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1 **Endosymbiont diversity in natural populations of *Tetranychus* mites is rapidly**  
2 **lost under laboratory conditions**

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14

15 **RUNNING TITLE**

16 Endosymbiont diversity: from the field to the lab

17 **ABSTRACT**

18 Although the diversity of bacterial endosymbionts in arthropods is well documented, whether and  
19 how such diversity is maintained remains an open question. We investigated the temporal changes  
20 occurring in the prevalence and composition of endosymbionts after transferring natural  
21 populations of *Tetranychus* spider-mites from the field to the laboratory. These populations,  
22 belonging to three different *Tetranychus* species (*T. urticae*, *T. ludeni* and *T. evansi*) carried variable  
23 infection frequencies of *Wolbachia*, *Cardinium*, and *Rickettsia*. We report a rapid change of the  
24 infection status of these populations after only 6 months of laboratory rearing, with an apparent loss  
25 of *Rickettsia* and *Cardinium*, while *Wolbachia* apparently either reached fixation or was lost. We  
26 show that *Wolbachia* had variable effects on host longevity and fecundity, and induced variable  
27 levels of cytoplasmic incompatibility (CI) in each fully infected population, despite no sequence  
28 divergence in the markers used and full CI rescue between all populations. This suggests that such  
29 effects are largely dependent upon the host genotype. Subsequently, we used these data to  
30 parameterize a theoretical model for the invasion of CI-inducing symbionts in haplodiploids, which  
31 shows that symbiont effects are sufficient to explain their dynamics in the laboratory. This further  
32 suggests that symbiont diversity and prevalence in the field are likely maintained by environmental  
33 heterogeneity, which is reduced in the laboratory. Overall, this study highlights the lability of  
34 endosymbiont infections and draws attention to the limitations of laboratory studies to understand  
35 host-symbiont interactions in natural populations.

36

37 **KEYWORDS**

38 Reproductive manipulation; cytoplasmic incompatibility; life-history traits; spider-mites;  
39 haplodiploids; microbial invasions; diversity loss.

40

41 **INTRODUCTION**

42 Vertically transmitted bacterial symbionts are extremely widespread in arthropods (Gibson and  
43 Hunter, 2010). While some symbiont-arthropod associations are essential for host survival and can  
44 persist for millions of years, others are facultative and are erratically distributed (reviewed in Moran  
45 *et al*, 2008). The maintenance of infection polymorphism of diverse facultative endosymbionts in  
46 host populations is thought to hinge mainly upon balancing selection between the costs and benefits  
47 of infection (Oliver *et al*, 2014). Such costs and benefits usually translate into changes in fecundity  
48 and longevity in the host. Moreover, some intracellular maternally inherited symbionts (e.g;  
49 *Wolbachia*, *Rickettsia*, *Cardinium*, *Arsenophonus* and *Spiroplasma*; Duron *et al*, 2008; Weinert *et al*,  
50 2015), are able to manipulate the reproduction of their hosts to enhance their own transmission  
51 (Engelstadter and Hurst, 2009), which has important consequences for their infection dynamics.  
52 Phenotypes of reproductive manipulation include feminization, induction of thelytokous  
53 parthenogenesis, male-killing, and (the most common and best studied) cytoplasmic incompatibility  
54 (CI; Engelstadter and Hurst, 2009).

55 In diploid species, CI leads to the embryonic mortality of part or all of the offspring resulting  
56 from crosses between infected males and uninfected females (or females infected by an  
57 incompatible strain). In contrast, crosses between infected females and both uninfected and  
58 infected males are fully viable, hence these females have a reproductive advantage relative to  
59 uninfected ones. This phenomenon thus allows the rapid spread of CI-inducing symbionts, as shown  
60 by many laboratory and field studies. For instance, only five generations were enough for the CI-  
61 inducing endosymbiotic bacteria *Wolbachia* to invade population cages of *Drosophila melanogaster*  
62 (Reynolds and Hoffmann, 2002), or of the mosquito *Aedes albopictus* (Dobson *et al*, 2002). This  
63 bacterium has also been shown to spread rapidly in field populations of different host species (e.g.  
64 Turelli and Hoffmann, 1995; Kriesner *et al*, 2013; Bakovic *et al*, 2018).

65 Despite the fact that such reproductive manipulation favors the spread of *Wolbachia*, stable  
66 infection polymorphisms are typical in nature, with some populations being fully infected, others

67 fully uninfected or infected with a different symbiont strain, and others harbouring intermediate  
68 symbiont frequencies (e.g. Vavre *et al*, 2002; Keller *et al*, 2004; Zhang *et al*, 2013b; Hamm *et al*,  
69 2014). This infection polymorphism may be associated with variation in the level of CI, the rate of  
70 maternal transmission and the relative fecundity of infected females compared to uninfected ones,  
71 which determines the threshold at which a given CI-inducing symbiont can invade a population  
72 (Hoffmann *et al*, 1990; Turelli and Hoffmann, 1995). Moreover, variability in infection frequencies  
73 between and within regions indicates benefits and costs of infection that vary across temporal and  
74 spatial gradients (e.g. Weeks *et al*, 2002; Oliver *et al*, 2014; Cass *et al*, 2016). However, the factors  
75 responsible for such variability remain largely elusive. In particular, the relative importance of  
76 environmental heterogeneity (e.g. Barton and Turelli, 2011; Hancock and Godfray, 2012; Schmidt *et*  
77 *al*, 2017), host diversity and biotic interactions (e.g. within-host interaction with other pathogens or  
78 parasites; reviewed in Oliver *et al*, 2014; Hopkins *et al*, 2017) in the maintenance of symbiont  
79 diversity remains poorly understood.

80         Laboratory studies may allow to disentangle the effect of the environment and of the host  
81 genetic background on symbiont diversity. However, drift and lab adaptation can also deeply impact  
82 natural variation. While this has been repeatedly demonstrated regarding nuclear variation (e.g.  
83 Hoffmann *et al*, 2001; Fragata *et al*, 2014; Francuski *et al*, 2014; Hoffmann and Ross, 2018), few  
84 studies have analyzed how laboratory acclimation affects symbiont diversity. Spider-mites are good  
85 candidates to investigate potential changes in infection polymorphism under laboratory conditions,  
86 as they often carry several endosymbiotic bacteria, usually maternally-inherited, with variable  
87 prevalence among natural populations. Among them, *Wolbachia* is the most prevalent (e.g. Liu *et al*,  
88 2006; Gotoh *et al*, 2007b; Zhang *et al*, 2013b; Zhang *et al*, 2016; Zélé *et al*, 2018a) and induces  
89 variable levels of CI, ranging from no CI to complete CI (Vala *et al*, 2002; Gotoh *et al*, 2007b; Xie *et al*,  
90 2011; Suh *et al*, 2015). In some cases, in spider-mites as in other haplodiploid species, CI involves a  
91 loss of the paternal set of chromosomes and diploid zygotes arising from incompatible matings may  
92 survive as haploid males (Male development - MD-CI; Perrot-Minnot *et al*, 2002; Gotoh *et al*, 2003).

93 In most cases, however, fertilized eggs from incompatible crosses fail to hatch as in diploid species,  
94 which leads to embryonic mortality of the females only (Female mortality - FM-CI; Breeuwer, 1997;  
95 Perrot-Minnot *et al*, 2002; Vala *et al*, 2002; Gotoh *et al*, 2003; Suh *et al*, 2015). Population-specific  
96 fitness effects of *Wolbachia* on spider-mite life history traits have also been reported, with costs  
97 (Perrot-Minnot *et al*, 2002; Suh *et al*, 2015), no effect (Breeuwer, 1997; Perrot-Minnot *et al*, 2002;  
98 Vala *et al*, 2002; Gotoh *et al*, 2007b), or benefits (Vala *et al*, 2002; Gotoh *et al*, 2007b; Xie *et al*, 2011)  
99 on spider-mite fecundity, but also variable effects on longevity and development time (Xie *et al*,  
100 2011). Note, however, that none of these studies (with the exception of Gotoh *et al*, 2007b) tested  
101 for coinfection with other endosymbionts, which may have confounding effects. Indeed, herbivorous  
102 spider-mites are often (co-)infected with *Cardinium* (Liu *et al*, 2006; Ros *et al*, 2012; Zhang *et al*,  
103 2016), which can also cause FM-CI (Gotoh *et al*, 2007a; Ros and Breeuwer, 2009; Xie *et al*, 2010; Zhu  
104 *et al*, 2012) without clear effect on other spider-mite life history traits reported to date (but see  
105 Zhao *et al*, 2013a; Zhao *et al*, 2013b; for *Wolbachia-Cardinium* coinfections); and occasionally with  
106 *Rickettsia* (e.g. Zhang *et al*, 2016; Zélé *et al*, 2018a) or *Spiroplasma* (e.g. Enigl and Schausberger,  
107 2007; Staudacher *et al*, 2017), whose effects in spider-mites are still unknown.

