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Conditional fitness benefits of the *Rickettsia* bacterial symbiont in an insect pest

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Abstract Inherited bacterial symbionts are common in arthropods and can have strong effects on the biology of their hosts. These effects are often mediated by host ecology. The *Rickettsia* symbiont can provide strong fitness benefits to its insect host, *Bemisia tabaci*, under laboratory and field conditions. However, the frequency of the symbiont is heterogeneous among field collection sites across the USA, suggesting that the benefits of the symbiont are contingent on additional factors. In two whitefly genetic lines collected from the same location, we tested the effect of *Rickettsia* on whitefly survival after heat shock, on whitefly competitiveness at different temperatures, and on whitefly competitiveness at different starting frequencies of *Rickettsia*. *Rickettsia* did not provide protection against heat shock nor affect the competitiveness of whiteflies at different temperatures or starting frequencies. However, there was a strong interaction between *Rickettsia* infection and whitefly genetic line. Performance measures indicated that *Rickettsia* was associated with significant female bias in both whitefly genetic lines, but in the second whitefly genetic line it conferred no significant fitness benefits nor conferred any competitive advantage to its host over uninfected whiteflies in population cages. These results help to explain other reports of variation in the phenotype of the symbiosis. Furthermore, they demonstrate the complex nature of these close symbiotic associations and the need to consider these interactions in the context of host population structure.

Keywords *Bemisia tabaci* · Temperature · Frequency dependence · Genetic line · Heat shock

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Introduction

The costs and benefits of interactions between species vary in space and time, often in predictable ways based on ecological conditions (Bronstein 1994). Insects are a substantial part of many ecological networks and often form close associations with maternally inherited, intracellular bacteria. Insect facultative symbionts are often reproductive manipulators, changing their host's reproduction in ways that increase their spread in host populations (Werren et al. 2008). Alternatively or in addition, they may conditionally mediate the ecological interactions of their insect hosts such that the evolution of the association depends on the net outcomes under a variety of ecological conditions (Hussa and Goodrich-Blair 2013). For example, some symbionts can influence the interactions with parasitoids (Oliver et al. 2003; Xie et al. 2014), insect pathogens (for example, Scarborough et al. 2005; Hedges et al. 2008; Teixeira et al. 2008; Jaenike et al. 2010; Lukasik et al. 2013), or the suite of plants on which the arthropod hosts feed (Ferrari et al. 2007; Hammer and Bowers 2015; Wagner et al. 2015). Although many examples of ecological effects of insect symbionts have emerged since the early 2000s, some records indicate that symbiont frequencies may vary more than predicted for a single selective agent

(Oliver et al. 2014). These reports of symbiont dynamics indicate that there are multiple ecological drivers of conditional costs or benefits of symbiont infection, many of which are still unknown.

Bemisia tabaci (Gennadius) (Hemiptera: Aleyrodidae) is a highly polyphagous herbivore that causes millions of dollars of agricultural losses worldwide each year by direct feeding, vectoring viruses, and the excetion of sticky honeydew that serves as a substrate for mold growth (Oliveira et al. 2001). It is considered a complex of cryptic species (Dinsdale et al. 2010; De Barro et al. 2011), and the putative species present in the field in the USA is referred to as the B (or B-biotype) or Middle East–Asia Minor 1 (De Barro et al. 2011). Hereafter, all references to *B. tabaci* refer to this putative species unless otherwise stated.

Bemisia tabaci has an obligate symbiont, *Portiera aleyrodidarum*, which provides its whitefly host with amino acids and carotenoids (Sloan and Moran 2012). Whiteflies also have a complex community of facultative endosymbionts, which can vary by species and geographical area. One of these facultative symbionts, *Rickettsia* sp. nr. *bellii* (Gottlieb et al. 2006), dramatically increases fitness and alters sex ratios under laboratory conditions for *B. tabaci* collected in Arizona, USA (Himler et al. 2011). *Rickettsia* occurs throughout the body of the whitefly and is maternally inherited by entering the oocytes (Gottlieb et al. 2006). Transmission through the plant has also been observed between feeding *B. tabaci*, and the presence of genetically similar *Rickettsia* in distantly related arthropods implies rare interspecific horizontal transmission (Caspi-Fluger et al. 2012). This symbiont swept through *B. tabaci* populations in the southwestern USA from 2000 to 2006 and has remained at high frequencies since (Himler et al. 2011; Cass et al. 2015). *Rickettsia*-infected whiteflies quickly outcompeted their uninfected counterparts in field cage trials in this region (Asiimwe et al. 2014), and in laboratory trials the *Rickettsia*- infected whiteflies were protected against an entomopathogenic bacterium that is common in the environment (Hendry et al. 2014).

