Heredity
https://doi.org/10.1038/s41437-020-0297-9

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ARTICLE

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

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Received: 6 September 2019 / Revised: 24 January 2020 / Accepted: 24 January 2020

Abstract

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

Introduction

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

In diploid species, CI leads to the embryonic mortality of part or all of the offspring resulting from crosses between infected males and uninfected females (or females infected by an incompatible strain). In contrast, crosses between infected

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Supplementary information The online version of this article (https://doi.org/10.1038/s41437-020-0297-9) contains supplementary material, which is available to authorized users.

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Published online: 11 February 2020

females and both uninfected and infected males are fully viable, hence these females have a reproductive advantage relative to uninfected ones. This phenomenon thus allows the rapid spread of CI-inducing symbionts, as shown by many laboratory and field studies. For instance, only five generations were enough for the CI-inducing endosymbiotic bacteria *Wolbachia* to invade population cages of *Drosophila melanogaster* (Reynolds and Hoffmann 2002), or of the mosquito *Aedes albopictus* (Dobson et al. 2002). This bacterium has also been shown to spread rapidly in field populations of different host species (e.g. Turelli and Hoffmann 1995; Kriesner et al. 2013; Bakovic et al. 2018).

Despite the fact that such reproductive manipulation favours the spread of symbionts, stable infection polymorphisms are typical in nature, with some populations being fully infected, others fully uninfected or infected with a different symbiont strain, and others harbouring intermediate symbiont frequencies (e.g. Vavre et al. 2002; Keller et al. 2004; Zhang et al. 2013b; Hamm et al. 2014). This infection polymorphism may be associated with variation in the level of CI, the rate of maternal transmission and the relative fecundity of infected females compared with uninfected ones, which determines the threshold at which a given CI-inducing symbiont can invade a population (Hoffmann et al. 1990; Turelli and Hoffmann 1995). Moreover, variability in infection frequencies between and within regions indicates benefits and costs of infection that vary across temporal and spatial gradients (e.g. Weeks et al. 2002; Oliver et al. 2014; Cass et al. 2016). However, the factors responsible for such variability remain largely elusive. In particular, the relative importance of environmental heterogeneity (e.g. Barton and Turelli 2011; Hancock and Godfray 2012; Schmidt et al. 2017), host diversity and biotic interactions (e.g. within-host interaction with other pathogens or parasites; reviewed in Oliver et al. 2014; Hopkins et al. 2017) in the maintenance of symbiont diversity remains poorly understood.

Laboratory studies may allow to disentangle the effect of the environment and of the host genetic background on symbiont diversity. However, drift and lab adaptation can also deeply impact natural variation. While this has been repeatedly demonstrated regarding nuclear variation (e.g. Hoffmann et al. 2001; Fragata et al. 2014; Francuski et al. 2014; Hoffmann and Ross 2018), few studies have analyzed how laboratory acclimation affects symbiont diversity. Spider mites are good candidates to investigate potential changes in infection polymorphism under laboratory conditions, as they often carry several endosymbiotic bacteria, usually maternally-inherited, with variable prevalence among natural populations. Among them, *Wolbachia* is the most prevalent (e.g. Liu et al. 2006; Gotoh et al. 2007b; Zhang et al. 2013b; Zhang et al. 2016; Zélé et al. 2018a) and induces variable levels of CI, ranging from no CI to complete CI (Vala et al. 2002; Gotoh et al. 2007b; Xie et al. 2011; Suh et al. 2015). In some cases, in spider mites as in other haplodiploid species, CI involves a loss of the paternal set of chromosomes and diploid zygotes arising from incompatible matings may survive as haploid males (Male development—MD-CI; Perrot-Minnot et al. 2002; Gotoh et al. 2003). In most cases, however, fertilized eggs from incompatible crosses fail to hatch as in diploid species, which leads to embryonic mortality of the females only (Female mortality—FM-CI; Breeuwer 1997; Perrot-Minnot et al. 2002; Vala et al. 2002; Gotoh et al. 2003; Suh et al. 2015). Population-specific fitness effects of *Wolbachia* on spider mite life-history traits have also been reported, with costs (Perrot-Minnot et al. 2002; Suh et al. 2015), no effect (Breeuwer 1997; Perrot-Minnot et al. 2002; Vala et al. 2002; Gotoh et al. 2007b), or benefits (Vala et al. 2002; Gotoh et al. 2007b; Xie et al. 2011) on spider mite fecundity, but also variable effects on longevity and development time (Xie et al. 2011). Note, however, that none of these studies (with the exception of Gotoh et al. 2007b) tested for coinfection with other endosymbionts, which may have confounding effects. Indeed, herbivorous spider mites are often (co-) infected with *Cardinium* (Liu et al. 2006; Ros et al. 2012; Zhang et al. 2016), which can also cause FM-CI (Gotoh et al. 2007a; Ros and Breeuwer 2009; Xie et al. 2010; Zhu et al. 2012) without clear effects on other spider mite life-history traits reported to date (but see Zhao et al. 2013a; Zhao et al. 2013b; for *Wolbachia*-*Cardinium* coinfections); and occasionally with *Rickettsia* (e.g. Zhang et al. 2016; Zélé et al. 2018a) or *Spiroplasma* (e.g. Enigl and Schausberger 2007; Staudacher et al. 2017), whose effects in spider mites are still unknown.