108 Here, we analyzed the temporal changes occurring in the prevalence and composition of  
109 endosymbionts after transferring spider-mite populations from the field to the laboratory. We  
110 observed very rapid changes in symbiont diversity, with an apparent loss of *Rickettsia* and  
111 *Cardinium*, while *Wolbachia* apparently reached fixation or was lost, after only 6 months  
112 (approximately 15 generations) of laboratory rearing. To understand fixation of *Wolbachia*, we  
113 measured its effects on spider-mite life history traits and the level of CI it induces in each fully  
114 infected population. Then, we used these data to parametrize a theoretical model for the invasion  
115 process of CI-inducing symbionts in haplodiploids. Finally, we discuss the potential factors that may  
116 explain the maintenance of symbiont diversity in the field compared to the laboratory.

117

118

119 **MATERIALS AND METHODS**

120 **Spider-mite populations and rearing**

121 Sixteen populations of Tetranychid mites were collected from September to December 2013 in the  
122 region of Lisbon, and adult spider-mite females from all populations were subsequently individually  
123 analyzed for species identification and for the presence of reproductive manipulators (Zélé *et al*,  
124 2018a). Three of these populations (Assaf, CVM and Alval) belonged to *Tetranychus ludeni*, three to  
125 *T. evansi* (GRA, GH and QL), and ten to the red form of *T. urticae* (AlRo, AlBe, FR, DF, LOU, COL, AMP,  
126 RF, DC and CH). The prevalence of five maternally-inherited endosymbiotic bacteria was previously  
127 estimated using genus-specific PCRs on 11-16 individual females per population (Zélé *et al*, 2018a).  
128 While *Wolbachia*, *Cardinium* and *Rickettsia* infection frequencies varied across populations (Fig. 1A),  
129 *Arsenophonus* and *Spiroplasma* were absent in all populations. These populations started with  
130 variable numbers of foundresses (AlBe: 25; FR: 30; AMP: 65; CH and GH: 80; COL: 100; Alval: 160;  
131 AlRo: 200; LOU and CVM: 300; DC: 400; DF, RF and QL: 500; Assaf: 600). They were then maintained  
132 in the laboratory under standard conditions (25 ± 2°C, 60% RH, 16/8 h L/D) at very high numbers  
133 (c.a. 500-1000 females per cage) in insect-proof cages containing either bean cv. Contender  
134 seedlings (obtained from Germisem, Oliveira do Hospital, Portugal) for *T. urticae* and *T. ludeni*, or  
135 tomato cv. Money Maker seedlings (obtained from Mr. Fothergill's Seeds, Kentford, UK) for the  
136 solanaceae specialist *T. evansi*.

137

138 **Screening for infection by endosymbionts and *Wolbachia* strain identification following laboratory**  
139 **rearing**

140 Six months after collection from the field (ca. 15 generations), infection by *Wolbachia*, *Cardinium*  
141 and *Rickettsia* was checked anew using 15-16 individual females per population (except for the  
142 population GRA that was lost during laboratory rearing) using the multiplex PCR described in Zélé *et*  
143 *al* (2018c). Subsequently, pools of 100 female per population were also checked for infection by  
144 these endosymbionts roughly 6, 12, 18 and 24 months after collection from the field (Fig. S1).

145 Previous sensitivity tests revealed that multiple symbionts can be detected in a single pool, even at  
146 low infection frequencies (up to 1/100 infected females; Zélé *et al*, 2018a). Finally, as the *wsp* gene  
147 was identical for all *Wolbachia* infecting these populations (Zélé *et al*, 2018a), we characterized the  
148 *Wolbachia* infections remaining in laboratory cultures six months after collection using a multilocus  
149 sequence typing (MLST; Baldo *et al*, 2006). MLST gene sequences were amplified from DNA  
150 extracted from a pool of 100 females per population using standard primers and PCR protocols  
151 (Baldo *et al*, 2006; Zélé *et al*, 2018a). Chromatograms were checked manually using MEGA version  
152 5.1 beta (Tamura *et al*, 2011) and we found no evidence for multiple infections within populations  
153 (as indicated by the absence of multiple peaks). All MLST sequences were then compared to entries  
154 in the PubMLST *Wolbachia* MLST database (available at <http://www.pubmlst.org/wolbachia/>) and  
155 novel sequences were submitted to the database curators for inclusion as new alleles. Each unique  
156 combination of MLST sequences was designated as an isolate, submitted to the PubMLST database,  
157 and assigned a unique ID number. Isolates with five-locus profiles that did not match an existing  
158 strain type were assigned a new strain type (Baldo *et al*, 2006).

159

#### 160 **Antibiotic treatments**

161 Roughly three months after collection from the field, a tetracycline solution (0.1 %, w/v) was used to  
162 treat mites (n=30 adult females initially) from each population for three successive generations  
163 (Breeuwer, 1997) to obtain uninfected populations. During the treatment, mites were maintained in  
164 petri dishes containing bean (or tomato for *T. evansi*) leaf fragments placed on cotton with the  
165 solution. At each generation, 50 adult mated daughters were transferred to a new petri dish  
166 containing fresh leaf fragments and solution. At the third generation after treatment, 14 individual  
167 females and a pool of 100 females per population were checked by PCR to confirm that they were  
168 uninfected. These populations were maintained in a mass-rearing environment without antibiotics  
169 for a minimum of five generations before performing experiments, to avoid potential side effects of  
170 antibiotic treatment (e.g. Ballard and Melvin, 2007; Zeh *et al*, 2012).



171

172 **Experiment 1: Effects of *Wolbachia* on *T. urticae* life-history traits and CI induction**

173 To test the effects of *Wolbachia* in each population that was still infected six months after field  
174 collection (all from *T. urticae*), the four possible crosses between Tetracycline-treated (T) and –  
175 untreated (W, *Wolbachia* infected) females and males were performed (i.e. TxT, TxW, WxT and WxW  
176 female x male crosses). An additional population (FR), fully uninfected (U) by *Wolbachia* after 6  
177 months, was also included as a control for the effect of the tetracycline treatment. Roughly two  
178 weeks prior to the experiment, age cohorts were created for each population by collecting ca. 100  
179 females from each mass culture, allowing them to lay eggs during five days on detached bean (or  
180 tomato) leaves placed on water-soaked cotton. The offspring from these cohorts was used in the  
181 experiments.

182 Two days prior to the onset of this experiment, quiescent virgin females with similar age  
183 were randomly collected from each cohort and placed separately on a leaf fragment to allow  
184 emergence while remaining virgin. Males were isolated from the same cohort one day before the  
185 beginning of the experiment to avoid potential sperm depletion. On the first day of the experiment  
186 (d0), 10 adult virgin females were placed with 10 males on a 9cm<sup>2</sup> bean leaf disc to allow mites to  
187 mate in panmixia. This procedure was chosen to increase potential conflicts over sex ratio between  
188 *Wolbachia* and its female host. Indeed, while *Wolbachia* always benefits from a higher proportion of  
189 daughters (i.e. due to its maternal mode of transmission; Hurst *et al*, 1996; Werren and Beukeboom,  
190 1998), the optimal sex ratio for female spider-mites depends on the number of foundresses in a  
191 patch, being more male biased as this number increases (Hamilton, 1967; Macke *et al*, 2011).