There is mounting evidence that the interaction between *Rickettsia* and *B. tabaci* is not spatially uniform. Despite the strong fitness benefits of *Rickettsia* from Arizona, frequencies of *Rickettsia* in *B. tabaci* collected from field sites across the USA were heterogeneous in 2011, ranging from 56 to 100 % (Cass et al. 2015). In Israel, *Rickettsia* frequencies are currently lower than in any USA population, and have declined in the last 15 years (Cass et al. 2015). In Israel, *Rickettsia* has few apparent fitness benefits under laboratory conditions (Chiel et al. 2009), and appears to increase whitefly susceptibility to insecticides (Kontsedalov et al. 2008). There have been mixed reports about the ability of *Rickettsia* to protect whiteflies against heat shock and extreme low or high temperatures (Mahadav et al. 2009; Brumin et al. 2011; Shan et al. 2014). There appears to be little genetic diversity in *Rickettsia* from these whiteflies, with samples from across the USA having 100 % sequence identity for an intergenic spacer region that is known to be highly variable in other *Rickettsia* (Cass et al. 2015). In the work described in the present paper, we explored the effect of whitefly genetic line and temperature on the interaction between *Rickettsia* and *B. tabaci* from Arizona.

Phenotypic differences are commonly associated with different symbiont isolates (for example, Oliver et al. 2005; Burke et al. 2010), and host genetic line has been shown to mediate symbiont phenotype, especially in reproductive manipulator symbionts (for example, Boyle et al. 1993; Fujii et al. 2001; McGraw et al. 2001; Bordenstein et al. 2003; Kondo et al. 2005). Less is known about the effects of host genetic background on a symbiont with a mutualist phenotype. We might expect less variation in host genetic effects when there is little or no conflict between host and symbiont genomes. The pattern shown so far has been mixed, with host variation found in some cases (for example, Ferrari et al. 2007) and not in others (for example, Oliver et al. 2005). We tested the fitness effect of *Rickettsia* in an additional whitefly genetic line to the one tested by Himler et al. (2011) in order to determine the role of host genetic background in the *Rickettsia*-whitefly phenotype.

We assessed how temperature (heat shock and constant hot or cold) affects the fitness benefits of *Rickettsia* in whiteflies collected in southern Arizona. We were especially interested in temperature effects because, in southern Arizona, where the strong fitness benefits were reported, summer temperatures regularly exceed 40 °C, with daily temperature fluctuations exceeding 25 °C (www.noaa.gov; www.cals.arizona.edu/azmet/06.htm). Other bacterial symbionts of insects have been shown to be positively or negatively affected by temperature or to affect their host's susceptibility to extreme temperatures (Rigaud et al. 1991; Chen et al. 2000; Montllor et al. 2002; Russell and Moran 2006; Jia et al. 2009; Wiwatanaratanabutr and Kittayapong 2009; Burke et al. 2010; Morag et al. 2012; Fan and Wernegreen 2013), and temperature change may be one of the cues needed to activate the pathogenicity of *Rickettsia* infecting humans (Policastro et al. 1997). Increasing global temperatures are likely to have profound effects on the interactions between symbionts and their insect hosts (Kiers et al. 2010; Wernegreen 2012) and variable performance of *Rickettsia*-infected whiteflies at different temperatures raises the possibility that climate change could affect the spread of *Rickettsia* and the ability of whiteflies to adapt to changing environmental conditions.

Materials and methods

Whitefly genetic lines

Experiments were performed with two inbred lines herein referred to as MAC1 and MAC2, originating from whiteflies collected in autumn 2006 (Himler et al. 2011) and autumn 2009, respectively, from cotton at the Maricopa Agricultural Center, AZ, USA. Approximately 100 individual field-collected female whiteflies were isolated in leaf disk arenas, their progeny collected, and the parent and some progeny sacrificed for diagnostic PCR to determine *Rickettsia* infection status. Whiteflies of the same status [*Rickettsia*-infected (R^+) or uninfected (R^-)] were pooled into culture cages. It is difficult to untangle the effects of symbionts in whiteflies, as they are refractive to antibiotic curing of symbionts and microinjection to transfer symbionts among host clonal genotypes. Therefore, introgression was used to homogenize the genetic background between R^+ and R^- sublines. Within each genetic line, an introgression series of six generations was made by backcrossing R^+ females and their descendants with R^- males, in 2009 for MAC1 (as described in Himler et al. 2011) and in 2010 for MAC2. Whitefly pupae were isolated to produce at least fifty virgin females in each generation to mate with males from the uninfected line. After completion of this series, >98 % of nuclear alleles were shared between the R^+ and R^- whiteflies of the same line (for example, see Brelsfoard et al. 2008).

