

## Endosymbiont diversity in natural populations of Tetranychus mites is rapidly lost under laboratory conditions

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### 1 Endosymbiont diversity in natural populations of *Tetranychus* mites is rapidly

#### 2 lost under laboratory conditions

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#### 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.

#### 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).

Despite the fact that such reproductive manipulation favors the spread of *Wolbachia*, stable 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, Cl 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-Cl; 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.

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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 (AIRo, AIBe, 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 (AIBe: 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

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

Six months after collection from the field (ca. 15 generations), infection by *Wolbachia*, *Cardinium* and *Rickettsia* was checked anew using 15-16 individual females per population (except for the population GRA that was lost during laboratory rearing) using the multiplex PCR described in Zélé *et al* (2018c). Subsequently, pools of 100 female per population were also checked for infection by 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 9 cm<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).

Three days later (d3), the daily female oviposition was estimated taking into account their daily mortality (daily oviposition per female over 3 days = total number of eggs laid on each leaf disc after 3 days / total number of alive females over the three days), and males were discarded. To determine the effect of *Wolbachia* on spider-mite longevity, females were transferred to new leaf 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 ( $CI_{obs}$  = number of unhatched eggs/[number of  $F_1$  females + 207 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<sub>corr</sub> = [(CI<sub>obs</sub> – CCM)/(1 – CCM)], 210 where CCM is the mean embryonic mortality observed in the control crosses (i.e. calculated as Cl<sub>obs</sub>). 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.

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

217

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

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

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

230 All experiments were conducted in a growth chamber under standard conditions (25 ± 2°C, 60% RH,

231 16/8 h L/D).

232

#### 233 Statistical analyses

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

Proportion data were computed using the function cbind, except for Cl<sub>corr</sub> (continuous variable bounded between 0 and 1) for which a "weights" argument was added in the model to account for the number of observations (i.e. number of unhatched eggs + number of adult daughters per disc). Proportion data were subsequently analysed with a binomial error distribution, or with a 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  $X^2$  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 Cl<sub>corr</sub>, 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 = mean daily 274 oviposition of infected females [incl. WxW and WxT crosses] over 3 days / 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 – (Cl<sub>corr</sub>/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 n290 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% Cls; 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 accross 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 lifespan than uninfected females, respectively (model 1.4,  $X_1^2 = 16.34$ , p<0.0001, and model 1.5,  $X_1^2 = 16.34$ ) 320 321 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,  $X_{1}^{2} = 5.08$ , p=0.02, and model 1.6,  $X_{1}^{2} = 17.81$ , 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 uninfected male in DC and RF (model 1.7,  $X_{1}^{2}$  = 5.04, p=0.02, and model 1.8,  $X_{1}^{2}$  = 11.98, p=0.0005, 325 326 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 AMP (model 2.1,  $X_{1}^{2}$  = 5.84, p=0.02), increased oviposition by 0.77 ± 0.36 in DF (model 2.2,  $X_{1}^{2}$  = 4.31, 337 338 p=0.04) and by 0.97  $\pm$  0.54 in CH (model 2.3,  $X_{1}^{2} = 6.41$ , 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:  $X_{1}^{2} = 0.40$ , 340 p=0.52, RF:  $X_{1}^{2}$  = 0.54, p=0.46, COL:  $X_{1}^{2}$  = 0.68, p=0.41, LOU:  $X_{1}^{2}$  = 0.15, p=0.70, FR:  $X_{1}^{2}$  = 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

males and females (model 6.0; Table S2). As the increased proportion of unhatched eggs in incompatible crosses led to a decrease in the production of females but not of males, these results indicate that CI induced by *Wolbachia* does not lead to haploidization of fertilized eggs (MD-type of CI) but to female early mortality (FM-type of CI) in all populations. Finally, the relative proportion of dead juveniles differed between populations and was affected by *Wolbachia* infection in females, with an overall decreased juvenile mortality of ca. 3% in the offspring of infected females, but no 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 Cl<sub>corr</sub> 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  $(X_{1}^{2})$ 368 =1.74, p=0.19) and among RF, COL, DF, LOU and CH ( $X_4^2$ =3.72, p=0.45), but a significantly lower level 369 of Cl in the latter than in the former group of populations (on average 33% and 61%, respectively;  $X_{1}^{2}$ 370 =38.37, p<0.0001). All infected populations differed significantly from the control FR ( $X_{1}^{2}$  =68.90, 371 p<0.0001).

372

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

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

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

The analysis of the proportions of unhatched eggs, daughters and sons in the brood revealed that *Wolbachia* induces a female mortality type of CI (FM-CI; Breeuwer, 1997; Vavre *et al*, 2000) in all populations. However, besides the sex ratio distortion observed in incompatible crosses due to CI, 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 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

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

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

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

534

#### 535 Future directions

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

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

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

577

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#### 833 FIGURE LEGENDS

834

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

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

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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 ± 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<sub>corr</sub> 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).

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

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











Population

Wolbachia-infected
 uninfected (Wolbachia lost)
 uninfected (Tetracycline-treated)



Cross type / population



Cross type / population

а

b

- adult males
- adult females
- dead juveniles
- unhatched eggs









Male / Female population

а

b



- adult males
- adult females
- dead juveniles
- unhatched eggs