192 Three days later (d3), the daily female oviposition was estimated taking into account their  
193 daily mortality (daily oviposition per female over 3 days = total number of eggs laid on each leaf disc  
194 after 3 days / total number of alive females over the three days), and males were discarded. To  
195 determine the effect of *Wolbachia* on spider-mite longevity, females were transferred to new leaf  
196 discs every three days until death and their daily survival was recorded. To determine the type of CI

197 induced by *Wolbachia* in this system (i.e. MD-CI and/or FM-CI; Vavre *et al*, 2000), the number of  
198 unhatched eggs and of adult offspring ( $F_1$  females +  $F_1$  males) obtained over the first three days of  
199 the experiment were counted 5 and 15 days after removing the parents, respectively (d8 and d18).  
200 This allowed computing the relative proportions of unhatched eggs (number of unhatched eggs /  
201 total number of eggs), dead juveniles ([total number of eggs - number of unhatched eggs - number  
202 of  $F_1$  adults] / total number of eggs), males (number of  $F_1$  males / total number of eggs), and females  
203 (number of  $F_1$  females / total number of eggs) in all populations.

204 Finally, as we found that *Wolbachia* induces FM-type of CI in all tested populations (cf.  
205 Results) we determined the level of CI induced by *Wolbachia*, as the proportion of embryonic death  
206 of females in incompatible crosses ( $CI_{obs} = \text{number of unhatched eggs} / [\text{number of } F_1 \text{ females} +$   
207  $\text{number of unhatched eggs}]$ ). To account for variation in background embryonic mortality (not  
208 related to CI and including both sons and daughters embryonic mortality), we used a corrected index  
209 of CI (Poinsot *et al*, 1998; Cattel *et al*, 2018) calculated as follows:  $CI_{corr} = [(CI_{obs} - CCM) / (1 - CCM)]$ ,  
210 where CCM is the mean embryonic mortality observed in the control crosses (i.e. calculated as  $CI_{obs}$ ).  
211 To control for an effect of infection on the background embryonic mortality, TxT and WxT crosses  
212 were used as controls for TxW and WxW crosses, respectively.

213 The entire experiment was done in three consecutive blocks, each including four replicates  
214 of each cross combination for each mite population, except for “DF”, for which all replicates were  
215 done in block three, due to contaminations detected in the previous blocks (i.e. these data were  
216 discarded).

217

## 218 **Experiment 2: CI rescue across *Wolbachia*-infected *T. urticae* populations**

219 To test whether *Wolbachia* infecting one population can rescue the CI induced by *Wolbachia*  
220 infecting another population, we performed all possible crosses between *Wolbachia*-infected  
221 populations. The experimental procedure was the same than for intra-populations crosses except  
222 that 20 adult virgin females were placed individually with one male on a 2cm<sup>2</sup> bean leaf disc.

223 Subsequently, both males and females were discarded and the number of eggs per individual disc  
224 was counted. The relative proportions of unhatched eggs, dead juveniles, males, and females were  
225 subsequently measured as previously described. To avoid biases arising from low number of eggs in  
226 proportion data, all females that laid less than five eggs within the first three days of the experiment  
227 were removed from statistical analyses (cf. final sample sizes in Table S3). Subsequently,  $CI_{corr}$  was  
228 calculated as above, using each intra-population cross as control for a given female population when  
229 crossed with males from all other populations.

230 All experiments were conducted in a growth chamber under standard conditions ( $25 \pm 2^\circ\text{C}$ , 60% RH,  
231 16/8 h L/D).

232

### 233 **Statistical analyses**

234 Analyses were carried out using the R statistical package (v. 3.6.0). The different statistical models  
235 built to analyse the phenotypic effects of *Wolbachia* in both intra- and inter-population crosses are  
236 described in the Supplementary materials, Table S1. The general procedure for building the  
237 statistical models was as follows: the status of females and their mates (i.e. treated with tetracycline  
238 or not in the first experiment, and the populations the individuals belonged to in the second  
239 experiment), were fit as fixed explanatory variables, whereas blocks (and leaf discs for survival  
240 analyses) were fit as random explanatory variables.

241 Survival data (models 1.0 to 1.8) were analysed using Cox proportional hazards mixed-effect  
242 models (coxme, kinship package). Hazard ratios (HR) were obtained from these models as an  
243 estimate of the difference between the rates of dying (i.e. the instantaneous rate of change in the  
244 log number of survivors per unit time; Crawley, 2007) between the control and the other crosses. All  
245 other response variables were analysed using generalized linear mixed models with the glmmTMB  
246 procedure (glmmTMB package; Brooks *et al*, 2017), which allows using a wide range of error  
247 distribution that are not implemented in the glmer procedure. Female daily oviposition was analysed  
248 with a gamma error distribution with a log link to account for heteroscedasticity (models 2.0 to 2.8).

249 Proportion data were computed using the function `cbind`, except for  $CI_{corr}$  (continuous variable  
250 bounded between 0 and 1) for which a "weights" argument was added in the model to account for  
251 the number of observations (i.e. number of unhatched eggs + number of adult daughters per disc).  
252 Proportion data were subsequently analysed with a binomial error distribution, or with a  
253 betabinomial error distribution to account for over-dispersed errors (models 3.0 to 12.0).

254 Maximal models, including all higher-order interactions, were simplified by sequentially  
255 eliminating non-significant terms and interactions to establish a minimal model, and the significance  
256 of the explanatory variables was established using chi-squared tests (Crawley, 2007). The significant  
257  $\chi^2$  values given in the text are for the minimal model (Crawley, 2007). When the variable  
258 "population" was found to interact significantly with other variables, each population was analysed  
259 separately to determine the effect of the status of both females and males, as well as their  
260 interactions. When a significant interaction between these explanatory variables was found, *a*  
261 *posteriori* orthogonal contrasts (Crawley, 2007) between crosses ("WxW", "WxT", "TxW" and "TxT")  
262 were carried out by aggregating factor levels together and by testing the fit of the simplified model  
263 using ANOVA. In the case of  $CI_{corr}$ , compatible and incompatible crosses were analysed separately to  
264 determine differences between populations.

265

## 266 **Modeling *Wolbachia* invasion under laboratory conditions**

267 To predict *Wolbachia* invasion in each population that was fully infected six months after collection,  
268 we used the data obtained for the phenotypic effects of *Wolbachia* to parameterize a mathematical  
269 model for FM-type CI (cf. Results) developed by Vavre *et al* (2000). This model allows estimating the  
270 value of the unstable equilibrium (i.e. the threshold for infection rates above which *Wolbachia* is  
271 expected to reach fixation, and below which it is predicted to go extinct; Hoffmann *et al*, 1990). The  
272 parameters of this model are the relative fecundity of infected versus uninfected females (*F*; this  
273 parameter is also weighted by the effect of *Wolbachia* on the female survival, so  $F = \text{mean daily}$   
274  $\text{oviposition of infected females [incl. WxW and WxT crosses]} / \text{mean daily oviposition of}$

275 uninfected females [incl. TxW and TxT crosses] over 3 days / hazard ratio of infection in females), the  
276 proportion of eggs that escape CI in the incompatible cross (H; i.e. the reverse of the CI level, so here  
277  $H = 1 - (CI_{corr}/100)$ ), and the proportion of uninfected eggs produced by infected females ( $\mu$ ; i.e. the  
278 reverse of the transmission rate). We assumed perfect maternal transmission as only a transmission  
279 rate of 100% may explain an observed infection frequency of 100% in females when CI is incomplete.  
280 Nevertheless, to account for potential inaccuracy of observed infection frequencies, we estimated  
281 the minimum transmission rate that can explain the maintenance of *Wolbachia* in each population  
282 (Table S5).

283

## 284 **RESULTS**

### 285 **Changes in endosymbiont prevalence under laboratory conditions**

286 The screen for endosymbiont infection following six months of laboratory rearing (c.a. 15  
287 generations) revealed a drastic change in symbiont prevalence found after field collection (Fig. 1A  
288 and described in Zélé *et al*, 2018a). Indeed, neither *Cardinium* nor *Rickettsia* were detected in any of  
289 the populations tested (prevalence < 11% with 95% CIs; Jeffreys interval recommended for small *n*  
290 by (Brown *et al*, 2001), whereas all females were found infected by *Wolbachia* in seven *T. urticae*  
291 populations (prevalence > 88-89% with 95% CIs), and none of them in eight populations, belonging  
292 to *T. urticae*, *T. evansi* and *T. ludeni* (prevalence < 11% with 95% CIs; Fig. 1B). Moreover, diagnostic  
293 PCRs performed on pools of 100 females 6, 12, 18 and 24 months after field collection (Fig. S1)  
294 confirmed the loss (prevalence < 1%) of endosymbionts in these populations. In general, there is a  
295 good correlation between the symbiont frequency in the original population and the probability of  
296 infection loss or fixation. Indeed, *Wolbachia* was lost in the populations in which its initial frequency  
297 was lower than 50%, while it reached fixation in the other populations.

