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

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

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13

## 14 **Abstract**

15 *Wolbachia pipientis* is an intracellular bacterium that infects many insects and other arthropods.  
16 Cytoplasmic incompatibility (CI) is one of several effects that *Wolbachia* has on its hosts. In its simplest  
17 form, CI causes host reproductive failure when an uninfected female mates with an infected male.  
18 Theoretically, this has two consequences. First, when an incompatible *Wolbachia* strain is introduced  
19 into a recipient host population, uninfected females are selected against, triggering *Wolbachia*  
20 invasion. Second, reproductive failures during *Wolbachia* invasion lead to a transient decrease in  
21 population growth rate. Modelling studies combining *Wolbachia* invasion and host population  
22 dynamics show that these two processes could interact to cause population decline and even  
23 extinction. However, these effects could be sensitive to density dependence, with competition  
24 reducing the demographic impact of CI and Allee effect increasing the probability of extinction. We  
25 tested these predictions with laboratory experiments in the fruit fly *Drosophila suzukii* and the  
26 exogenous *Wolbachia* strain *wTei*. Surprisingly, the introduction of *wTei* into *D. suzukii* populations at  
27 carrying capacity did not result in the expected invasion of *wTei* and transient population decline. In  
28 parallel, we found strong negative density dependence, but no Allee effect. From these results, we  
29 propose that negative density dependence is an important but underappreciated driver of the eco-  
30 evolutionary feedback underlying *Wolbachia* invasion and insect population dynamics. If future  
31 models and data support this hypothesis, pest management strategies using *Wolbachia*-induced CI  
32 should target early-season populations with negligible competition but a possible Allee effect.

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

## 35 **Introduction**

36 *Wolbachia pipientis* is an endosymbiotic bacterium found in a large number of nematode and  
37 arthropod species, including insects [1,2]. The overall record of interactions between *Wolbachia* and

38 their hosts is highly variable, ranging from parasitism to mutualism. The ability of *Wolbachia* to  
39 manipulate the reproduction of their hosts at the advantage of its own transmission is the most  
40 widespread facet of these interactions [3]. Cytoplasmic incompatibility (CI) is one such manipulation.  
41 CI is the consequence of a sperm modification that occurs during spermatogenesis in the infected  
42 male. When an infected male mates with an infected female, the modification is restored and the cross  
43 produces offspring. If the female is uninfected, the modification is not restored which, in diploid  
44 species, causes a malfunction during the first mitotic divisions, resulting in embryo death [4]. Hence,  
45 infected females have more offspring on average than uninfected females because they can produce  
46 offspring with all types of males, be they infected or not. Since *Wolbachia* is transmitted maternally  
47 and vertically, these females are more likely to transmit the incompatible strain, thus promoting its  
48 spread [5].

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

59 In order to optimize the success of such *Wolbachia* replacement programs, it is necessary to identify  
60 the factors that influence *Wolbachia* invasion dynamics. Historically, most theoretical models of CI  
61 dynamics are analogous to population genetic models and have focused on the evolution of the  
62 frequencies of an incompatible strain within the host population, neglecting the specificities of host  
63 population dynamics [9-13]. Only a limited number of host demographic parameters directly affecting  
64 changes in frequencies were considered, and assumed to be constant [8,13,14]. These models predict  
65 the existence of a critical infection frequency, referred to as the invasion threshold, above which an

66 incompatible strain will deterministically invade the host population. The threshold depends on three  
67 parameters: the intensity of infection-induced CI, the success of maternal transmission and the costs  
68 resulting from infection, such as reduced fertility. More recently, Hancock *et al.* [15–17] added density  
69 dependence on host demographic parameters in order to study *Wolbachia* invasion dynamics with  
70 more realistic assumptions. They concluded from different models that the intensity of competition,  
71 by varying the reproductive success of individuals, significantly reduces the ability of *Wolbachia* to  
72 invade a population [16,17].

73 The ability of *Wolbachia* to invade may serve another pest management strategy, alternative to the  
74 drive of interesting characters. In a landmark publication, Dobson *et al.* [18] have proposed a novel CI-  
75 based insect pest suppression strategy based on *Wolbachia* invasion. They extended classical CI  
76 models by assuming realistic host population dynamics with density dependent survival of immature  
77 stages and parameter values taken from the insect literature. They simulated the introduction of an  
78 incompatible *Wolbachia* strain into a host population that had reached carrying capacity. While the  
79 incompatible strain spreads through the population, the frequency of incompatible crosses increased,  
80 resulting in a transient reduction in host population size. Population size declined to a minimum when  
81 the proportions of infected and uninfected individuals were similar, and then increased again when  
82 the incompatible strain became the majority. One of their predictions is that the minimum population  
83 size reached during invasion is influenced not only by *Wolbachia*'s own invasion parameters, but also  
84 by demographic parameters intrinsic to host populations. For instance, under conditions of high host  
85 reproductive rate and intraspecific competition, the demographic consequences of CI may go  
86 unnoticed.

87 The transient population decline caused by *Wolbachia*-induced cytoplasmic incompatibility, as  
88 predicted by Dobson *et al.* [18], is a form of demographic destabilization that, in conjunction with other  
89 processes, could cascade towards population extinction. As other process, the Allee effect is an  
90 excellent candidate. It is characterized by population growth rate decreasing with the decrease of the  
91 population size, which triggers an extinction vortex if the growth rate becomes negative below a  
92 critical number of individuals (the Allee threshold) or if the growth rate remains positive but combines  
93 with stochasticity. The Allee effect has thus been envisioned as a catalyst for extinction upon which

94 novel tactics could be developed to eradicate pest populations [19-23]. Building on these ideas,  
95 Blackwood *et al.* [24] assumed an Allee effect to investigate the conditions under which CI expression  
96 would serve pest eradication. To this end, they simulated *Wolbachia* introductions into populations  
97 affected by a more or less intense Allee effect. When the Allee effect was strong and the population  
98 reproduction rate low, the model predicted that the minimum population size reached during  
99 *Wolbachia* invasion would fall below the Allee threshold, leading to population extinction. On the  
100 other hand, at high reproduction rates, the transient reduction induced by CI was not large enough to  
101 bring the population size below the Allee threshold.

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

108 There are several reasons for choosing *Drosophila suzukii* as a model organism. First, *D. suzukii* is a  
109 major invasive pest of berry and stone fruit crops, including cherries, blueberries, raspberries,  
110 blackberries, peaches, nectarines, apricots, grapes, etc... [25]. Native from Southeast Asia, *D. suzukii*  
111 was first reported in the USA and Europe in the late 2000s and continues to spread worldwide (e.g.  
112 Africa; [26-28]). Unlike other *Drosophila* species, the serrated ovipositor of *D. suzukii* allows females  
113 to lay eggs under the skin of ripening or mature fruits, causing significant damage to cultivated fruits  
114 and consequently, huge economic losses [25]. This calls for research to develop environmentally  
115 friendly methods to control this major agricultural pest. Second, *D. suzukii* is naturally infected with  
116 *Wolbachia*. Contrary to the common observation of "most-or-few" infection frequencies, the  
117 prevalence of the resident *wSuz* strain in European populations is highly variable, averaging 46% [29].  
118 This infection polymorphism could be a consequence to the fact that *wSuz* induced no or weak CI  
119 [29,30] as well as the variable benefits and costs of *wSuz* protection from viruses [31]. Third, we  
120 capitalized on a strong body of experimental work based on the transinfection of *D. suzukii* with various  
121 *Wolbachia* strains, including *wHa* and *wTei*, which produce cytoplasmic incompatibility [32-34]. Finally,

122 the expression of negative density dependence on individual performance has already been observed  
123 in *D. sukuzii* [35]. Under experimental conditions with high larval densities, some individuals were  
124 observed to leave the breeding site, which was interpreted as a response to intraspecific competition  
125 for resources [36]. While the Allee effect has not yet been demonstrated in *D. sukuzii*, it has been  
126 documented in other closely related *Drosophila* species [37].