Whitefly rearing and *Rickettsia* infection status

Whitefly cultures were maintained in the laboratory on cowpea plants, *Vigna unguiculata*. Experiments were performed at 16 h light/8 h dark, 65 % relative humidity, 27 °C control temperature in climate-controlled rooms or in climatic incubators (E-30B, Percival Scientific, Perry, IA, USA), with temperature conditions confirmed by data loggers (HOBO, Bourne, MA, USA). Experiments began with adult whiteflies less than 24 h post-emergence: leaves with fourth-instar nymphs from the culture cages were kept abaxial side up on 1 % agar in 85-mm-diameter petri dishes with ventilated lids, inverted for adult whiteflies to emerge overnight. Whiteflies were counted and sexed under a stereomicroscope. *Rickettsia* infection status was tested in individual female whiteflies using diagnostic PCR, as described previously (Cass et al. 2015). Samples of whitefly cultures were regularly tested to confirm that they maintained the correct infection status.

Interaction of *Rickettsia* and host genetic lines

The effects of *Rickettsia* were compared in the two whitefly genetic lines by measuring whitefly fecundity (number of adult progeny), development time, developmental survival, and the sex ratio of the R^+ and R^- sublines. The experiment was initiated in cages made from ventilated, plastic gallon jars, inverted over a potted plant with two or three seven-day-old cowpea seedlings bearing two leaves. Six (for the $MAC1R^+$, $MAC2R^+$, $MAC2R^-$) and nine (for the $MAC1R^-$) replicate cages were used for each subline. Newly emerged adults were quickly sexed on ice blocks and checked for recovery in glass vials. Twenty females and twenty males were released into each replicate cage for six days of oviposition, after which they were removed. Nine days later, when many fourth-instar nymphs were present but no adults had yet emerged, the two oldest leaves were removed from each plant, all eggs and nymphs on the abaxial side were counted, and then the leaves were placed on 1 % agar in inverted petri dishes with ventilated lids for the adults to emerge. The next day the counting procedure was repeated for the two newer leaves with the highest number of nymphs for each plant. It was necessary to stagger the counting over two days due to the time it took to count all whitefly progeny. The number and sex of adult whiteflies that emerged from each leaf were recorded daily until no more emerged. Developmental survival was calculated as the total number of adults emerged divided by the total number of eggs and nymphs counted at the time the leaves were removed.

Developmental survival and the sex ratio were analyzed in a logistic regression with *Rickettsia* infection and whitefly genetic line as explanatory variables, using R (R Development Core Team 2010). A quasibinomial model was used when the residual deviance was greater than the degrees of freedom. The number of adult progeny per cage was analyzed in an ANOVA with *Rickettsia* infection and whitefly genetic line as explanatory variables. Development time in days was analyzed in JMP version 8.0 in a standard least squares restricted maximum likelihood linear model with *Rickettsia* infection, sex, and whitefly genetic line as explanatory variables and replicate cage as a nested random effect.

Mortality after heat shock

Heat shock was performed in climatic incubators (E-30B, Percival Scientific) with temperature conditions during the experimental period confirmed by data loggers (HOBO). The heat shock conditions were based on the temperatures observed in the cotton-growing regions of Arizona in 2010 (the Arizona Meteorological Network database, ag.arizona.edu/azmet), optimized in a series of trials at different

temperature–time combinations using more than 2000 whiteflies. Heat shock was performed with individual adult whiteflies aged 24–48 h post-emergence either in glass vials or on leaf discs, as described below. The glass vials and leaf disc cups were closed with mesh caps to allow air, heat, and moisture circulation.

Heat shock in glass vials

Whiteflies, each in an individual glass vial, were exposed to 40 °C for 3 h, after which the survivorship and sex of each whitefly was scored. For each of two experimental blocks, approximately 50 female whiteflies and approximately 25 male whiteflies from each of the four sublines were exposed to heat, i.e., a total of more than 600 whiteflies were exposed to heat shock in glass vials. An approximately equivalent number of whiteflies for each experimental block and subline were kept as controls at 27 °C. Proportional mortality was analyzed in JMP version 8.0 using logistic regression, with temperature, sex, block, whitefly genetic line, and *Rickettsia* infection explored as explanatory variables. The data were simplified for visualization using the Henderson–Tilton formula (1955) to adjust the heat-shock proportional mortality to include the corresponding control-temperature mortality.

Heat shock on leaf discs

Leaf discs were placed on 10 mL 1 % agar in 30-mL plastic cups. Whiteflies on leaf discs were sexed without anesthetization in the cups. Leaf discs bearing an individual female only were then exposed to 44.5 °C for 3 h, after which mortality was scored. For each of two experimental blocks, approximately 100 female whiteflies from each of the four sublines were exposed to heat, meaning that a total of more than 800 female whiteflies were exposed to heat shock on leaf discs. An approximately equivalent number of whiteflies for each experimental block and subline were kept as controls at 27 °C. Proportional mortality was analyzed in JMP version 8.0 using logistic regression. No mortality was observed in the control treatment, so analysis was performed only on the whiteflies exposed to heat, where block, whitefly genetic line, and *Rickettsia* infection served as explanatory variables.