298

### 299 ***Wolbachia* diversity in the laboratory**

300 The MLST sequences were the same for all *Wolbachia* that reached fixation in *T. urticae* populations.

301 This confirms the results previously obtained using the *wsp* gene (i.e. only one *wsp* sequence was  
302 found across all populations, GenBank: DQ910771; Zélé *et al*, 2018a) although we cannot rule out  
303 that diversity existed in field collected samples, and that the same (or a similar) *Wolbachia* variant  
304 reached fixation in all populations under our laboratory conditions. Most sequences found were  
305 already present in the PubMLST database (*gatB*: allele 9; *coxA*: allele 38; *hcpA*: allele 143, and *ftsZ*:  
306 allele 23), but we identified a new allele for *fbpA*: the allele 444, which presents one SNP with the  
307 existing allele 4. Consequently, we defined a new strain of *Wolbachia*, ST491, which is very similar to  
308 strain ST219 belonging to supergroup B and found in China by Zhang *et al* (2013a).

309

### 310 **Experiment 1: Effects of *Wolbachia* on *T. urticae* life-history traits and CI induction**

#### 311 *Effects of Wolbachia on spider-mite longevity*

312 As all symbionts were lost in *T. evansi* and *T. ludeni*, the following results were obtained only in the  
313 *T. urticae* populations in which *Wolbachia* reached fixation in the laboratory. Daily female survival  
314 was significantly affected by the status (treated with tetracycline or not) of both the females and  
315 their mates, but in a population-specific manner (model 1.0 in Table S1, see also Table S2 for log  
316 hazard ratios and the significance of all fixed effects and their interactions; Fig. S2 for survival  
317 curves). Indeed, the independent analysis of each population showed that the tetracycline  
318 treatment did not affect longevity in the populations AMP, DF and the uninfected control FR (model  
319 1.1 to 1.3) while in CH and COL *Wolbachia*-infected females had a ca. 1.5 and 1.3 times shorter  
320 lifespan than uninfected females, respectively (model 1.4,  $X^2_1 = 16.34$ ,  $p < 0.0001$ , and model 1.5,  $X^2_1 =$   
321  $6.40$ ,  $p = 0.01$ , respectively). In addition, females mated with a *Wolbachia*-infected male survived 1.3  
322 and 1.6 times less than those mated with an uninfected male in COL and LOU, respectively (model  
323 1.5,  $X^2_1 = 5.08$ ,  $p = 0.02$ , and model 1.6,  $X^2_1 = 17.81$ ,  $p < 0.0001$ , respectively). Conversely, females  
324 mated with a *Wolbachia*-infected male survived 0.8 and 0.7 times longer than those mated with an  
325 uninfected male in DC and RF (model 1.7,  $X^2_1 = 5.04$ ,  $p = 0.02$ , and model 1.8,  $X^2_1 = 11.98$ ,  $p = 0.0005$ ,  
326 respectively).

327

328 *Effects of Wolbachia on spider-mite fecundity*

329 The analysis of daily female oviposition over 3 days revealed no significant 3-way interaction  
330 between populations, female and male infection status (model 2.0, see Table S2 for the significance  
331 of all fixed effects and their interactions). Sequential removals of non-significant factors (including  
332 their interactions) from the model unveiled no significant interaction between female and male  
333 infection status and between population and male infection status, nor significant effect of male  
334 infection status. However, a significant interaction between population and female infection status  
335 was found (Fig. 2). The independent analysis of each population further revealed variable effects of  
336 *Wolbachia* infection in females depending on the population: decreased oviposition by  $0.93 \pm 0.45$  in  
337 AMP (model 2.1,  $X^2_1 = 5.84$ ,  $p=0.02$ ), increased oviposition by  $0.77 \pm 0.36$  in DF (model 2.2,  $X^2_1 = 4.31$ ,  
338  $p=0.04$ ) and by  $0.97 \pm 0.54$  in CH (model 2.3,  $X^2_1 = 6.41$ ,  $p=0.01$ ), but no significant effect of  
339 *Wolbachia* infection in the other populations, including the control (models 2.4 to 2.8, DC:  $X^2_1 = 0.40$ ,  
340  $p=0.52$ , RF:  $X^2_1 = 0.54$ ,  $p=0.46$ , COL:  $X^2_1 = 0.68$ ,  $p=0.41$ , LOU:  $X^2_1 = 0.15$ ,  $p=0.70$ , FR:  $X^2_1 = 0.36$ ,  $p=0.55$ ).

341

342 *Effects of Wolbachia on offspring development*

343 Overall, the relative proportion of unhatched eggs varied according to the tested population and the  
344 infection status of both males and females (model 3.0, see Table S2 for the significance of all fixed  
345 effects and their interactions; Fig. 3A). Indeed, in all populations, except in the control FR, the  
346 proportion of unhatched eggs was higher in crosses between uninfected females mated with  
347 infected males than in other crosses, which indicates the induction of CI by *Wolbachia* (models 3.1 to  
348 3.8; see Table S2 for the results of the contrasts analyses). The relative proportion of females also  
349 varied according to the tested population and the infection status of both males and females (model  
350 5.0, Table S2), and in all populations, except in the control FR, the proportion of females was lower  
351 in incompatible than in compatible crosses (models 5.1 to 5.8; Table S2). Conversely, the relative  
352 proportion of males only differed between populations independently of *Wolbachia* infection in

353 males and females (model 6.0; Table S2). As the increased proportion of unhatched eggs in  
354 incompatible crosses led to a decrease in the production of females but not of males, these results  
355 indicate that CI induced by *Wolbachia* does not lead to haploidization of fertilized eggs (MD-type of  
356 CI) but to female early mortality (FM-type of CI) in all populations. Finally, the relative proportion of  
357 dead juveniles differed between populations and was affected by *Wolbachia* infection in females,  
358 with an overall decreased juvenile mortality of ca. 3% in the offspring of infected females, but no  
359 significant interaction was found (model 4.0; Table S2).

360

#### 361 *CI level induced by Wolbachia in each population*

362 Females were produced in all incompatible crosses showing that CI was incomplete. Moreover, the  
363 analysis of the level of  $CI_{corr}$  in incompatible crosses showed a significant interaction between the  
364 tested population and the infection status of both males and females (model 7.0, Table S2). While no  
365 difference was found between compatible crosses of all populations (model 7.1, Table S2), a  
366 significant difference was found between populations for incompatible crosses (model 7.2, Fig. 3B  
367 and Table S2). The contrast analysis revealed no significant difference between AMP and DC ( $X^2_1$   
368 =1.74,  $p=0.19$ ) and among RF, COL, DF, LOU and CH ( $X^2_4=3.72$ ,  $p=0.45$ ), but a significantly lower level  
369 of CI in the latter than in the former group of populations (on average 33% and 61%, respectively;  $X^2_1$   
370 =38.37,  $p<0.0001$ ). All infected populations differed significantly from the control FR ( $X^2_1=68.90$ ,  
371  $p<0.0001$ ).

372

#### 373 **Experiment 2: CI rescue across *Wolbachia*-infected *T. urticae* populations**

374 The ability of *Wolbachia* infection in females from each population to rescue CI induced by  
375 *Wolbachia* infection in males from all other populations was tested by crossing all infected  
376 populations with each other. As previously, we summarized the effect of *Wolbachia* on the  
377 development of *T. urticae* eggs by computing the relative proportions of unhatched eggs, dead  
378 juveniles, males and females (Fig. 4A), as well as  $CI_{corr}$  (Fig. 4B) for each combination of crosses. For



379 all proportions, the statistical analyses did not reveal any significant interaction between females  
380 and males from different populations (models 8.0 to 12.0, see Table S3 for the significance of all  
381 fixed effects and their interactions). The proportions of unhatched eggs and of males were not  
382 significantly higher in inter-population crosses than in intra-population controls, indicating that CI  
383 induced by *Wolbachia*-infected males from any population is rescued by *Wolbachia* infection in  
384 females from any other population.