127 The aim of this study is to estimate the strength of positive and negative density dependence in *D.*  
128 *sukuzii* and to test the effect of *Wolbachia*-induced CI on the dynamics of experimental populations.  
129 In this article, we report the absence of an Allee effect under our laboratory conditions. In contrast, we  
130 found strong negative density dependence resulting in logistic population growth. Finally, we found  
131 no effect of introducing an incompatible *Wolbachia* strain on *D. sukuzii* population dynamics. We  
132 discuss these results in the light of the models developed by Dobson *et al.* [18] and Blackwood *et al.*  
133 [24] and hypothesize that negative density dependence may have much stronger influences than  
134 expected on *Wolbachia* invasion and the demographic consequences of cytoplasmic  
135 incompatibilities.

## 136 **Materials and methods**

### 137 **Insect rearing**

138 Two lines of *D. sukuzii* have been maintained in the laboratory since 2018, *i.e.*, for around 24  
139 generations before experiments. These lines were infected with either *wSuz*, the strain naturally  
140 present in *D. sukuzii* [29,30,38] or the exogenous *Wolbachia* strain *wTei*, the natural symbiont of *D.*  
141 *teissieri*. The latter was selected because of the incompatibility induced by transinfected *D. sukuzii*  
142 males when crossed with *wSuz*-infected females[32,34]. The three lines were reared on cornmeal-  
143 based food medium containing, per 1 liter of water: 10 g agar, 80 g corn flour, 80 g brewer's yeast  
144 and 60 ml moldex (100 g methyl hydroxybenzoate in 1L 70% ethanol). Rearing, as well as all

145 experiments, occurred in a climatic chamber at 23-24°C, 50-60% humidity and a photoperiod of 12h-  
146 12h.

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

148 In order to estimate accurately the number of flies in population cages, we developed a rapid method  
149 based on insect counts on photographed cages. Prior to experiments, we assessed the relevance of  
150 this method. For this, between 4 and 396 individuals were introduced into 59 cubic experimental  
151 cages (21 cm x 21 cm x 21 cm) made of fine-mesh fabric and a transparent plastic wall. The cages were  
152 then photographed using a Nikon N750D camera and a Nikon lens (AF-S Micro Nikkor 60mm f/2.8G  
153 ED). Visible Individuals were counted on a computer using the software ImageJ 1.52 [39] on three  
154 predefined areas (S1 Supporting information) : (i) individuals appearing only on the back wall (area  
155 n°1), (ii) individuals appearing on the whole surface except the left side and the cage floor (area n°2),  
156 or (iii) only individuals appearing on the front wall (area n°3) . Areas n°1 and n°2 were respectively  
157 used for the experiments 2 and 3. Area n°3 was not used in the present study, but it will be used in  
158 follow-up experiments. For each area, linear regressions between the numbers of individuals on  
159 pictures and the total numbers in the cage were fitted, and the coefficients of determination  $R^2$   
160 estimated. Kolmogorov-Smirnoff tests, from the DHARMA package in R, were used to test normality of  
161 the residual distribution. These and the following statistical analyses were carried out using R 4.2.2  
162 software (core team 2022).

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

### 164 **Founding populations**

165 This experiment aimed at quantifying a demographic Allee effect, i.e., positive density dependence at  
166 low population size. To this end, we founded *D. sukikii* populations with a range of initial number of  
167 individuals and estimated subsequent growth rates. Fifty-two populations were initiated with pairs of  
168 <24 hour-old virgin individuals. We manipulated the number of pairs: 1 ( $N=13$ ,  $N$  being the number



169 of replicated populations), 3 ( $N=13$ ), 10 ( $N=10$ ), 30 ( $N=8$ ) and 100 ( $N=8$ ). For each population, the  
170 pairs were introduced into a cage as described above. Replicates were distributed across four blocks  
171 spaced one week apart. Weekly, flies were provided with a new oviposition patch consisting of 100 ml  
172 (75 mm diameter) rearing medium supplemented with a fresh raspberry. As the generation time was  
173 around two weeks in our rearing conditions, each patch was exposed three weeks to ascertain that  
174 eggs laid in the patch yielded new adults emerging into the cages. Immature drosophila developed  
175 by feeding on the rearing medium whereas adult flies were supplied with water and honey three times  
176 a week. To estimate population size, each cage was photographed once a week. Populations were  
177 followed nine weeks post foundation; generations were completely overlapping, but if they were  
178 discrete, nine weeks would represent 4 to 5 generations of *D. suzukii*.

## 179 **Fitting an exponential growth model**

180 The early dynamics of populations founded with a small number of individuals is expected to depend  
181 on initial population size and the strength of an Allee effect. In the absence of an Allee effect,  
182 exponential population growth is expected. With a weak Allee effect, exponential growth is also  
183 expected, but the growth rate should be lower for initially small populations (e.g., 1 pair, 3 pairs, etc.)  
184 than for larger ones (e.g., 100 pairs). With a strong Allee effect, populations of initial size smaller than  
185 the Allee threshold should go extinct whereas populations initially above the Allee threshold should  
186 increase exponentially, with a growth rate again depending on population size. We tested these  
187 predictions by counting populations that went extinct and estimating the growth rate of those that  
188 persisted. The growth rate ( $r$ ) was estimated by fitting a simple exponential growth model such as  
189  $N_t = N_0 \exp(r t)$  to each population time series, excluding the last week at which the largest  
190 populations had stopped increasing. Also, due to missing values, 5 populations out of the 52 founded  
191 were discarded. In practice, we fitted weekly counts with a generalized linear model based on a  
192 Poisson distribution and a log link function, i.e.,  $\log(N_t) = \log(N_0) + r t$ . The initial number of  
193 individuals observed just after the populations had been founded was defined as an offset ( $\log N_0$ ),  
194 that is, a parameter included in the model but not estimated. Growth curves and estimated growth

195 rates  $r$  were obtained for each population. A linear regression was then used to analyze the effect of  
196 the number of founders on growth rate.