Competition at constant temperatures

The relative fitnesses of R^+ and R^- whiteflies were compared in a population cage experiment run at temperatures of 22 °C ("cold"), 27 °C ("control"), and 32 °C ("hot"). These temperatures were chosen based on the typical highs and lows of a cotton canopy in summer (Brown 1998) and an unsuccessful pilot experiment carried out at more extreme temperatures (19 and 35 °C), as detailed below in the "Results" section. Performance was measured via frequencies of *Rickettsia* infection over two generations (F1 and F2). Six replicate cages per temperature were used for each of the two whitefly genetic lines. The experimental design involved transferring a constant number of whiteflies to a new cage each generation, and was chosen to prevent overcrowding, death of the plants, and any confounding effect of varying degrees of density-dependent selection in different generations. The transfer day was chosen based on the midpoint of adult emergence measurements in previous experiments (Himler et al. 2011) to try to best capture a variable and representative cohort. Each cage contained two six-day-old cowpeas and was infested with 50 mating pairs of whiteflies, with a starting frequency of 14 % R^+, consistent with a similar experiment by Himler et al. (2011) using the MAC1 line only. In all cages, the parental whiteflies were removed after four days of egg-laying at 27 °C and the plants were moved to the treatment temperatures for F1 development. Seven days after the first F1 adults emerged at each temperature, 50 F1 mating pairs were collected and introduced to a new cage. The F1 mating pairs were removed from the plants when fourth-instar nymphs of the F2 were visible. The experiment was terminated seven days after the first adults of the F2 generation emerged for each temperature, except for the 32 °C treatment, in which the F2 eggs did not develop.

At each generation, a sample of approximately 30 (range 20–50) female progeny from each cage was kept to screen for *Rickettsia*. One MAC2 cold and one MAC2 control cage were excluded due to plant death. Mean slopes of infection frequencies from the parental to F1 generation were compared among temperatures and between whitefly genetic lines using multiple linear regression in JMP version 8.0. The F2 frequencies were included in the graphical representation of the data but not in the statistical analysis because the hot treatment was not represented (F1 females failed to reproduce in this treatment). After setting up the experiment, some contamination of R^+ whiteflies was found in the MAC1R^- stock line. Therefore, the parental generation of MAC1 whiteflies was screened to determine the actual starting frequency of *Rickettsia* infection for each cage. This led to a slightly higher mean starting frequency in the MAC1 line (overall mean of 25 % R^+, range of 14–45 %) than in the MAC2 line, where no contamination was found (starting frequency 14 % R^+ in each cage).

Frequency dependence of spread

To distinguish between the effects of *Rickettsia* starting frequency and whitefly genetic line in the experiment

described above, we conducted an additional experiment to test for an interaction between these two variables after the MAC1R$^-$ stock line was restored. Population cages were set up at 27 °C as per the "Competition at constant temperatures" section (above) except with R$^+$ starting frequencies of either 10 or 30 %. Four replicate cages for each of the two genetic backgrounds at each frequency were used, for a total of 16 cages, set up over two days. One 10 % MAC2 cage was excluded due to plant death. Parental whiteflies were removed after five days of oviposition. The F1 generation was collected seven days after the first F1 adult emerged in each cage. Fifty F1 females per cage were screened for *Rickettsia* and the mean slopes of infection frequencies were compared among whitefly genetic lines and starting frequencies using multiple linear regression in JMP version 8.0.

Results

Fitness of whitefly genetic lines

Rickettsia and whitefly genetic line influenced whitefly performance (Fig. 1), and the fitness benefits of *Rickettsia* in the MAC1 were generally consistent with previous estimates (Himler et al. 2011). *Rickettsia* infection significantly reduced whitefly development time (Fig. 1a; $F_{1,46.7} = 104.20$, $P < 0.0001$), and development time differed between whitefly genetic lines ($F_{1,46.7} = 55.13$, $P < 0.0001$). A significant interaction between these terms indicated that the *Rickettsia* phenotype varied between the whitefly genetic lines ($F_{1,46.7} = 88.51$, $P < 0.0001$). *Rickettsia* significantly reduced MAC1 development time by an average of 3.5 days ($F_{1,25.8} = 164.42$, $P < 0.0001$) but