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

Materials and methods

Spider mite populations and rearing

Sixteen populations of Tetranychid mites were collected from September to December 2013 in the region of Lisbon,

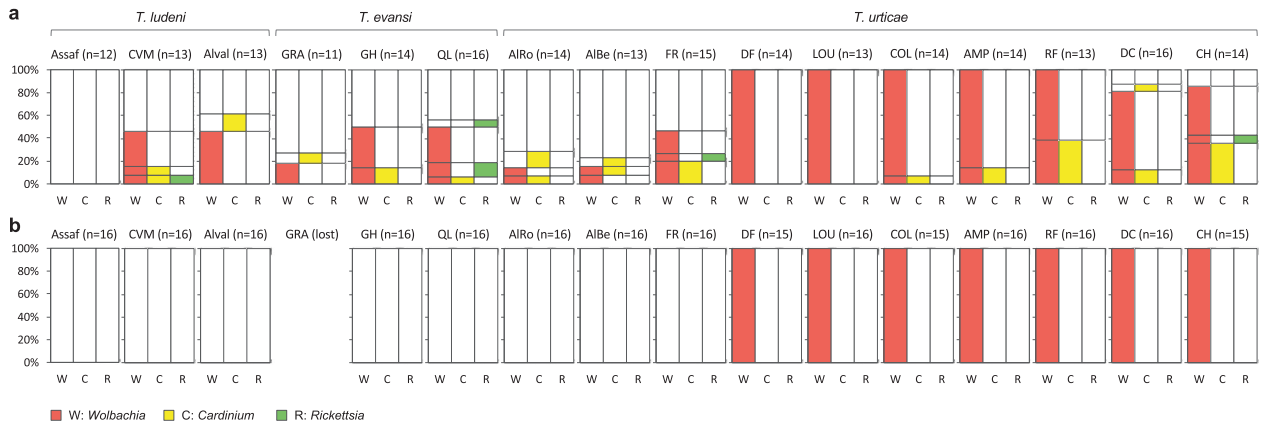

Fig. 1 Changes in endosymbiont infection frequency in each spider mite population following laboratory rearing. Each box represents a population, and within each graph, columns represent the infection status by W: *Wolbachia* (red cells); C: *Cardinium* (yellow cells); and R: *Rickettsia* (green cells)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

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

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). Previous sensitivity tests revealed that multiple symbionts can be detected in a single pool, even at low infection frequencies (up to 1/100 infected females; Zélé et al. 2018a). Finally, as the *wsp* gene was identical for all *Wolbachia* infecting these populations (Zélé et al. 2018a), we characterized the *Wolbachia* infections remaining in laboratory cultures 6 months after collection using a multilocus sequence typing (MLST; Baldo et al. 2006). MLST gene sequences were amplified from DNA extracted from a pool of 100 females per population using standard primers and PCR protocols (Baldo et al. 2006; Zélé et al. 2018a). Chromatograms were checked manually using MEGA version 5.1 beta (Tamura et al. 2011) and we found no evidence for multiple infections within populations (as indicated by the absence of multiple peaks). All MLST sequences were then compared with entries in the PubMLST *Wolbachia* MLST database (available at http://www.pubmlst.org/wolbachia/) and novel sequences were submitted to the database curators for inclusion as new alleles. Each unique combination of MLST sequences was designated as an isolate, submitted to the PubMLST database, and assigned a unique ID number. Isolates with five-locus profiles that did not match an existing strain type were assigned a new strain type (Baldo et al. 2006).