## 197 **Testing density dependence**

198 A complementary method to test for positive density dependence is to assess the relation between  
199 the proportion  $p$  of populations growing between  $t$  and  $t+1$  and the population size at time  $t$ . Analyzing  
200  $p$  rather than individual counts circumvents the high sampling variance at low numbers and the  
201 consequent spurious estimations of growth rates [40]. With an Allee effect, we expect  $p$  to increase  
202 with population size at low population size, with  $p=0.5$  being the Allee threshold. Independently of  
203 the experimental manipulation of initial population size, we associated all possible pairs of  $N_t$  and  $N_{t+1}$   
204 for each of the 48 populations at each weekly interval. A population was considered stable or growing  
205 for  $N_{t+1} \geq N_t$  (further referred to as a growing population given the low number of pairs, 14 of 410 pairs,  
206 with  $N_{t+1} = N_t$ ) or decreasing otherwise. We then calculated the proportion  $p$  of growing populations  
207 within 18 classes of population sizes (1-49, 50-99, ..., 850-899). This proportion was fitted with a logistic  
208 regression to test three alternative hypotheses: a null model suggesting no relation between  
209 population size and growth ( $p = \beta_0$ ); a standard model suggesting a linear relation ( $p = \beta_0 + \beta_1 N_t$ ); a  
210 polynomial model allowing a non-linear relationship ( $p = \beta_0 + \beta_1 N_t + \beta_2 N_t^2$ ). The third model is well  
211 suited to describe bell-shaped curves that characterize populations with both an Allee effect and  
212 negative density dependence. The AIC was used to select the best model.

## 213 **Experiment 3. *Wolbachia* invasion and consequent** 214 **population dynamics**

### 215 **Experimental populations**

216 The aim of this experiment was to assess changes in *D. sukukii* population dynamics caused by the  
217 introduction of individuals infected with an incompatible *Wolbachia* strain. To this end, we founded

218 eight populations with 100 *wSuz*-infected individuals (sex ratio 1:1) aged 3 to 5 days. Each population  
219 was studied continuously across three distinct periods, for a total of 23 weeks: a first period of  
220 population growth (5 to 8 week); a seven-week period of dynamic equilibrium around the carrying  
221 capacity, starting upon observation of the first decrease in number; a post-introduction period (8 to  
222 11 weeks) starting upon the introduction of infected flies. Populations were maintained as in the  
223 previous experiment, with a new reproduction patch provided weekly and exposed during three  
224 weeks, and water and honey to feed adults three times a week. Cages were again photographed every  
225 week for estimation of population sizes. The experiment could have last longer but was terminated  
226 due to the covid crisis.

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

228 Individuals introduced after the seven-week period of dynamic equilibrium were infected by either  
229 *wSuz* for the four "Control" populations or *wTei* for the four "IC" populations. The number introduced  
230 was equal to 10% of the mean number of individuals across the period of equilibrium. Before  
231 introduction, we verified the fixation of *wSuz* for all populations by genotyping 20 individuals per  
232 population (methods below). After introduction, we monitored the evolution of *wTei* frequencies over  
233 time in the four CI populations. For this, we randomly sampled 20 individuals per week and stored  
234 them in 99% ethanol at -20°C until analysis. Individual infection status was assessed via HRM (High  
235 Resolution Melting; [41], a genotyping method that allows the detection of changes in the melting  
236 profiles of double-stranded DNA. For each individual, DNA was extracted using Macherey Nagel's  
237 NucleoSpin kit. *Wolbachia*-specific primers 81f (5'- TGGTCCAATAAGTGATGAAGAAAC-3'; Braig *et al*,  
238 1998) and 2R (5'- CAGCAATTTTCAGGATTAG -3'; [41]) were used to amplify the *wsp* ("*Wolbachia*  
239 Surface Protein") gene. PCRs were performed in a 10 µL volume containing 500nM primers, 1X  
240 Precision Melt Supermix (Biorad), containing dNTPs, iTaq™ DNA polymerase, MgCl<sub>2</sub>, EvaGreen  
241 buffer, stabilizers, and 2 µL of 10-fold diluted DNA. Cycle conditions were 95°C for 2 min, then 30 sec  
242 at 95°C, 30 sec at 54°C and 30 sec at 72°C for 40 cycles, followed by 30 sec at 95°C and 1 min at 60°C  
243 (Biorad CFX96).

## 244 **Estimating carrying capacity**

245 The eight populations were studied over enough time to estimate the carrying capacity  $K$  in our  
246 environmental conditions. For this, we fitted non-linear regressions to the number of adults observed  
247 each week, from foundation to the introduction of infected flies (first and second time periods). Three  
248 candidate population growth models were fitted: Gompertz, logistic, and Weibull. SSlogis,  
249 SSgompertz and SSweibull functions in R were used to obtain the initial parameter values for each  
250 candidate model (so-called "selfStart" models). All these models share one parameter, the asymptotic  
251 population size defined as the carrying capacity. The AIC and root-mean-square error (RMSE) were  
252 then used to select the best of the three models fitted to each population.

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

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

$$263 \quad N_{ijt} = \beta_0 + \beta_1 \cdot t + \beta_2 \cdot intro + \beta_3 \cdot t \cdot intro + \beta_4 \cdot t \cdot intro \cdot strain + \epsilon_{ijt}$$

264 where  $N_{ijt}$  is the number of individuals in populations of introduction status  $i$  (before vs after  
265 introduction; variable *intro* in the model) having undergone treatment  $j$  (introduction of wTei or wSuz;  
266 variable *strain* in the model) at time  $t$  relative to the introduction (rescaled so that  $t=0$  upon  
267 introduction). This model allowed to test four predictions (Fig 1).

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

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

## 277 Results

### 278 Estimation of population size (experiment 1)

279 In order to estimate population size, we counted adult flies on pictures of three different areas of the  
280 experimental cages. Whatever the area considered, we found a clear relationship between the number  
281 counted on pictures and the total number of flies in the cages (Fig 2). Linear regressions were  
282 characterized by high coefficients of determination:  $R^2=0.99$  for the first two areas and  $R^2=0.86$  for the  
283 last area. Kolmogorov-Smirnov tests confirmed the normal distribution of errors, and hence, an  
284 absence of sampling bias (Fig 2; KS test:  $p=0.83$ ,  $0.85$ , and  $0.34$  for areas 1, 2 and 3 respectively).

### 285 Early dynamics of small populations (experiment 2)

286 None of the newly founded populations went extinct, even introducing a single pair. Population  
287 growth during the first eight weeks was well described by an exponential model, regardless of the  
288 number of founding pairs (Fig 3). The estimated growth rate  $r$  was influenced by initial population size

289 (Anova,  $F_{4,42} = 14.6$ ,  $p = 1.47 \cdot 10^{-7}$ ). Contrary to expectation with an Allee effect, the growth rate  $r$  was  
290 highest at smallest population sizes, from  $1.73 \pm 0.03$  for single pairs to  $1.4 \pm 0.04$  for 100 pairs.

291 The detailed analysis of week-to-week variations in population sizes confirmed the absence of Allee  
292 effect. Among the three alternative models, the second order polynomial logistic regression best fitted  
293 the proportion of increasing populations (AIC=61.3, versus 82.9 for the null model and 63.5 for linear  
294 regression). The resulting model ( $\text{logit } p = 1.814 + 2.014 \cdot 10^{-3} N_t - 1.056 \cdot 10^{-5} N_t^2$ ) suggests that the  
295 proportion of populations growing between consecutive weeks ( $p$ ) peaks at a population size of about  
296  $N_t=95$  individuals. This is the population size at which the derivative of the above equation equals zero.  
297 However, the predicted value of  $p$  for this "optimal" population size ( $p=0.87$ ) is not different from that  
298 predicted for  $N_t=0$ , i.e. the intercept:  $p=0.86$  [0.77; 0.92]. Thus, despite a second-order logistic  
299 regression best fitting the data, there is no evidence of an Allee effect. This also appears clearly in Fig  
300 4.