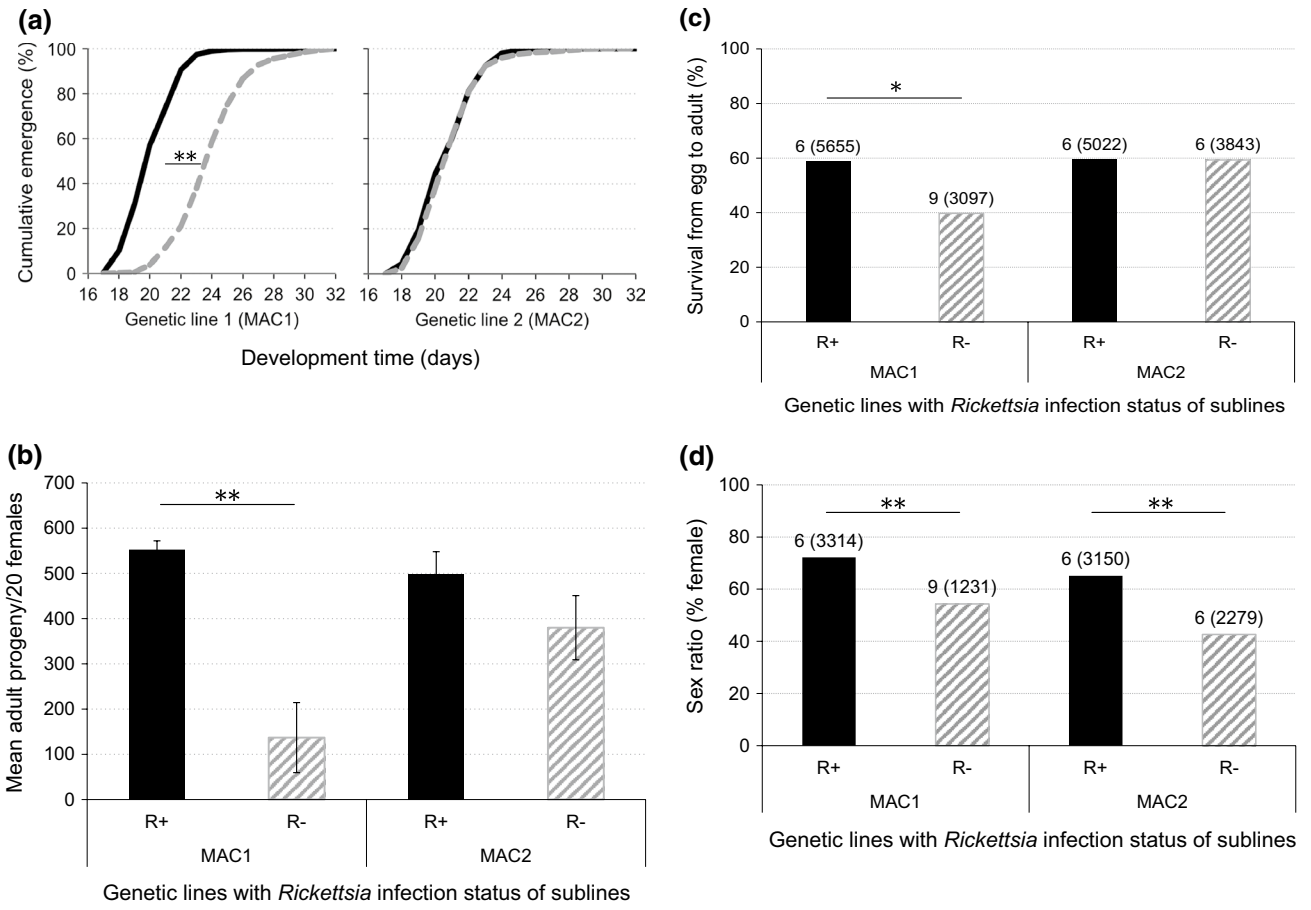

Fig. 1 Mean fitness measures for two genetic lines of *Bemisia tabaci* (MAC1 and MAC2) collected in Maricopa, AZ, USA. Measurements were made from the offspring of 20 mating pairs in each of six (MAC1R$^+$, MAC2R$^+$, MAC2R$^-$) or nine (MAC1R$^-$) replicate cages. *Error bars* show standard errors. *Asterisks* indicate significantly different groups (*$P < 0.02$; **$P < 0.0001$). *Black solid lines* and *bars* denote *Rickettsia*-infected R$^+$ and *gray dashed lines* and *bars* denote *Rickettsia*-uninfected R$^-$ sublines; **a** development time of females (males showed similar trends), **b** number of adult progeny, **c** developmental survival, and **d** sex ratio (percent female). The number of replicates and absolute numbers of whiteflies are included *above each bar* for **c** and **d**

had no significant influence on MAC2 development time ($F_{1,20.6} = 0.43$, $P = 0.520$; Fig. 1a).