Antibiotic treatments

Roughly 3 months after collection from the field, a tetracycline solution (0.1%, w/v) was used to treat mites ($n = 30$ adult females initially) from each population for three

successive generations (Breeuwer 1997) to obtain uninfected populations. During the treatment, mites were maintained in petri dishes containing bean (or tomato for *T. evansi*) leaf fragments placed on cotton with the antibiotic solution. At each generation, 50 adult mated daughters were transferred to a new petri dish containing fresh leaf fragments and solution. At the third generation after treatment, 14 individual females and a pool of 100 females per population were checked by PCR to confirm that they were uninfected. These populations were maintained in a mass-rearing environment without antibiotics for a minimum of five generations before performing experiments, to avoid potential side effects of antibiotic treatment (e.g. Ballard and Melvin 2007; Zeh et al. 2012).

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

To test the effects of *Wolbachia* in each population that was still infected 6 months after field collection (all from *T. urticae*), the four possible crosses between Tetracycline-treated (T) and -untreated (W, *Wolbachia*-infected) females and males were performed (i.e. T × T, T × W, W × T and W × W, female × male crosses). An additional population (FR), fully uninfected (U) by *Wolbachia* 6 months after field collection, was also included as a control for the effect of the tetracycline treatment. Roughly 2 weeks prior to the experiment, age cohorts were created for each population by collecting ca. 100 females from each mass culture, allowing them to lay eggs during 5 days on detached bean (or tomato) leaves placed on water-soaked cotton. The offspring from these cohorts was used in the experiments.

Two days prior to the onset of this experiment, quiescent virgin females with similar age were randomly collected from each cohort and placed separately on a leaf fragment to allow emergence while remaining virgin. Males were isolated from the same cohort 1 day before the beginning of the experiment to avoid potential sperm depletion. On the first day of the experiment (d0), 10 adult virgin females were placed with 10 males on a 9 cm^2 bean leaf disc to allow mites to mate in panmixia. This procedure was chosen to increase potential conflicts over sex ratio between *Wolbachia* and its female host. Indeed, while *Wolbachia* always benefits from a higher proportion of daughters (i.e. due to its maternal mode of transmission; Hurst et al. 1996; Werren and Beukeboom 1998), the optimal sex ratio for female spider mites depends on the number of foundresses in a 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 3 days), and males were discarded. To determine the effect of *Wolbachia* on spider mite longevity, females were transferred to new leaf discs every 3 days until death and their daily survival was recorded. To determine the type of CI induced by *Wolbachia* in this system (i.e. MD-CI and/or FM-CI; Vavre et al. 2000), the number of unhatched eggs and of adult offspring (F_1 females + F_1 males) obtained over the first 3 days of the experiment were counted 5 and 15 days after removing the parents, respectively (d8 and d18). This allowed computing the relative proportions of unhatched eggs (number of unhatched eggs/total number of eggs), dead juveniles ([total number of eggs − number of unhatched eggs − number of F_1 adults]/total number of eggs), males (number of F_1 males/total number of eggs), and females (number of F_1 females/total number of eggs) in all populations.

Finally, as we found that *Wolbachia* induces FM-type of CI in all tested populations (cf. "Results") we determined the level of CI induced by *Wolbachia*, as the proportion of embryonic death of females in incompatible crosses (CI_{obs} = number of unhatched eggs/[number of F_1 females + number of unhatched eggs]). To account for variation in background embryonic mortality (not related to CI and including both sons and daughters embryonic mortality), we used a corrected index of CI (Poinsot et al. 1998; Cattel et al. 2018) calculated as follows: $CI_{corr} = [(CI_{obs} - CCM)/(1 - CCM)]$, where CCM is the mean embryonic mortality observed in the control crosses (i.e. calculated as CI_{obs}). To control for an effect of infection on the background embryonic mortality, T × T and W × T crosses were used as controls for T × W and W × W 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 the third block, due to contaminations detected in the previous blocks (i.e. these data were discarded).

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 2 cm^2 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 3 days of the experiment were removed from statistical analyses (cf. final sample sizes in Table S3). Subsequently,

CI_{corr} was calculated as above, using each intra-population cross as control for a given female population when crossed with males from all other populations.

All experiments were conducted in a growth chamber under standard conditions (25 ± 2 °C, 60% RH, 16/8 h L/D).

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.

Survival data (models 1.0–1.8) were analysed using Cox proportional hazards mixed-effect models (coxme, kinship package). Hazard ratios (HR) were obtained from these models as an estimate of the difference between the rates of dying (i.e. the instantaneous rate of change in the log number of survivors per unit time; Crawley 2007) between the control and the other crosses. All other response variables were analysed using generalized linear mixed models with the glmmTMB procedure (glmmTMB package; Brooks et al. 2017), which allows using a wide range of error distribution that are not implemented in the glmer procedure. Female daily oviposition was analysed with a gamma error distribution with a log link to account for heteroscedasticity (models 2.0–2.8). Proportion data were computed using the function cbind, except for CI_{corr} (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 3.0–12.0).