385

### 386 **Consequences of the phenotypic effects of *Wolbachia* for its invasion under laboratory conditions**

387 The data obtained for the phenotypic effects of *Wolbachia* allowed us to parameterize the model of  
388 Vavre *et al* (2000) to predict *Wolbachia* invasion in the populations in which it reached fixation (Fig.  
389 5). The estimated values taken for the relative fecundity of infected versus uninfected females  
390 accounting for survival differences (F), and for the proportion of eggs that escape CI in the  
391 incompatible cross (H), are provided in Table S4. As we could not detect uninfected females in the  
392 infected populations, this should indicate that transmission is perfect when CI is incomplete.  
393 However, because this parameter is difficult to assess precisely and because the outcome of the  
394 model is very sensitive to its value, we estimated the minimum transmission rate under which  
395 *Wolbachia* should be lost. It was of 83.6% in DC, 91.9% in AMP, 90.3% in RF, 98.5% in COL, 80.9% in  
396 DF, 92.5% in LOU, and 98.4% in CH (Table S5). The population-specific effects of *Wolbachia*, ranging  
397 from costs to benefits, and its ability to exert different levels of cytoplasmic incompatibility affected  
398 the model predictions. Assuming perfect maternal transmission, *Wolbachia* is expected to invade in  
399 the populations DC, RF, DF and LOU, whatever its initial infection frequency (i.e., unstable  
400 equilibrium  $< 0$ ), as no fecundity and longevity costs associated with infection were detected. For the  
401 populations AMP, COL and CH, the model predicts the existence of an unstable equilibrium above  
402 which infection should spread. Due to fitness costs of infection (on oviposition and/or longevity), this  
403 unstable equilibrium was relatively high, especially in the populations COL and CH in which it was  
404 above 50% (Fig. 5 and Table S4). As the initial frequency of *Wolbachia* infection in each of these

405 population was above their respective unstable equilibrium, the rapid invasion of *Wolbachia*  
406 observed in the laboratory is in accordance with theoretical predictions.

407

## 408 **DISCUSSION**

409 In a previous study conducted in southwest Europe on 16 natural populations of *Tetranychus* spider-  
410 mites, we detected *Wolbachia*, *Cardinium*, and *Rickettsia* with highly variable prevalence (Z  l   et al,  
411 2018a). Here, we report a rapid change of the infection status of these populations after only 6  
412 months of laboratory rearing (ca. 15 generations of lab evolution), from an apparent loss of  
413 *Rickettsia* and *Cardinium* to apparent fixation or loss of *Wolbachia*. In the seven populations where  
414 *Wolbachia* remained (all from *T. urticae*), we found variable effects of infection on host traits.

415

### 416 **Variability in *Wolbachia* effects and level of cytoplasmic incompatibility**

417 *Wolbachia* affected differently the longevity of females from different populations, with either no  
418 effect or a cost of infection on survival. Moreover, we found variable effects of mating with  
419 *Wolbachia*-infected males on this trait, with both positive and negative effects, as previously found  
420 in *T. urticae* populations in China (Xie et al, 2011). *Wolbachia* also affected female fecundity  
421 differently depending on the population, ranging from no effect to costs or benefits, as in many  
422 spider-mite populations worldwide (Breeuwer, 1997; Perrot-Minnot et al, 2002; Vala et al, 2002;  
423 Gotoh et al, 2007b; Xie et al, 2011; Suh et al, 2015). These effects, although of relatively low  
424 amplitudes may still have important consequences for the invasion dynamics of *Wolbachia* (e.g. the  
425 existence of an invasion threshold when *Wolbachia* induces a fecundity or a longevity cost,  
426 independently of the level of CI it induces; Fig. 5).

427 The analysis of the proportions of unhatched eggs, daughters and sons in the brood revealed  
428 that *Wolbachia* induces a female mortality type of CI (FM-CI; Breeuwer, 1997; Vavre et al, 2000) in  
429 all populations. However, besides the sex ratio distortion observed in incompatible crosses due to CI,  
430 we did not find any effect of *Wolbachia* on the offspring sex ratio in compatible crosses. This

431 suggests that sex ratio distortion induced by *Wolbachia* in absence of CI, as observed by Vala *et al*  
432 (2003), is not a common feature of *Wolbachia* in spider-mites.

433 Finally, we found that the level of CI induced by *Wolbachia* also varies depending on the  
434 population (ca. 33% in the populations RF, COL, DF, LOU and CH, and c.a. 61% in AMP and DC), albeit  
435 *Wolbachia wsp* (Zélé *et al*, 2018a) and MLST sequences at the time of the experiment did not differ  
436 among populations. Such variability of FM-CI levels induced by *Wolbachia*, without clear association  
437 with different *Wolbachia wsp* sequences, has been previously reported in spider-mites (Vala *et al*,  
438 2002; Gotoh *et al*, 2003; Gotoh *et al*, 2007b; Xie *et al*, 2011; Suh *et al*, 2015). However, although the  
439 use of *wsp* and of the MLST approach is a standard in the community of *Wolbachia* researchers,  
440 these genes may not be particularly suited to discriminate between closely related strains (Ishmael  
441 *et al*, 2009; Atyame *et al*, 2011; Conner *et al*, 2017), or to accurately reflect the properties of a  
442 *Wolbachia* strain (Bleidorn and Gerth, 2018) including different level of CI induction (Hamm *et al*,  
443 2014; Kaur *et al*, 2017). In particular, genes responsible for CI induction (the *cidA-cidB* or *cifA-cifB*,  
444 and *cinA-cinB* operons) have recently been identified in different *Wolbachia* strains infecting  
445 different hosts (Beckmann *et al*, 2017; LePage *et al*, 2017; Bonneau *et al*, 2018; Lindsey *et al*, 2018).  
446 It has been proposed that CI strength could be adjusted via the level of expression of these genes, or  
447 the ratio of *cifA* and *cifB* transcripts across development (Lindsey *et al*, 2018). Our populations could  
448 thus be infected with different but closely-related *Wolbachia* strains differing for these genes.  
449 Unfortunately, we failed to amplify the *cidA* and *cidB* genes of *Wolbachia* in *T. urticae* (see Box S1)  
450 and future work should focus on sequencing the entire genome of *Wolbachia* from spider-mites to  
451 improve our understanding of this system. Still, the absence of sequence divergence among  
452 *Wolbachia* from different populations is in agreement with our finding that all populations were  
453 compatible with each other (i.e. full CI-rescue between populations). Therefore, variations across *T.*  
454 *urticae* populations in fitness effects and in the strength of reproductive phenotypes may be due to  
455 the hosts specific genetic backgrounds as shown in some drosophila species (e.g. Reynolds and  
456 Hoffmann, 2002; Mercot and Charlat, 2004; Cooper *et al*, 2017), but also in *T. urticae* (Sun *et al*,

457 2016).

458

459 **Loss or fixation of endosymbionts in the laboratory**

460 We found contrasting evolutionary dynamics of invasion of *Wolbachia* across the sixteen  
461 populations, with rapid invasion leading to fixation in seven populations, and its loss in all others.  
462 *Cardinium* and *Rickettsia* were also lost in all populations. Stochastic effects (i.e. random genetic  
463 drift) may play an important role in the fate of endosymbionts in the laboratory, especially for low  
464 initial infection frequencies or small host population sizes (Jansen *et al*, 2008; Reuter *et al*, 2008;  
465 Oliver *et al*, 2014). In this study, founder effects may thus explain the loss of infection in some  
466 populations that were started from few individuals (e.g. AlBe and FR), or very low initial symbiont  
467 infection frequencies (Fig. 1A). However, most populations were founded with relatively high  
468 numbers of individuals, and all were subsequently maintained at very high numbers. Moreover, the  
469 deterministic model of Vavre *et al* (2000) parameterized with our data predicted a rapid invasion of  
470 *Wolbachia* in all populations in which we could study its effects, even from low or mid initial  
471 infection frequencies (e.g. in the populations COL, DF and LOU, and in the populations DC, AMP and  
472 RF, respectively). It suggests that the fixation of *Wolbachia* observed in the laboratory were mostly  
473 determined by CI, rather than by the fitness effects of this symbiont and/or by drift.