Rickettsia infection significantly increased the number of adult whitefly progeny produced ($F_{1,23} = 30.09$, $P < 0.0001$), and whitefly genetic line marginally affected whitefly fecundity ($F_{1,23} = 4.10$, $P < 0.055$), with a significant interaction between these terms ($F_{1,23} = 7.68$, $P < 0.05$). *Rickettsia* increased the number of MAC1 adult progeny by an average of 416 per 20 R^+ females ($F_{1,13} = 79.76$, $P < 0.0001$), but did not significantly increase the number of adult progeny produced by the $MAC2R^+$ whiteflies ($F_{1,10} = 1.27$, $P < 0.29$). The MAC2 genetic line average fecundity (439.1 ± 53.2 SE) was intermediate between the low $MAC1R^-$ (136.8 ± 19.6 SE) and the high $MAC1R^+$ (552.3 ± 49.6 SE; Fig. 1b).

Whitefly survivorship to adulthood varied among replicates, especially in the MAC2. In general, there was no significant difference in survivorship associated with *Rickettsia* (Fig. 1c; quasibinomial analysis, $F_{1,23} = 1.30$, $P < 0.27$), whitefly genetic line ($F_{1,23} = 1.58$, $P < 0.22$), or a *Rickettsia* by whitefly genetic line interaction ($F_{1,23} = 1.55$, $P < 0.23$). However, in separate analyses, *Rickettsia* increased the $MAC1R^+$ whitefly survival to adulthood (quasibinomial analysis, $F_{1,13} = 9.00$, $P < 0.02$) but not the $MAC2R^+$ whitefly survival to adulthood (quasibinomial analysis, $F_{1,10} = 0.0003$, $P < 0.99$; Fig. 1c). The reason for this difference is unknown, but the slower development time of the $MAC1R^-$ subline may also have contributed to its reduced developmental success. This is because whiteflies would generally have been less mature at the time the leaves were removed from the plants.

Sex ratio was strongly influenced by *Rickettsia* (Fig. 1d; quasibinomial analysis, $F_{1,25} = 142.08$, $P < 0.0001$) and whitefly genetic line ($F_{1,23} = 82.63$, $P < 0.001$). *Rickettsia* consistently increased the female bias in both whitefly genetic lines (interaction term $F_{1,23} = 1.35$, $P < 0.26$; Fig. 1d).

Mortality after heat shock

In general, *Rickettsia* did not affect whitefly mortality after heat shock (Fig. 2). In glass vials, there was no interaction between temperature and *Rickettsia* after controlling for the effects of whitefly genetic line and sex (Fig. 2a; $\chi^2 = 1.23$, $P = 0.27$). MAC1 whiteflies were 2.6 times more likely to survive than MAC2 whiteflies (95 % CI 1.99–3.48, $\chi^2 = 48.21$, $P < 0.0001$). There was an interaction between whitefly genetic line and *Rickettsia* ($\chi^2 = 29.28$, $P < 0.0001$), suggesting *Rickettsia* influenced survival differently in the two lines, irrespective of temperature. There was no effect of block in these experiments (nominal logistic regression, $\chi^2 = 1.13$, $P = 0.29$), so this term was removed from the model.

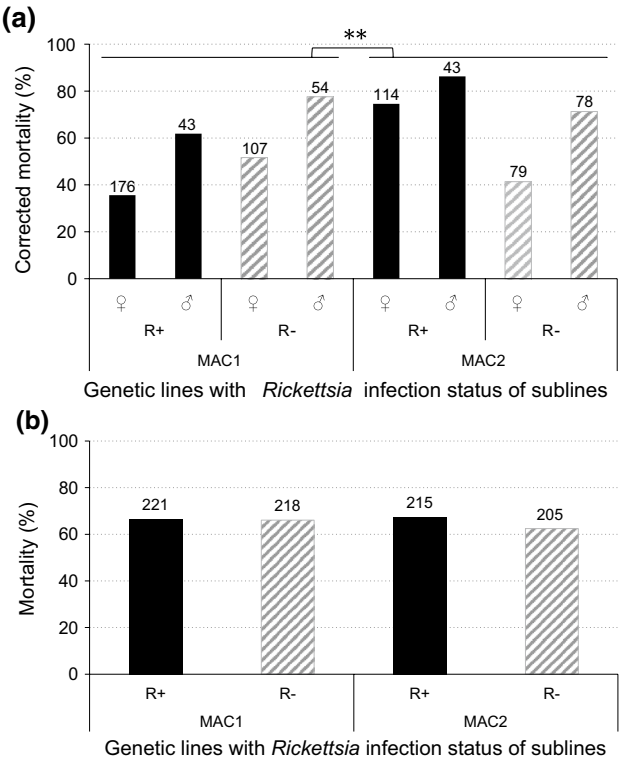

Fig. 2 Mortality after heat shock for *Bemisia tabaci* **a** adults in individual glass vials (40 °C, 3 h), and **b** adult females on individual leaf discs (44.5 °C, 3 h). Mortality for heat shock in glass vials was corrected for mortality in the no-heat shock controls (Henderson and Tilton 1955). *Asterisks* indicate significantly different groups (**$P < 0.0001$). *Black solid bars* denote *Rickettsia*-infected R^+ and *gray dashed bars* denote *Rickettsia*-uninfected R^- whitefly sublines. *Numbers above the bars* indicate the number of whiteflies tested. An approximately equal number of control whiteflies were kept at 27 °C