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

Modelling *Wolbachia* invasion under laboratory conditions

To predict *Wolbachia* invasion in each population that was fully infected 6 months after collection, we used the data obtained for the phenotypic effects of *Wolbachia* to parameterize a mathematical model for FM-type CI (cf. "Results") developed by Vavre et al. (2000). This model allows estimating the value of the unstable equilibrium (i.e. the threshold for infection rates above which *Wolbachia* is expected to reach fixation, and below which it is predicted to go extinct; Hoffmann et al. 1990). The parameters of this model are the relative fecundity of infected versus uninfected females (F; here this parameter was also weighted by the effect of *Wolbachia* on the female survival, so F = mean daily oviposition of infected females [incl. W × W and W × T crosses] over 3 days/mean daily oviposition of uninfected females [incl. T × W and T × T crosses] over 3 days/hazard ratio of infection in females), the proportion of eggs that escape CI in the incompatible cross (H; i.e. the reverse of the CI level, so here $H = 1 - (CI_{corr}/100)$), and the proportion of uninfected eggs produced by infected females (μ; i.e. the reverse of the transmission rate). We assumed perfect maternal transmission as only a transmission rate of 100% may explain an observed infection frequency of 100% in females when CI is incomplete. Nevertheless, to account for potential inaccuracy of observed infection frequencies, we estimated the minimum transmission rate that can explain the maintenance of *Wolbachia* in each population (Table S5).

Results

Changes in endosymbiont prevalence under laboratory conditions

The screen for endosymbiont infection following 6 months of laboratory rearing (ca. 15 generations) revealed a drastic change in symbiont prevalence after field collection (Fig. 1a and described in Zélé et al. 2018a). Indeed, neither *Cardinium* nor *Rickettsia* were detected in any of the populations tested (prevalence <11% with 95% confidence intervals; Jeffreys interval recommended for small *n* by Brown et al. 2001), whereas all females were found infected by *Wolbachia* in seven *T. urticae* populations (prevalence >88–89% with 95% confidence intervals), and none of them in eight

populations, belonging to *T. urticae*, *T. evansi* and *T. ludeni* (prevalence <11% with 95% confidence intervals; Fig. 1b). Moreover, diagnostic PCRs performed on pools of 100 females 6, 12, 18 and 24 months after field collection (Fig. S1) confirmed the loss (prevalence <1%) of endosymbionts in these populations. In general, there is a good correlation between the symbiont frequency in the original population and the probability of infection loss or fixation. Indeed, *Wolbachia* was lost in the populations in which its initial frequency was lower than 50%, while it reached fixation in the other populations.

Wolbachia diversity in the laboratory

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

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

Effects of *Wolbachia* on spider mite longevity

As all symbionts were lost in *T. evansi* and *T. ludeni*, the following results were obtained only in the *T. urticae* populations in which *Wolbachia* reached fixation in the laboratory. Daily female survival was significantly affected by the status (treated with tetracycline or not) of both the females and their mates, but in a population-specific manner (model 1.0 in Table S1, see also Table S2 for log HRs and the significance of all fixed effects and their interactions; Fig. S2 for survival curves). Indeed, the independent analysis of each population showed that the tetracycline treatment did not affect longevity in the populations AMP, DF and the uninfected control FR (model 1.1–1.3), 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, $X^2_1 = 16.34$, $p < 0.0001$, and model 1.5, $X^2_1 = 6.40$, $p = 0.01$, respectively). In addition, females mated with a *Wolbachia*-infected male survived 1.3 and 1.6 times less than those mated with an uninfected male in COL and LOU, respectively (model 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 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, $X^2_1 = 5.04$, $p = 0.02$, and model 1.8, $X^2_1 = 11.98$, $p = 0.0005$, respectively).

Effects of *Wolbachia* on spider mite fecundity

The analysis of daily female oviposition over 3 days revealed no significant 3-way interaction between populations, female and male infection status (model 2.0, see Table S2 for the significance of all fixed effects and their interactions). Sequential removal of non-significant factors (including their interactions) from the model unveiled no significant interaction between female and male infection status and between population and male infection status, nor a significant effect of male infection status. However, a significant interaction between population and female infection status was found (Fig. 2). The independent analysis of each population further revealed variable effects of *Wolbachia* infection in females depending on the population: decreased oviposition by 0.93 ± 0.45 in AMP (model

