capacity  $K$ .**

Population	Model	AIC	RMSE	K estimate
1	Gompertz	197.9	135.7	597.2 (452.6,741.8)
	<b>Logistic</b>	<b>197.3</b>	<b>133.3</b>	<b>593.5 (466.3,720.6)</b>
	Weibull	–	–	–
2	<b>Gompertz</b>	<b>164.2</b>	<b>162.1</b>	<b>455.7 (317.1,594.3)</b>
	Logistic	–	–	–
	Weibull	–	–	–
3	Gompertz	–	–	–
	Logistic	154.7	109.4	572.7 (478.5,666.9)
	<b>Weibull</b>	<b>155.7</b>	<b>104.9</b>	<b>570.7 (475.5,665.9)</b>
4	Gompertz	165.3	66.6	489.0 (433.7,544.3)
	Logistic	–	–	–
	<b>Weibull</b>	<b>158.1</b>	<b>47.9</b>	<b>488.2 (449.6,526.8)</b>
5	Gompertz	–	–	–
	Logistic	–	–	–
	<b>Weibull</b>	<b>169.0</b>	<b>109.6</b>	<b>527.4 (436.2,618.7)</b>
6	Gompertz	182.6	81.5	543.3 (464.9,621.7)
	<b>Logistic</b>	<b>181.7</b>	<b>79.2</b>	<b>533.4 (467.4,599.4)</b>
	Weibull	183.4	78..2	523.8 (462.0,585.5)
7	Gompertz	179.5	110.6	520.0 (400.2,639.9)
	Logistic	178.6	106.9	510.4 (415.5,605.3)
	<b>Weibull</b>	<b>177.0</b>	<b>94.2</b>	<b>504.5(427.3,581.7)</b>
8	Gompertz	185.2	135.7	579.3 (459.6,699.0)
	<b>Logistic</b>	<b>184.7</b>	<b>133.4</b>	<b>577.6 (464.9,690.3)</b>
	Weibull	–	–	–

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632  $K$  (referred as *Asym* in the models) is the parameter common to all three models and that corresponds to the  
 633 asymptotic value of the population size when time tends to  $\infty$ , or the carrying capacity. Values of  $K$  and 95%  
 634 confidence intervals were estimated. Models in bold are those that best fit the observed data (lowest AIC and  
 635 RMSE).