The leaf disc experiments showed little variation in mortality (Fig. 2b). Mortality after heat shock was not affected by *Rickettsia* (nominal logistic regression, $\chi^2 = 1.50$, $P = 0.22$) nor whitefly genetic line (nominal logistic regression $\chi^2 = 0.52$, $P = 0.47$). Experimental blocks were significantly different such that whiteflies were 5.7 times more likely to die in the second block (nominal logistic regression, 95 % CI for this multiplicative change was 4.1–7.9, $\chi^2 = 107.0$, $P < 0.0001$), although the trends were the same between blocks. This block effect was likely due to slight differences in the timing of when whitefly arenas were removed from the incubator. Pilot studies had shown there was only a small window of time to achieve partial mortality of the population, so small differences in heat exposure duration could have caused differences between block results. There was no mortality in the control whiteflies kept at 27 °C.

Competition in population cages at constant temperatures

Whitefly genetic line strongly affected the change in frequency of *Rickettsia* from the P to F1 generations (Fig. 3; multiple linear regression, $F_{3,30} = 150.4$, $P < 0.0001$) but temperature did not (multiple linear regression, $F_{3,30} = 1.3$, $P = 0.28$). These trends continued into the F2 generation for the cold and control treatments that remained after mortality of whiteflies in the hot treatment. *Rickettsia* frequency increased rapidly in the MAC1 regardless of temperature, with a mean slope (change in *Rickettsia* frequency) from the P to F1 generations of 49.6 (95 % CI 42.5–56.9). *Rickettsia* frequency did not change in the MAC2, with a mean slope from the P to F1 generations of −0.7 (95 % CI −5.3 to 3.9). There was no significant interaction between temperature and whitefly genetic line (multiple linear regression, $F_{5,28} = 0.63$, $P = 0.54$).

Overall, the whiteflies were very susceptible to both high and low constant temperature stress. In a pilot experiment with more extreme temperatures, F1 eggs did not hatch after five weeks at 19 °C but did hatch when moved to 22 °C. In the first trial of a hot treatment, F2 eggs did not hatch after three weeks at 35 °C, appeared brown in color and were few in number (range 3–135, mean 36 eggs per cage). At the more moderate temperatures chosen for the current experiment, we also saw evidence of temperature stress, with few offspring produced at 22 °C and the whiteflies unable to develop and continue to a third generation at 32 °C. At 32 °C, there were 260–582 F2 eggs per cage (mean 418) but they had not hatched after three weeks, while the normal hatching time at the control temperature of 27 °C is 6 days (Butler et al. 1983).

Frequency dependence of spread

The potentially confounding difference in starting frequency between the whitefly genetic lines in the constant-temperature experiment (Fig. 3) prompted another experiment to test for frequency dependence. This experiment showed *Rickettsia* starting frequency had only a slight effect on *Rickettsia* increase (Fig. 4; multiple linear regression, $t = 2.0$, $P = 0.07$, $df = 12$), confirming that the differences in *Rickettsia* spread in the constant temperature experiment were largely due to differences between whitefly genetic lines. Overall, the MAC1 had a change in *Rickettsia* frequency 25.7 times higher than the MAC2 (multiple linear regression, $t = 9.0$, $P < 0.0001$, $df = 12$; 95 % CI for slope 19.5–31.9). Although there was no significant interaction in the overall analysis between *Rickettsia* starting frequency and whitefly genetic line (multiple linear regression, $F_{3,11} = 0.12$, $P = 0.73$), *Rickettsia* frequency increased sharply in the MAC1 at both starting frequencies (two-sided t test, $t = 0.92$, $P = 0.40$, $df = 6$), whereas the frequency of *Rickettsia* in the MAC2 line remained almost constant in the 30 % treatment and declined slightly in the 10 % treatment (Fig. 4; two-sided t-test, $t = 3.1$, $P = 0.03$, $df = 5$; slope 13.6 times steeper at 30 % than 10 %, 95 % CI 2.3–25.0).