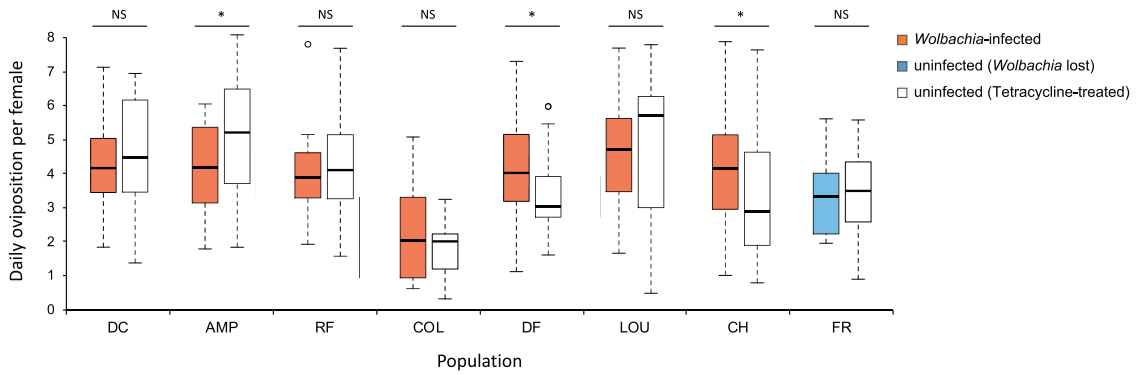

Fig. 2 *Wolbachia* effects on oviposition of *T. urticae* females. Orange boxes: *Wolbachia*-infected females, white boxes: tetracycline-treated females. The statistical significances are given above bars: *$p <$ 0.05; ns not significantly different at the 5% level. The population FR (blue box) lost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