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

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

304 The eight experimental populations studied over a longer time period were well described by  
305 Gompertz, Logistic or Weibull population growth models (Table 1). Notwithstanding slightly different  
306 shapes, these three models are characterized by a plateau, the carrying capacity,  $K$  (Fig 5). Although  
307 the estimated values of  $K$  varied between populations, they were of similar order of magnitude,  
308 between 455 and 593 (Table 1). This carrying capacity was reached after five to eight weeks.

### 309 **Consequences of cytoplasmic incompatibilities at** 310 **carrying capacity (Experiment 3)**

311 To test the effect of introducing CI into a recipient population of *D. suzukii*, we fitted a generalized  
312 linear model to population size, with a negative binomial distribution of errors and a log link function  
313 (Fig 6). The variance in numbers within replicate was low ( $5.3 \times 10^{-12}$ ) so we added no random effect.  
314 Hypotheses H1 to H4 were tested via likelihood ratio tests for comparisons of models with and without  
315 parameters under scrutiny.

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

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

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

## 339 **Invasion dynamics of the incompatible strain**

### 340 **(Experiment 3)**

341 After introducing flies infected by *wTei* at an initial frequency of 10% of the estimated population size,  
342 the prevalence of *wTei* did not change consistently over time ( $\chi^2=3.5473$ ,  $df=1$ ,  $p=0.059$ ). The  
343 prevalence of *wTei* reached up to 25% in two populations, which probably produced the marginally  
344 significant test, but it was below the initial frequency introduced in the other two (Fig 7). For  
345 populations with weak evidence for invasion of *wTei*, weekly variations in the frequency of infected  
346 individuals were high, which could reflect random effects such as sampling variance, drift, or the  
347 combination of both processes.

## 348 **Discussion**

349 The endosymbiotic bacterium *Wolbachia pipientis*, widespread in insects, often causes cytoplasmic  
350 incompatibility, a reproductive failure that occurs when males and females do not have the same  
351 infection status. This has led to novel and interesting ideas for insect pest management based on the  
352 introduction of an incompatible *Wolbachia* strain into a target population, with the assumption that  
353 the resulting incompatibilities will affect insect population dynamics. The reasoning is that if the  
354 incompatible strain is introduced at an initial frequency above its invasion threshold, it will invade the  
355 population, which should then lead to an increase in the proportion of incompatible crosses and, in  
356 turn, a decrease in the population growth rate. This basic idea has been formalized in the theoretical  
357 models of Dobson *et al.* [18] and Blackwood *et al.* [24] which predict that the introduction of one or  
358 more incompatible *Wolbachia* strains into a target population could lead to a transient decrease in



359 population size [18]. Furthermore, assuming that a pre-existing Allee effect is enhanced by  
360 complementary tactics such as mating disruption, the unwanted population could be driven to  
361 extinction [24]. Although these models are of great interest for their efforts to link *Wolbachia* invasion  
362 to insect population dynamics, their hypotheses and predictions have not been tested with  
363 appropriate experiments. Using *Drosophila suzukii* as a model organism, we present here a first step  
364 in this direction.

365 Our main result is that introducing the incompatible *Wolbachia* strain *wTei* into a recipient *Drosophila*  
366 *suzukii* population at carrying capacity does not result in an observable decrease in population size.  
367 This finding, with this specific *Wolbachia* strain and insect host, contradicts theoretical predictions  
368 [18,24], and moderates the perspective on using cytoplasmic incompatibility as a lever for insect pest  
369 management. In the remainder of the discussion, we explore the various hypotheses that could explain  
370 this result.

371 The most trivial hypothesis to explain the lack of influence of *Wolbachia*-induced IC on population  
372 dynamics is that contrary to expectations, the introduced incompatible *Wolbachia* did not invade the  
373 recipient populations and therefore, did not produce enough incompatible crosses to produce an  
374 effect. This is a hypothesis that we cannot firmly reject. Although the prevalence of individuals infected  
375 with *wTei* reached up to 25% in some populations, weekly estimates revealed important variations,  
376 with frequencies that were sometimes lower than the 10% fixed at introduction. This result contrasts  
377 with the published data, which show that incompatible *Wolbachia* strains introduced above their  
378 invasion threshold easily invade recipient populations. In *Aedes aegypti*, the introduction of 20  
379 *Wolbachia* (*wAlbB*)-infected females into an experimental population of 100 uninfected individuals  
380 resulted in the fixation of the introduced strain in seven generations [42]. In another trial on the same  
381 species, 65% of *wMel*-infected mosquitoes were introduced in two populations of 1680 uninfected  
382 individuals, and again, the introduced strain became fixed 30 and 80 days after introduction [43]. In  
383 *Drosophila simulans*, cage populations were established with a mixture of uninfected individuals and  
384 either 35 % of *wAu*-infected individuals or 4 % of *wRi*-infected individuals (for a total of 650 and 1440  
385 adult females, respectively). A significant increase in *wAu* and *wRi* frequencies was observed for all  
386 populations from the 10<sup>th</sup> generation onwards, except in two cages where *wRi* was either lost or at low

387 frequency [44]. With a similar invasion dynamic, our seven-to-ten-week experiment (i.e., 4-5  
388 generations) should have revealed the invasion of wTei more clearly than it did. In this respect, our  
389 results are surprising.

390 Invasion may have failed if the number of infected drosophila introduced was too low to trigger  
391 invasion. Theoretically, *Wolbachia* invasion depends on the intensity of CI, the rate of transmission,  
392 and the cost of infection [9-13]. Assuming perfect transmission, the incompatible strain should invade  
393 if it is introduced above a threshold frequency equal to the ratio of infection cost ( $sf$  in the above-  
394 mentioned models) to incompatibility ( $sh$ ). In our system of *D. sukukii* and wTei, incompatibility is only  
395 incomplete, with an egg hatch rate of about 33% after incompatible crosses (i.e.,  $sh = 0.67$ ; [34]. In  
396 parallel, the analyses of traits such as fecundity, longevity, egg hatch rate and developmental time all  
397 suggest an absence of cost of wTei infection on *D. sukukii* ([32,34]). This apparent absence of infection  
398 costs ( $sf = 0$ ), even with incomplete incompatibility, suggests a theoretical invasion threshold of 0. This  
399 means that in practice the introduction of very few infected individuals infected with wTei should result  
400 in the invasion of this strain. Under these conditions, the number of flies we introduced experimentally  
401 - between 76 and 100 individuals in populations that were an order of magnitude larger - should have  
402 been high enough to induce invasion.

403 The observed frequencies of wTei in the experimental populations with CI can alternatively be  
404 interpreted as a successful but slow invasion. In this context, the generational overlap inherent in our  
405 biological model could have allowed older males, inducing lower CI, to participate in reproduction.  
406 Indeed, CI intensity decreases rapidly with male age in several *Drosophila* species [45-47]. In *D.*  
407 *sukukii*, CI intensity is reduced to 27% in mass crosses between wSuz-infected females and wTei-  
408 infected males aged 9-10 days, compared to 53% when males are 2-3 days old [32,34]. If older males  
409 are as competitive for access to females as younger ones, the negative relationship between CI  
410 intensity and male age could reduce the demographic consequences of CI, particularly in species such  
411 as *D. sukukii*, where individuals can survive for over 58 days at 23-24°C [34].