474         The spread of CI-inducing symbionts is predicted to be more likely than that of a comparable  
475 neutral genetic element, even in the face of an invasion threshold (Jansen *et al*, 2008). Therefore,  
476 the loss of endosymbionts in populations with high population density, and when the initial infection  
477 frequency was close to 50% (e.g. *Wolbachia* in CVM, Alval, GH and QL, or *Cardinium* in RF and CH),  
478 suggests that the lost symbionts did not induce high CI levels that could compensate for fitness costs  
479 (e.g. due to fitness costs of infection, the populations AMP, COL and CH are also expected to lose the  
480 infection for an initial infection frequency below 36%, 70% and 59%, respectively; Fig. 5) and/or drift  
481 effects. Indeed, not only variability in CI levels is a common feature in spider-mites, but several  
482 studies have also reported infections by non CI-inducing *Wolbachia* (Perrot-Minnot *et al*, 2002; Vala

483 *et al*, 2002; Gotoh *et al*, 2003; Gotoh *et al*, 2007b; Xie *et al*, 2011; Suh *et al*, 2015) and *Cardinium*  
484 (Gotoh *et al*, 2007a) strains in spider-mites. Moreover, although *Wolbachia* and *Cardinium*  
485 transmission rates were found to be often close to one in arthropods (e.g. Rasgon and Scott, 2003;  
486 Narita *et al*, 2007; Perlman *et al*, 2008), this might not be the case for all symbiont strains, and in all  
487 host species/populations. Unfortunately, the transmission rate of *Cardinium*, *Rickettsia*, and of  
488 *Wolbachia* infecting the populations in which they were lost is unknown here.

489 Hence, although the invasion by *Wolbachia* can easily be explained by its phenotypic effects  
490 on the host, its loss and that of *Cardinium* and *Rickettsia*, can be attributed to any factor (e.g.  
491 inefficient maternal transmission, absence or low CI induction, high fitness costs, stochastic effects).

492

#### 493 **What explains the maintenance of symbiont diversity in the field compared to the lab?**

494 It should be noticed that we did not find an effect of collection date on the probability of infection  
495 by *Wolbachia* in these field populations (Zélé *et al*, 2018a). Moreover, another field collection of *T.*  
496 *urticae* populations, conducted two years later in the same region in Portugal, shows that the  
497 prevalence of the three endosymbionts remained relatively similar (Zélé *et al*, 2018b). Diversity and  
498 polymorphism thus seem stable in field populations. If symbionts in the lab rapidly reached fixation  
499 or extinction, then what maintains different prevalence levels between populations in the field and  
500 polymorphism within populations? A few, non-exclusive, hypotheses can be put forward.

501 Different prevalence levels between populations might be explained by spatial variation of  
502 environmental conditions in the field, which may impact the effects of endosymbionts on host  
503 fitness. For example, temperature is known to affect endosymbiont transmission, their fitness  
504 effects on hosts and the strength of reproductive manipulation (e.g. Clancy and Hoffmann, 1998;  
505 Anbutsu *et al*, 2008; Carrington *et al*, 2010; Bordenstein and Bordenstein, 2011; Ross *et al*, 2017b).  
506 In line with this, *Wolbachia* prevalence varies with temperature in the field (e.g. Toju and Fukatsu,  
507 2011; Sumi *et al*, 2017; Ferguson *et al*, 2018). In spider-mites, *Wolbachia* prevalence is also  
508 associated with temperature: a field study shows that prevalence increases with temperature (e.g.

509 Zhu *et al*, 2018), but a too high temperature cures mites from *Wolbachia* (e.g. Van Opijnen and  
510 Breeuwer, 1999). Spatial variation in other environmental factors such as host nutrition (e.g. Clancy  
511 and Hoffmann, 1998), including the host plant of herbivorous arthropods (reviewed in Frago *et al*,  
512 2012), and/or the presence of host pathogens or natural enemies (reviewed in Oliver *et al*, 2014;  
513 Hopkins *et al*, 2017), may affect the prevalence of symbionts and explain differences between  
514 populations. Similarly, temporal (seasonal and/or circadian) variations in all these factors may lead  
515 to temporal variations in endosymbiont prevalence within populations and, hence, may explain the  
516 maintenance of infection polymorphism at the population level.

517 Another possible means to maintain variation in prevalence levels between populations is  
518 spatial structure of different host genotypes (i.e. limited gene flow between populations), which may  
519 be more or less pervasive to CI or other fitness effect of the symbionts (see above). Many studies  
520 have shown the existence of population structure in spider-mites (reviewed in Sousa *et al*, 2019).  
521 Hence, migrations among populations with variable infection prevalence should blur differences in  
522 prevalence levels between populations. However, they may also allow the maintenance of infection  
523 polymorphism within populations. Indeed, several models predict that (positive) frequency-  
524 dependent selection on CI prevents stable coexistence of infected and uninfected hosts in a  
525 panmictic population, but enables it in structured populations, in which migration rate falls below a  
526 critical value (reviewed in Engelstadter and Telschow, 2009).

527 Finally, infection polymorphism within field populations may be maintained by horizontal  
528 transfers of symbiont between hosts from different populations or species. Evidences of horizontal  
529 transfers come from incongruences between phylogenies of host and symbionts in spider-mites (e.g.  
530 Yu *et al*, 2011; Ros *et al*, 2012), as in many other arthropod hosts (e.g. Vavre *et al*, 1999;  
531 Raychoudhury *et al*, 2009; Ahmed *et al*, 2016; Conner *et al*, 2017). If such horizontal transfers are  
532 frequent enough in field populations, they could play a role in the infection dynamics of the  
533 symbionts and allow the maintenance of some symbionts at low frequency.

534

535 **Future directions**

536 We observed a rapid loss of endosymbionts diversity following colonization in a laboratory  
537 environment. Such lability of endosymbionts can be particularly useful to develop and  
538 experimentally test theoretical models of symbiont invasion. However, such laboratory studies may  
539 also not reflect the processes at play in the field, thereby hampering a good understanding of host-  
540 symbiont interactions.

541         Important efforts have recently been developed to understand the effect of the transition  
542 from the laboratory to the field on the dynamic of *Wolbachia* within mosquito populations due to its  
543 implication for disease control (e.g. Hoffmann *et al*, 2014; Nguyen *et al*, 2015). In particular, our  
544 observations highlight the relevance of the new methods that are currently developed to minimize  
545 laboratory adaptation and, hence, to increase the relevance of laboratory experiments for the  
546 understanding of natural populations (Leftwich *et al*, 2016; Ross *et al*, 2017a).

547         Although some studies report rapid genetic changes in arthropods during a transition from  
548 the field to the laboratory (e.g. Hoffmann *et al*, 2001; Fragata *et al*, 2014; Francuski *et al*, 2014),  
549 changes in symbiotic communities are still largely understudied. This is at odds with the relevance  
550 they may have for implementing existing studies of host adaptation to novel environment (e.g.  
551 Matos *et al*, 2015; Fragata *et al*, 2016; Hoffmann and Ross, 2018). Whether the loss or fixation of  
552 particular symbionts (strains or species) under laboratory conditions is adaptive for the host, or  
553 whether it is a by-product of the host environment on the symbiotic community, remains elusive.

554

555 **AUTHORS' CONTRIBUTIONS**

556 Designed the project: FZ and SM, with discussions with MM, MW and FV. Designed experiments: FZ,  
557 SM; Population maintenance: IS; molecular analyses: FZ, MW; performed the experiments: FZ and IS;  
558 statistical analyses and model application: FZ; paper writing: FZ, FV and SM with input from all  
559 authors. All authors read and approved the final version of the manuscript.