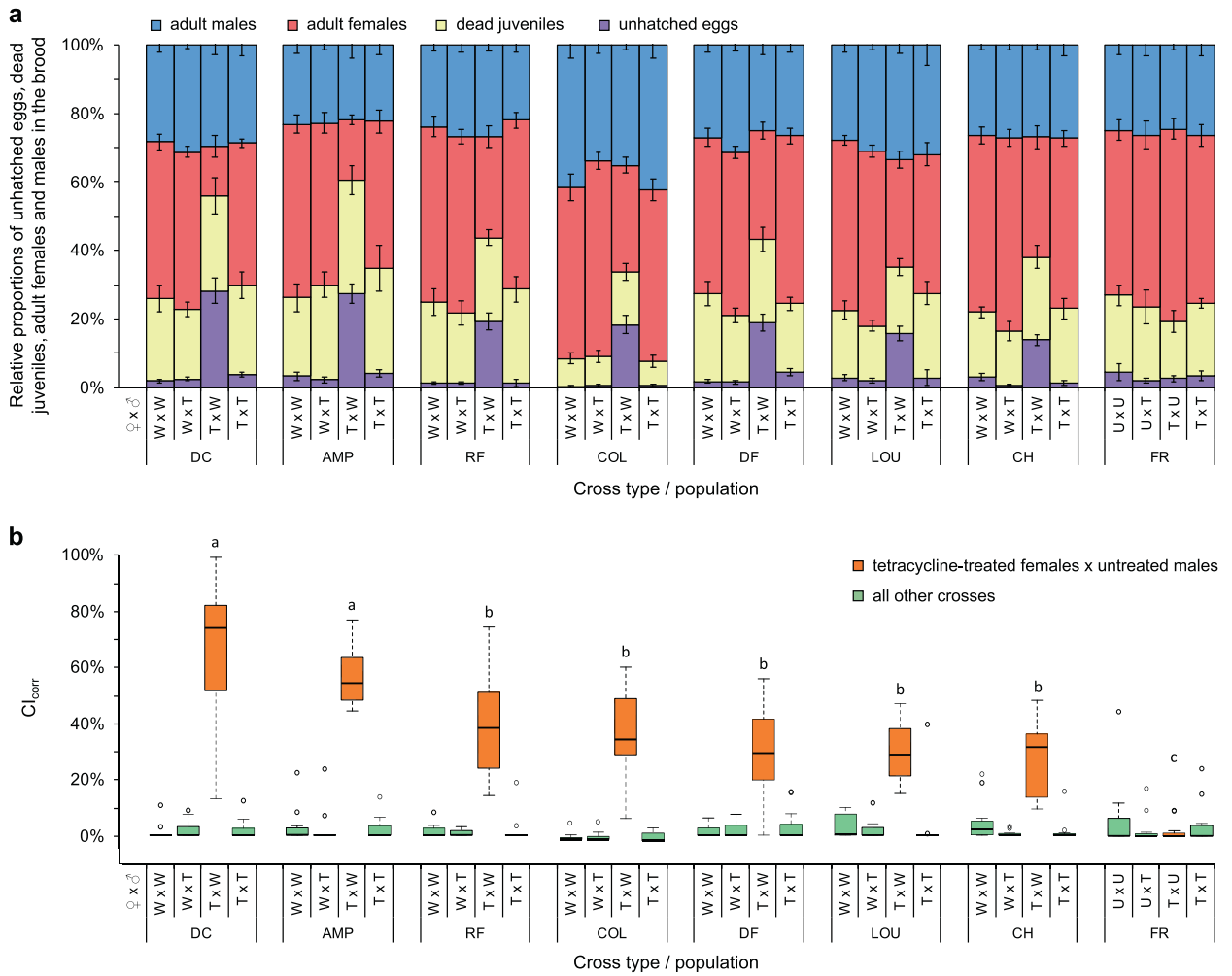

Fig. 3 Summary of the development of *T. urticae* eggs and cytoplasmic incompatibility (CI) levels in intra-population crosses between *Wolbachia*-infected and uninfected mites. a Relative proportions of unhatched eggs (purple bars), dead juveniles (yellow bars), adult females (red bars) and adult males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

2.1, $X^2_1 = 5.84$, $p = 0.02$), increased oviposition by 0.77 ± 0.36 in DF (model 2.2, $X^2_1 = 4.31$, $p = 0.04$) and by 0.97 ± 0.54 in CH (model 2.3, $X^2_1 = 6.41$, $p = 0.01$), but no significant effect of *Wolbachia* infection in the other populations, including the control population FR (models 2.4–2.8, DC: $X^2_1 = 0.40$, $p = 0.52$, 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: $X^2_1 = 0.36$, $p = 0.55$).