412 A final hypothesis, not exclusive to the previous arguments, is that the influence of negative density  
413 dependence on host population dynamics overwhelms that of *Wolbachia*-induced CI. In our

414 experiment, after an initial phase of exponential growth, the populations were well described by  
415 logistic-type models characterized by a carrying capacity estimated between 450 and 600 adults. This  
416 upper limit to population growth is probably a consequence of competition among larvae for limited  
417 food resources. Indeed, intraspecific competition certainly occurs in natural populations of *D. sukukii*.  
418 Our data suggest that the number of larvae developing in small fruits can be high (for instance, we  
419 could find up to 21 individuals at emergence from raspberry or blackberry exposed 48 h in the field;  
420 S2 Supporting information). This suggests that larval intraspecific competition may be severe *in natura*,  
421 in particular when fruit abundance is weak, *i.e.*, at the beginning or at the end of the season. Laboratory  
422 experiments are consistent. According to Bezerra Da Silva *et al.* [36], the recurrent observation of *D.*  
423 *sukukii* pupae outside oviposition patches is a sign of high sensitivity to larval competition. The number  
424 of eggs and larvae developing in damaged fruits affects development time and further reduces adult  
425 size and fecundity [48,49]. In adults, increasing density results in females laying in fewer eggs laid per  
426 fruit, a behavior interpreted as an adaptation to alleviate competition among immature stages [50].

427 If larval survival depends on the intensity of competition within the fruit, a reduction in egg hatch rate  
428 resulting from *Wolbachia*-induced CI should, at high densities, alleviate competition and therefore,  
429 cause an immediate increase in larval survival. Reduced competition would offset incompatibility, and  
430 if this were true, CI would not cause the predicted decline in population size. Such a verbal argument  
431 echoes a specific section of the theoretical analysis of Dobson *et al.* ([18], Fig. 2b), where the effect of  
432 density on immature survival is scaled by the parameter  $\gamma$  [51]. Increasing the influence of density by  
433 increasing  $\gamma$  made the effects of introducing incompatible strains disappear [18]. Other modelling  
434 studies have highlighted similar influence of competition among hosts on *Wolbachia* invasion [15-17].  
435 More generally, the strength of competition may alter the benefits of gene drive [52] and of the sterile  
436 insect technique [53] with undesirable consequences such as increasing (rather than decreasing) the  
437 size of pest populations.

438 The extent to which negative density dependent survival may jeopardize attempts to reduce pest  
439 reproduction appears to be a key issue. However, empirical evidence for the critical role of density  
440 dependence in *Wolbachia* invasion and consequences for host population dynamics is completely  
441 lacking. In *D. sukukii*, we suggest that negative density dependence may explain the discrepancy

442 between prior expectations and observed population dynamics. However, further species-specific  
443 models and experiments are needed to better understand the interplay between density dependence,  
444 the invasion of incompatible *Wolbachia* strains and the resulting population dynamics.

445 Positive density dependence, *i.e.*, the Allee effect, is also being proposed as an important process in  
446 pest management. The basic reasoning is that eradication can be achieved via a combination of  
447 treatments that reduce population size (pesticides, predators, etc.) and tactics that enhance a pre-  
448 existing Allee effect (for instance, mating disruption). Theoretical models, verbal arguments and  
449 reviews are numerous [19,20,54,55] and, for insect pests, well supported by widespread evidence of  
450 Allee effects [21,56]. A keystone example is that of the gypsy moth *Lymantria dispar*, whose spread is  
451 slowed by a combination of insecticides, sterile insect technique and mating disruption [57]. From this  
452 perspective, an important finding of our study is the absence of a demographic Allee effect in *D.*  
453 *suzukii*. Despite founding populations with the smallest possible number of individuals (one male and  
454 one female) we observed no positive relationship between population size and growth rate, and no  
455 extinction. This finding contrasts with observations of Allee effects in closely related species such as *D.*  
456 *melanogaster* [37,58]. In our experimental conditions with population cages, relatively high densities  
457 may have prevented an Allee effect. Alternatively, a component Allee effect could have occurred at  
458 the individual level, but was offset by negative density dependence and thus did not translate to the  
459 population level.

460 In conclusion, many of our results are puzzling with respect to prior expectations. The absence of a  
461 transient decrease in *D. suzukii* population size after the introduction of the incompatible *Wolbachia*  
462 strain *wTei* is most easily explained by the slow invasion of this introduced strain, which, in itself, lacks  
463 clear explanations. Nevertheless, there were some incompatible crosses in populations where the  
464 proportion of *wTei* was sometimes higher than 20%, but these did not influence the host population  
465 dynamics. We argue that the strong negative density dependence could be a key driver of insect  
466 population dynamics, counterbalancing the planned effect of cytoplasmic incompatibility, and  
467 possibly hampering pest management programs based on reproductive failure.

468

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471 discussion on data.