560

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571

572 **COMPETING INTERESTS**

573 We declare that we do not have any conflict of interest.

574

575 **DATA ARCHIVING**

576 Full datasets have been deposited in the Dryad data repository ([doi.org/ 10.5061/dryad.pk0p2ngjg](https://doi.org/10.5061/dryad.pk0p2ngjg)).

577

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- 832

833 **FIGURE LEGENDS**

834

835 **Figure 1. Endosymbiont infection frequency in each spider-mite population following (a) 0-3**  
836 **months, and (b) 6 months of laboratory rearing after collection in the field.** Each box represents a  
837 population, and within each graph, columns represent the infection status by W: *Wolbachia* (red  
838 cells); C: *Cardinium* (yellow cells); and R: *Rickettsia* (green cells). White cells represent uninfected  
839 individuals. Coinfections within the same individuals are indicated by more than one shaded region  
840 on the same horizontal plane.

841

842 **Figure 2. *Wolbachia* effects on oviposition of *T. urticae* females.** Orange boxes: untreated females,  
843 white boxes: *Wolbachia*-free females. The statistical significances are given above bars: \* $p < 0.05$ ;  
844 ns, not significantly different at the 5% level. The population FR (blue box) lost *Wolbachia* in the  
845 laboratory and is used here as control for the tetracycline treatment.

846

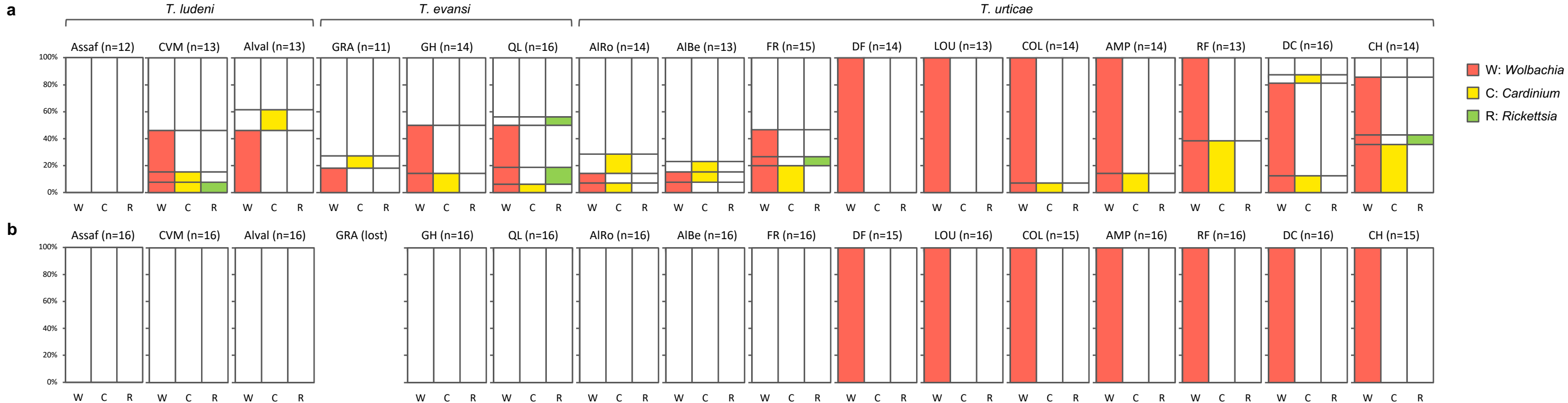
847 **Figure 3. Summary of the development of *T. urticae* eggs and cytoplasmic incompatibility (CI)**  
848 **levels in intra-population crosses between *Wolbachia*-infected and uninfected mites.** (a) Relative  
849 proportions of unhatched eggs (purple bars), dead juveniles (yellow bars), adult females (red bars)  
850 and adult males (blue bars) for each type possible cross. Bar plots represent means  $\pm$  s.e. (values  
851 provided in Table S2). T: tetracycline-treated; W: *Wolbachia*-infected; U: naturally *Wolbachia*-  
852 uninfected. The population FR lost *Wolbachia* in the laboratory and is used as control for tetracycline  
853 treatment. (b) Boxplot of CI-related mortality estimated using the  $CI_{corr}$  index, which removes the  
854 basal embryonic mortality (estimated in control crosses). Identical or absent superscripts indicate  
855 nonsignificant differences at the 5% level among populations for crosses between tetracycline-  
856 treated females and untreated males (“T x W/U”; orange boxes). No significant differences were  
857 found between all other crosses (“T x T”, “U/W x T”, “U/W x U/W”; green boxes).