Effects of *Wolbachia* on offspring development

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

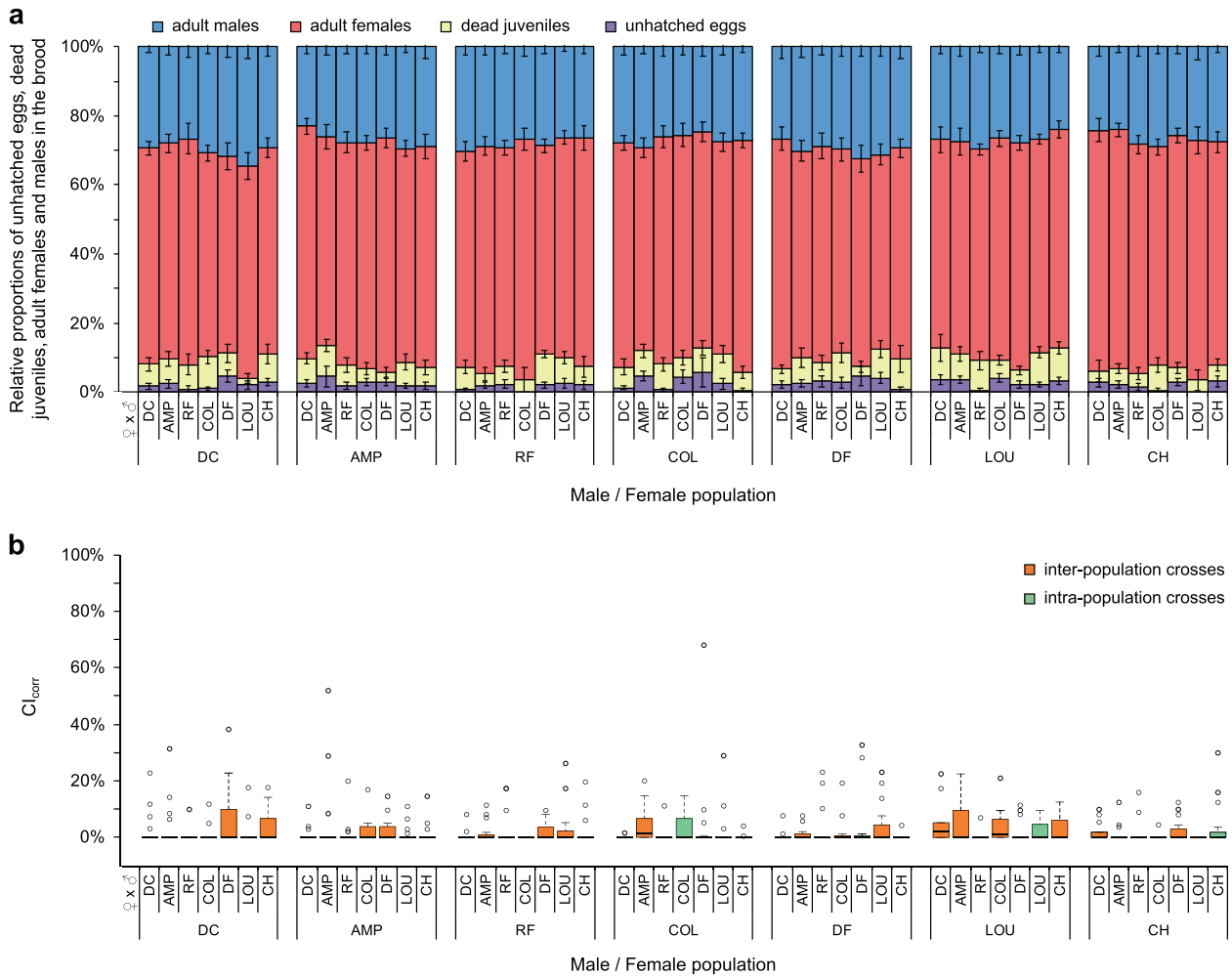

Fig. 4 Summary of the development of *T. urticae* eggs and cytoplasmic incompatibility (CI) levels in inter-population crosses using *Wolbachia*-infected mites. a Relative proportions of unhatched eggs (purple bars), dead juveniles (yellow bars), adult females (red bars) and adult males (blue bars) for 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_{corr} 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).

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).

CI level induced by *Wolbachia* in each population

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

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

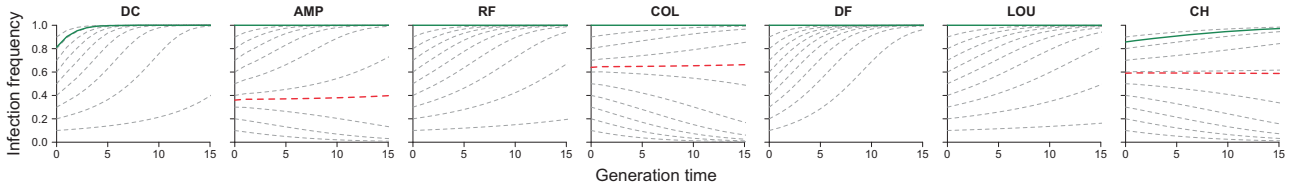

Fig. 5 Expected invasion of *Wolbachia* based on its phenotypic effects in each population. We used the data obtained for the phenotypic effects of *Wolbachia* to parametrize the model for each population that fixed the infection under laboratory rearing (parameter values provided in Table S4). Dashed 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.

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 CI_{corr} (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 8.0–12.0, 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.

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

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

Discussion

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

Variability in *Wolbachia* effects and level of CI

Wolbachia affected differently the longevity of females from different populations, with either no effect or a cost of infection on survival. Moreover, we found variable effects of mating with *Wolbachia*-infected males on this trait, with both positive and negative effects, as previously found in *T. urticae* populations in China (Xie et al. 2011). *Wolbachia* also affected female fecundity differently depending on the population, ranging from no effect to costs or benefits, as in many spider mite populations worldwide (Breeuwer 1997;

Perrot-Minnot et al. 2002; Vala et al. 2002; Gotoh et al. 2007b; Xie et al. 2011; Suh et al. 2015). These effects, although of relatively low amplitudes may still have important consequences for the invasion dynamics of *Wolbachia* (e.g. the existence of an invasion threshold when *Wolbachia* induces a fecundity or a longevity cost, 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 suggests that sex ratio distortion induced by *Wolbachia* in absence of CI, as observed by Vala et al. (2003), is not a common feature of *Wolbachia* in spider mites.

Finally, we found that the level of CI induced by *Wolbachia* also varies depending on the population (ca. 33% in the populations RF, COL, DF, LOU and CH, and ca. 61% in AMP and DC), albeit *Wolbachia wsp* (Zélé et al. 2018a) and MLST sequences at the time of the experiment did not differ among populations. Such variability of FM-CI levels induced by *Wolbachia*, without clear association with different *Wolbachia wsp* sequences, has been previously reported in spider mites (Vala et al. 2002; Gotoh et al. 2003; Gotoh et al. 2007b; Xie et al. 2011; Suh et al. 2015). However, although the use of *wsp* and of the MLST approach is a standard in the community of *Wolbachia* researchers, these genes may not be particularly suited to discriminate between closely-related strains (Ishmael et al. 2009; Atyame et al. 2011; Conner et al. 2017), or to accurately reflect the properties of a *Wolbachia* strain (Bleidorn and Gerth 2018), including different level of CI induction (Hamm et al. 2014; Kaur et al. 2017). In particular, genes responsible for CI induction (the *cidA-cidB* or *cifA-cifB*, and and *cinA-cinB* operons) have recently been identified in different *Wolbachia* strains infecting different hosts (Beckmann et al. 2017; LePage et al. 2017; Bonneau et al. 2018; Lindsey et al. 2018). It has been proposed that CI strength could be adjusted via the level of expression of these genes, or the ratio of *cifA* and *cifB* transcripts across development (Lindsey et al. 2018). Our populations could thus be infected with different but closely-related *Wolbachia* strains differing for these genes. Unfortunately, we failed to amplify the *cidA* and *cidB* genes of *Wolbachia* in *T. urticae* (see Box S1) and future work should focus on sequencing the entire genome of *Wolbachia* from spider mites to improve our understanding of this system. Still, the absence of sequence divergence among *Wolbachia* from different populations is in agreement with our finding that all populations were compatible with each other (i.e. full CI rescue between populations). Therefore, variations across *T. urticae* populations in fitness effects and in the strength of reproductive phenotypes may be due to the hosts specific genetic backgrounds as shown in some drosophila species (e.g. Reynolds and Hoffmann 2002; Mercot and Charlat 2004; Cooper et al. 2017), but also in *T. urticae* (Sun et al. 2016).