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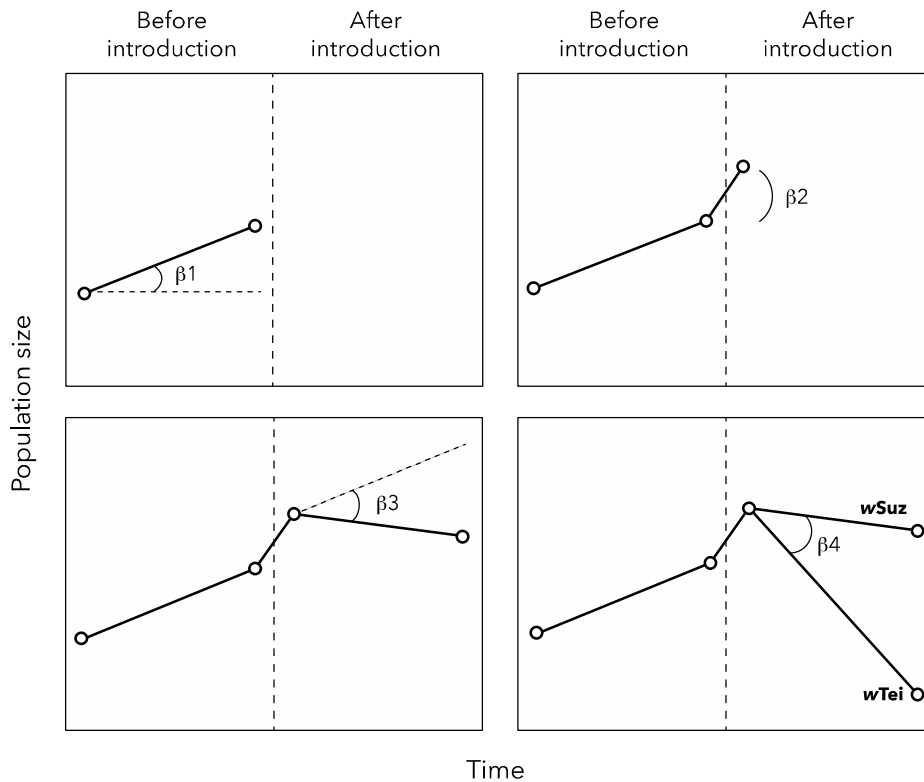
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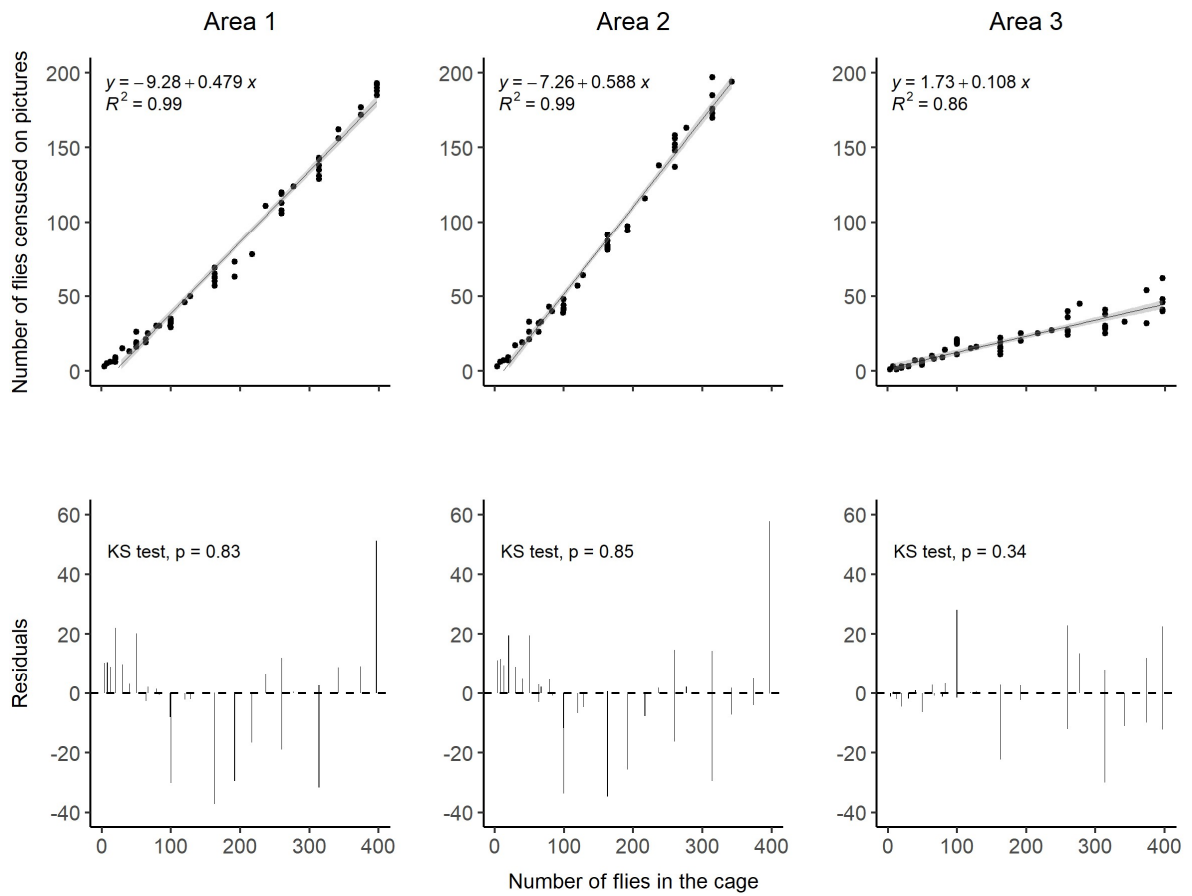
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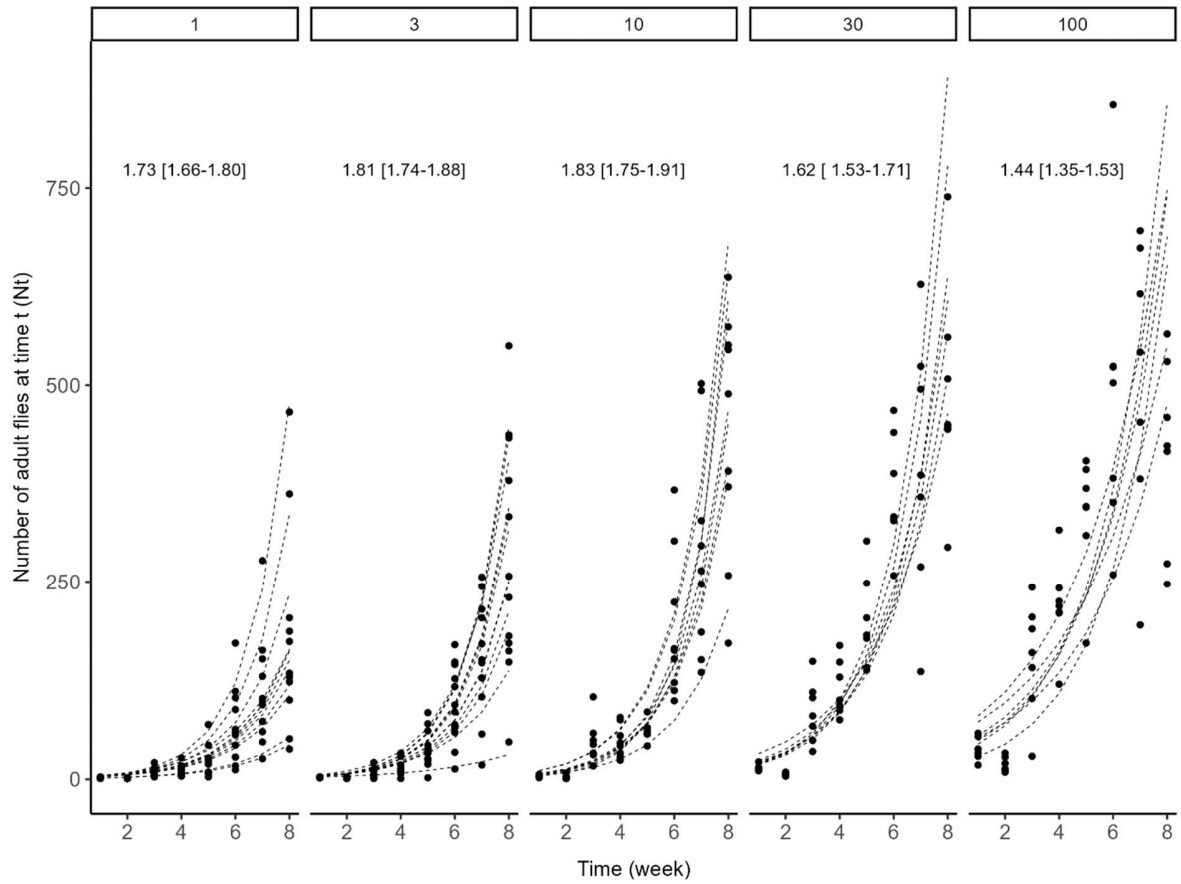
599 **Figure 1.** Schematic representation of the statistical model fitted to time variations in number of *D.*  
600 *sukuzii* in order to test the effect of introducing insects infected with either incompatible (*wTei*) or  
601 compatible (*wSuz*) *Wolbachia* into a recipient population. Four hypotheses were tested, based on  
602 estimation of four model parameters. H1: parameter  $\beta_1$  population growth before introduction;  
603 populations were at carrying capacity and we therefore expected  $\beta_1=0$ ; H2: parameter  $\beta_2$  represents  
604 the immediate effect of introduction on population size; the number of flies introduced was set at 10%  
605 of the carrying capacity so we expected  $\beta_2>0$ . H3: parameter  $\beta_3$  represents the effect of introduction  
606 on time variation of population size; if  $\beta_1=0$  and  $\beta_2>0$ , we expected  $\beta_3<0$  reflecting a return to carrying  
607 capacity. H4: parameter  $\beta_4$  represents the effect of the *Wolbachia* strain introduced on the variation  
608 of population size after introduction; theoretical models predict that the introduction of an  
609 incompatible strain (*wTei*) should result in a transient decrease in the population size, which should  
610 not be observed in control populations (introduction of *wSuz*), so that we expect  $\beta_4<0$ .