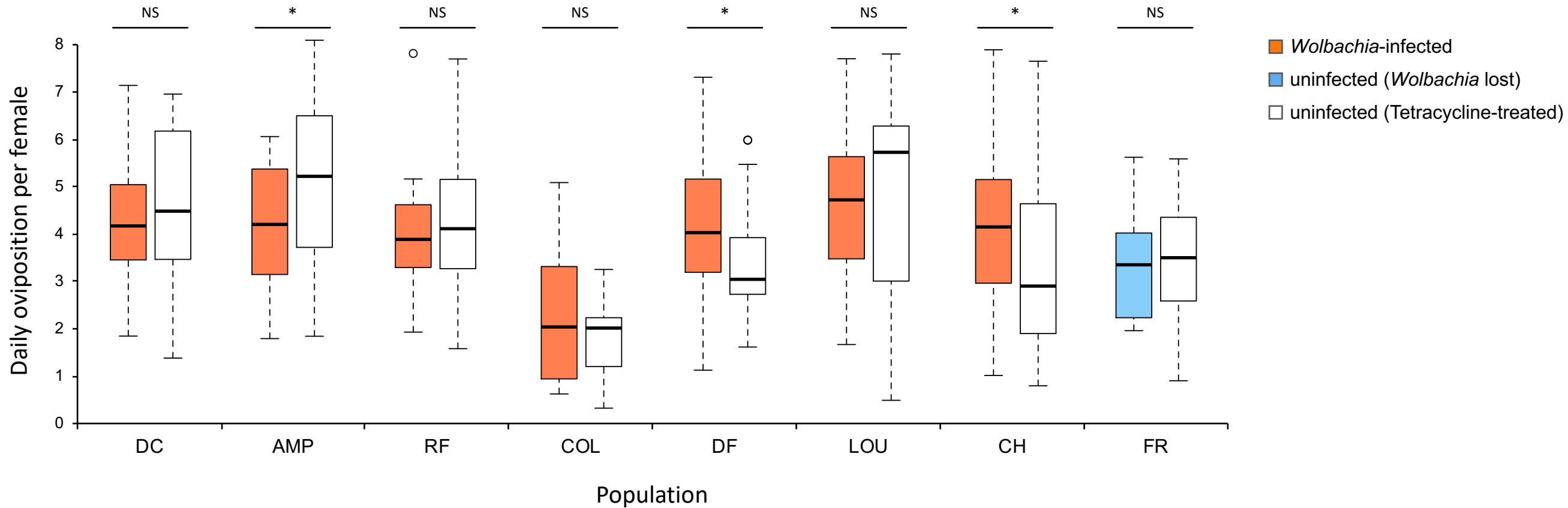
858

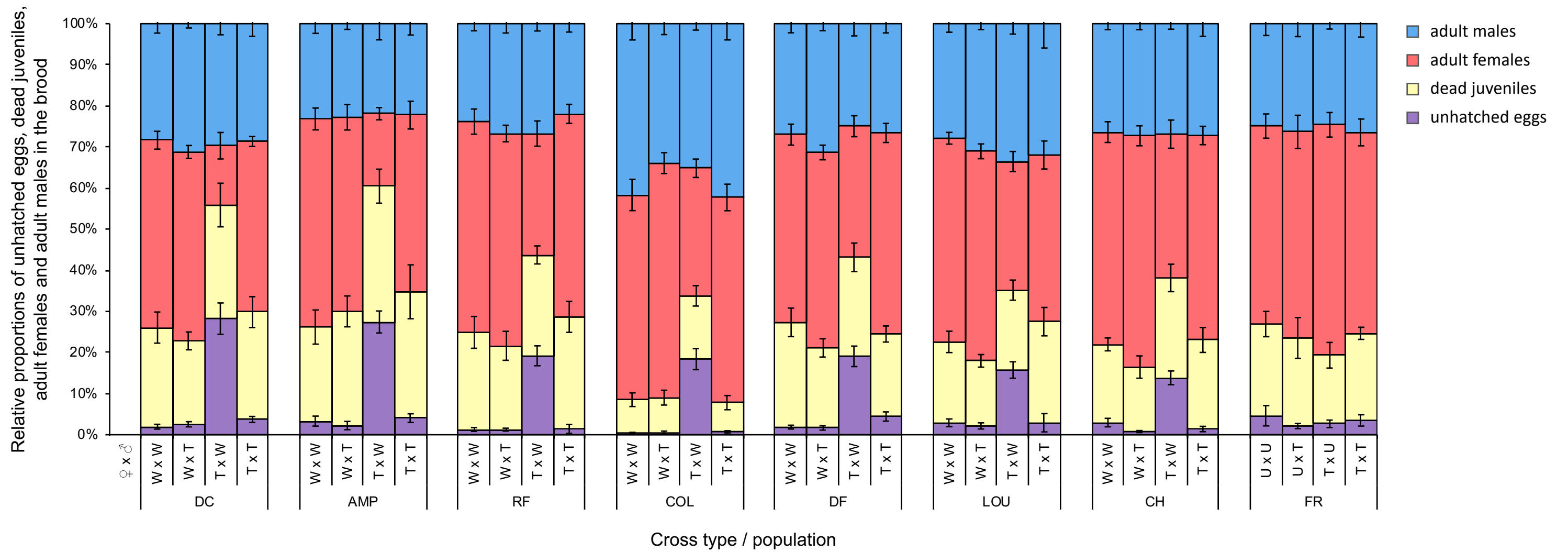
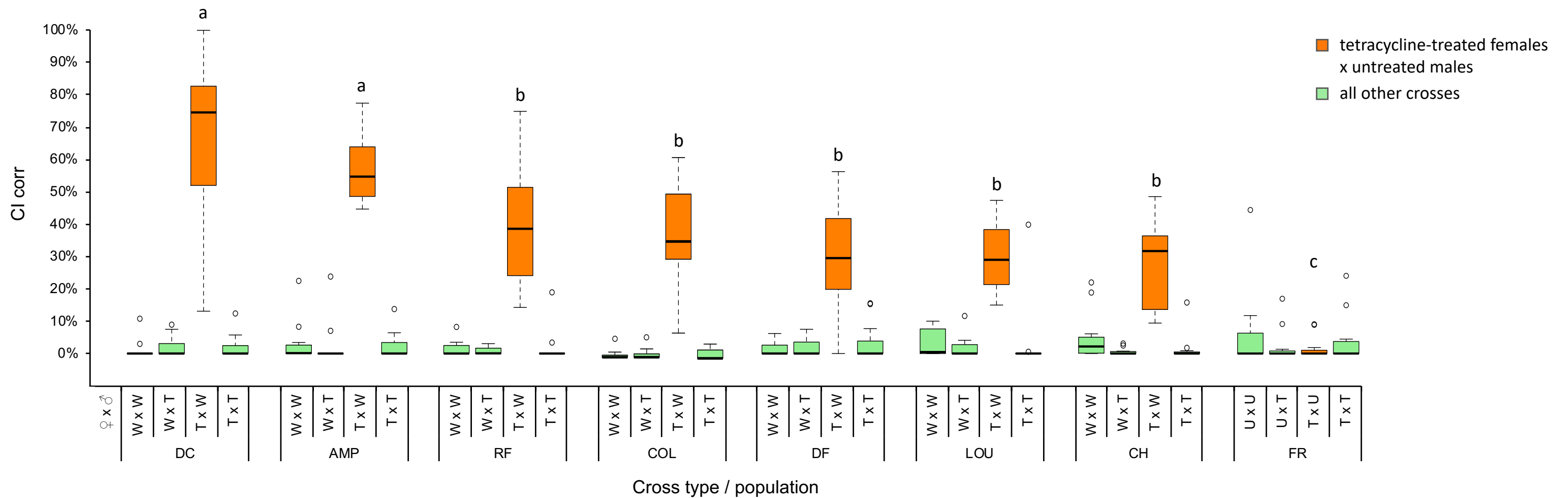
859 **Figure 4. Summary of the development of *T. urticae* eggs and cytoplasmic incompatibility (CI)**  
860 **levels in inter-population crosses using *Wolbachia*-infected mites.** (a) Relative proportions of  
861 unhatched eggs (purple bars), dead juveniles (yellow bars), adult females (red bars) and adult males  
862 (blue bars) for each type possible cross. Bar plots represent means  $\pm$  s.e. (values provided in Table  
863 S3). (b) Boxplot of CI-related mortality estimated using the  $CI_{corr}$  index, which removes the basal  
864 embryonic mortality (estimated in control crosses). No significant differences were found among  
865 crosses (green boxes: intra-population crosses; orange boxes: inter-population crosses).

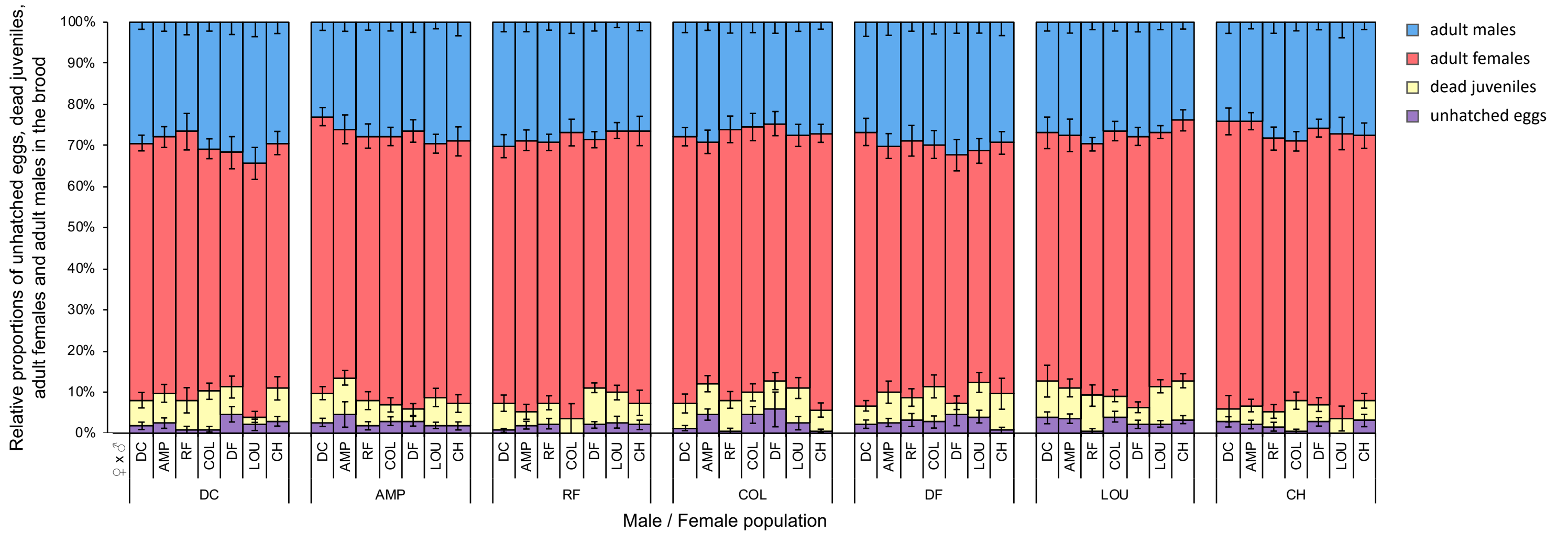
866

867 **Figure 5. Expected invasion of *Wolbachia* based on its phenotypic effects in each population.** We  
868 used the data obtained for the phenotypic effects of *Wolbachia* to parametrize the model for each  
869 population that fixed the infection under laboratory rearing (parameter values provided in Table S4).  
870 Dashed grey lines represent the course of infection frequencies through generations for initial  
871 infection frequencies ranging from 0.1 to 0.9. Green line: course of infection that took place in the  
872 laboratory following the prediction of the model; Dashed red line: threshold for invasion.





**a****b**

**a****b**