Loss or fixation of endosymbionts in the laboratory

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

The spread of CI-inducing symbionts is predicted to be more likely than that of a comparable neutral genetic element, even in the face of an invasion threshold (Jansen et al. 2008). Therefore, the loss of endosymbionts in populations with high population density, and when the initial infection frequency was close to 50% (e.g. *Wolbachia* in CVM, Alval, GH and QL, or *Cardinium* in RF and CH), suggests that the lost symbionts did not induce high CI levels that could compensate for fitness costs (e.g. due to fitness costs of infection, the populations AMP, COL and CH are also expected to lose the infection for an initial infection frequency below 36, 70 and 59%, respectively; Fig. 5) and/or drift effects. Indeed, not only variability in CI levels is a common feature in spider mites, but several studies have also reported infections by non CI-inducing *Wolbachia* (Perrot-Minnot et al. 2002; Vala et al. 2002; Gotoh et al. 2003, 2007b; Xie et al. 2011; Suh et al. 2015) and *Cardinium* (Gotoh et al. 2007a) strains. 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 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.

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

What explains the maintenance of symbiont diversity in the field compared with 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 2 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.

Different prevalence levels between populations might be explained by spatial variation of environmental conditions in the field, which may impact the effects of endosymbionts on host fitness. For example, temperature is known to affect endosymbiont transmission, their fitness effects on hosts and the strength of reproductive manipulation (e.g. Clancy and Hoffmann 1998; Anbutsu et al. 2008; Carrington et al. 2010; Bordenstein and Bordenstein 2011; Ross et al. 2017b). In line with this, *Wolbachia* prevalence varies with temperature in the field (e.g. Toju and Fukatsu 2011; Sumi et al. 2017; Ferguson et al. 2018), and, in spider mites, a field study shows that *Wolbachia* prevalence increases with temperature (e.g. Zhu et al. 2018), but a too high temperature cures mites from this symbiont (e.g. Van Opijnen and Breeuwer 1999). Spatial variation in other environmental factors such as host nutrition (e.g. Clancy and Hoffmann 1998), including the host plant of herbivorous arthropods (reviewed in Frago et al. 2012), and/or the presence of host pathogens or natural enemies (reviewed in Oliver et al. 2014; Hopkins et al. 2017), may affect the prevalence of symbionts and explain differences between populations. Similarly, temporal (seasonal and/or circadian) variations in all these factors may lead to temporal variations in endosymbiont prevalence within populations and, hence, may explain the maintenance of infection polymorphism at the population level.

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

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 host–symbiont 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.

Data availability

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

Acknowledgements We are grateful to Joaquin Calatayud, Salomé Clémente, Diogo Godinho and Leonor Rodrigues for their help in collecting data from the intra-population crosses; to André Alves, Catarina Bota, Jéssica Paulo, José Leitão, Andreia Oliveira and Luís Silva for the inter-population crosses. We also thank Patrick Makoundou for his attempts to amplify *cidA* and *cidB* gene fragments from *T. urticae*. Finally, we thank Olivier Duron, Inês Fragata, Michael Turelli and Filipa Vala for useful discussions and suggestions. This work was funded by an FCT-ANR project (FCT-ANR//BIA-EVF/0013/2012) to SM and Isabelle Olivieri, and by an FCT-Tubitak project (FCT-TUBITAK/0001/2014) to SM and Ibrahim Cakmak. FZ was funded through an FCT Post-Doc fellowship (SFRH/BPD/125020/2016). Funding agencies did not participate in the design or analysis of experiments.

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

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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