611



612

613 **Figure 2.** Estimation of population size. Linear regressions between the number of adult *D. sukuzii*  
614 censused on pictures of three different areas of the experimental cages and the total number of  
615 individuals in the cage.

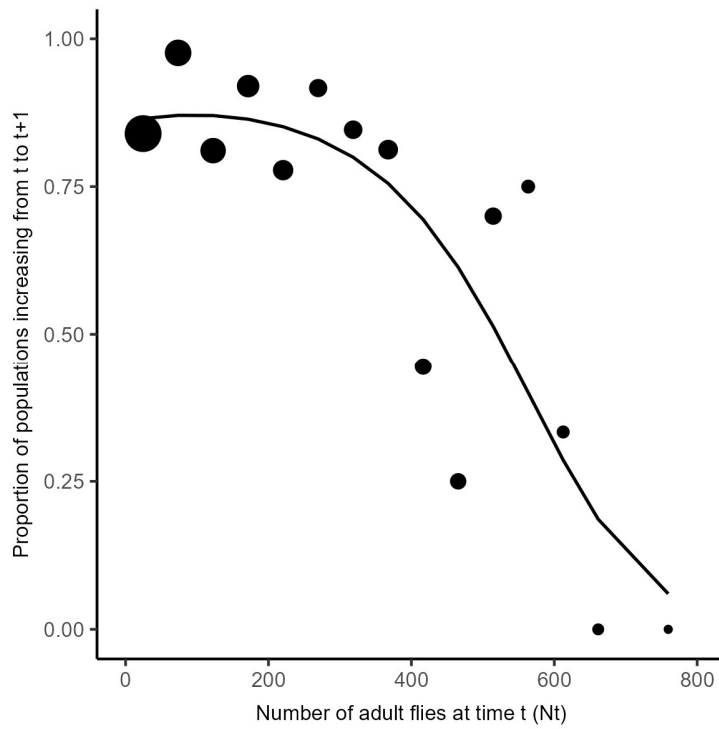


616

617 **Figure 3.** Early dynamics of small *D. sukikii* populations initiated with 1, 3, 10, 30 or 100 male-female  
618 pairs. For each replicated population, the eight-week time series of observed number of individuals  
619 (circles) was fitted with an exponential growth model  $N_t = N_0 \exp(r t)$  (lines). Estimated marginal means  
620 of population growth rate  $r$  and their 95% confidence intervals are shown.

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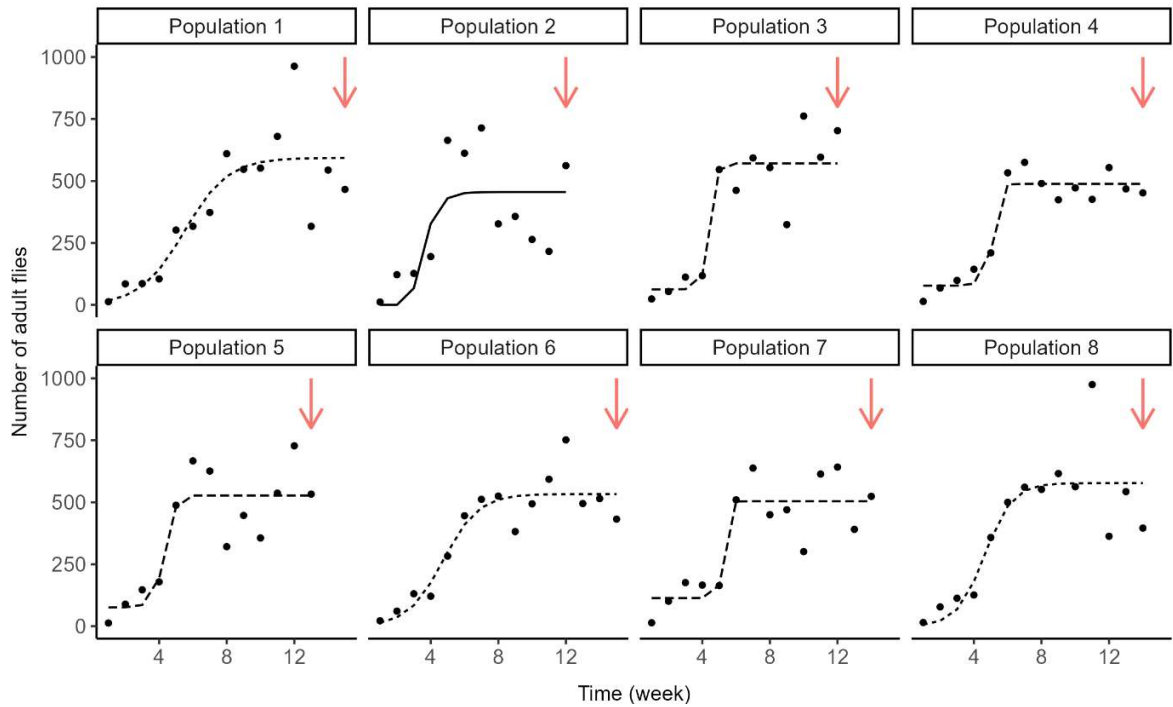


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624 **Figure 4.** Early dynamics of small *D. sukii* populations. For each range of population size at time *t*  
625 ( $N_t$ : 0-49, 50-99, etc.) circles represent the proportion  $p$  of pairs for which the number of individuals  
626 increased ( $N_{t+1} \geq N_t$ ). Circle size represent the number of pairs available to estimate each proportion  
627 (largest circle:  $x$  pairs; smallest circle:  $y$  pairs). The curve represents the second-order polynomial  
628 regression that best fitted the data ( $\text{logit } p = 1.814 + 2.014 \cdot 10^{-03} N_t - 1.056 \cdot 10^{-05} N_t^2$ ).

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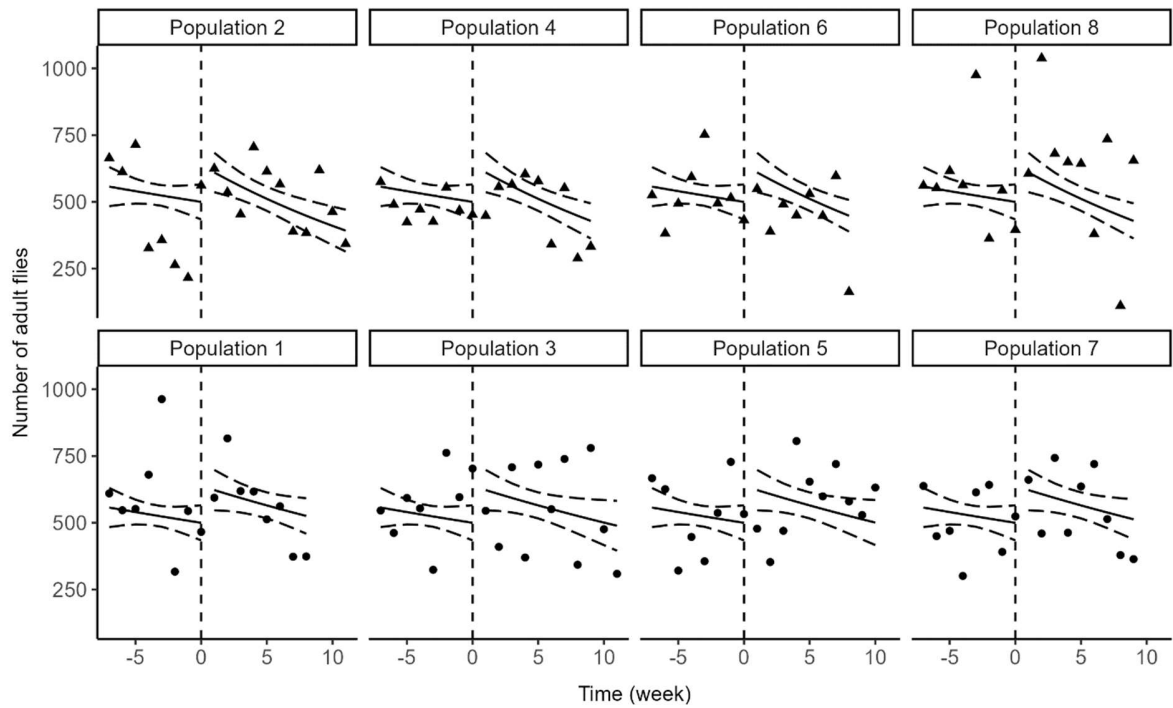
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632 **Figure 5.** Reaching the carrying capacity. Observed and predicted time variations of *D. sukuzii*  
633 population size for eight replicated populations before the introduction of Wolbachia-infected  
634 individuals. Line types represent the logistic population growth model that best fitted the data (dashed  
635 = Weibull, solid= Gompertz, dotted= logistic). Red arrows indicate the time at which infected flies were  
636 introduced, i.e., seven weeks after the first observed decrease in population size.

637



638

639 **Figure 6.** Impacts of introduced CI-inducing flies. Observed and predicted number of adult *D. sukuzii*  
640 in populations with CI (triangles; introduction of individuals infected by *wTei*) and without CI (circle;  
641 introduction of individuals infected by *wSuz*). Time is scaled so that introductions occur at  $t=0$ .

642 *l.*

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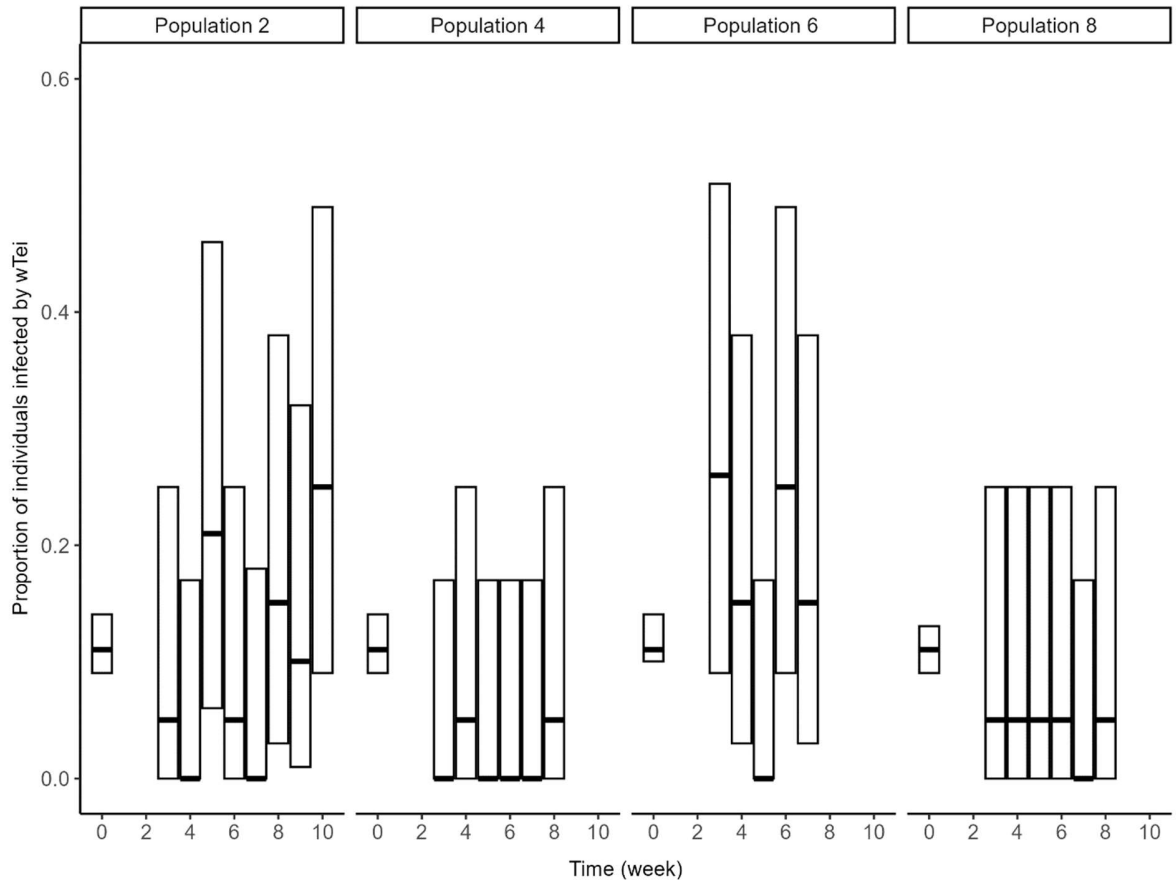
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649 **Figure 7.** Dynamics of Wolbachia infection. Evolution of the proportion of *D. sukikii* infected by the  
650 introduced *Wolbachia* strain *wTei*. Each proportion is presented with its theoretical 95% confidence  
651 interval, based on the binomial distribution. The first bar at  $t=0$  corresponds to the proportion of *wTei*-  
652 infected flies actually introduced (10 %). Subsequent prevalence was estimated from the third week  
653 post-introduction, after emergence of offspring from the introduced individuals.

654

655 **Table 1-** Gompertz, logistic and Weibull population growth models fitted to each of the eight  
 656 experimental population. K (referred as Asym in the models) is the parameter common to all three  
 657 models and that corresponds to the asymptotic value of the population size when time tends to  $\infty$ , or  
 658 the carrying capacity. Values of K and 95% confidence intervals were estimated. Models in bold are  
 659 those that best fit the observed data (lowest AIC and RMSE).

660

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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