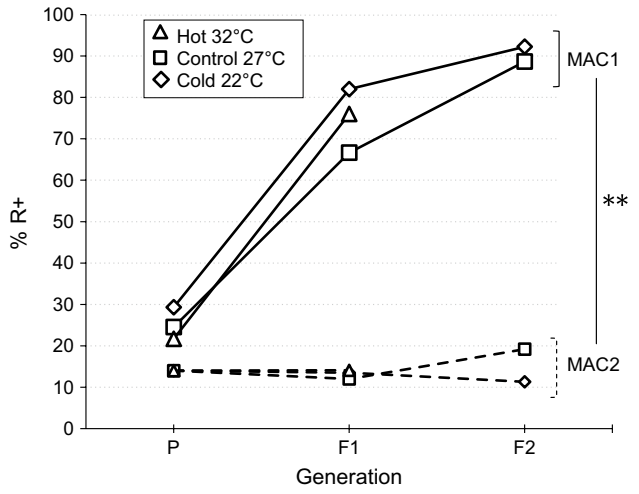

Fig. 3 Competition of R^+ and R^- *Bemisia tabaci* at cold (*diamonds* 22 °C), control (*squares* 27 °C), and hot (*triangles* 32 °C) temperatures for the two whitefly genetic lines (MAC1 *solid lines*, MAC2 *dashed lines*). *Asterisks* indicate significantly different groups (**$P < 0.0001$). *Rickettsia* frequency was measured in a sample of ~30 (range 2–50) female offspring for each generation from 5–6 replicate cages per treatment

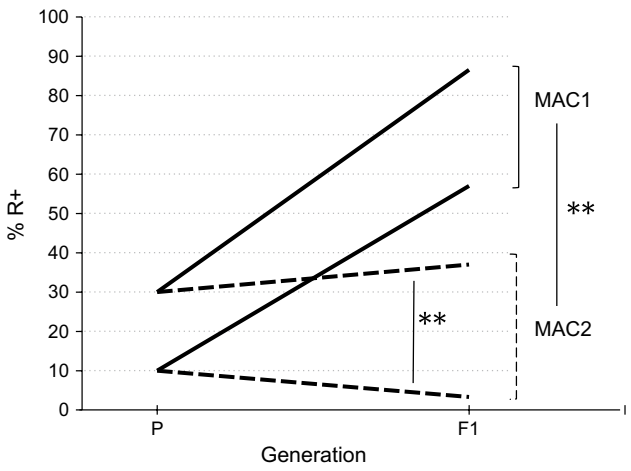

Fig. 4 Competition of R^+ and R^- *Bemisia tabaci* at different starting frequencies (10 or 30 % R^+) for the two whitefly genetic lines (MAC1 *solid lines*, MAC2 *dashed lines*). *Asterisks* indicate significantly different groups (*$P = 0.02$; **$P < 0.0001$). *Rickettsia* frequency was measured in a sample of 50 female offspring from 3–4 replicate cages per treatment

Discussion

These results provide strong evidence that the facultative symbiont *Rickettsia* has different effects in two whitefly genetic lines. In all cases, the MAC1 line benefited more from *Rickettsia* infection than did the MAC2 line. It is interesting that the significant variation in the degree to which *Rickettsia* benefits whiteflies occurred in two lines collected at different times from a single location where the frequency of *Rickettsia* is high. Host genetic background has been shown to affect the phenotypes of other mutualist symbionts in some instances; for example, the facultative symbiont *Regiella insecticola* can positively, negatively, or not affect the performances of different aphid lines on particular plant hosts (Ferrari et al. 2007). It is unlikely that genetic differences in *Rickettsia* underlie the observed differences between our whitefly genetic lines because the *Rickettsia* appears to be highly similar (if not identical) in both lines, based on the 100 % sequence identity of an intergenic spacer region known to be highly variable in other *Rickettsia* (Cass et al. 2015). The differences observed between whitefly genetic lines are probably due to host nuclear genes, although we cannot rule out effects of mitochondrial genes or those of other maternally inherited symbionts.

The MAC2 genetic line, like the MAC1, showed a significant female bias associated with *Rickettsia* in the performance assays (Fig. 1d). Based on these results, we expected an increase in the frequency of R^+ (assayed only with females) to be evident in the MAC2 population cages in the F1. Instead, there was no evidence of an increase in *Rickettsia* infection in this whitefly background, and hence no evidence of *Rickettsia*-influenced female bias (Figs. 3, 4). That we did not see an increase in R^+ in the MAC2 line in the population cage assays might be due to as-yet-undetected fitness costs associated with *Rickettsia*, although equivalent numbers of offspring were produced in the MAC2R^+ and R^- sublines in the performance assays. These results suggest different conditions faced by the whiteflies in the population cage assay, in which R^+ and R^- whiteflies interact directly and thus could influence each other's fitness, compared to the performance experiments in single subline cages. Several other studies have also found different outcomes in population cages than were expected from directly testing fitness parameters of insects bearing bacterial symbionts in isolation (Xi et al. 2005; Oliver et al. 2008; Harris et al. 2009). In general, population cage assays may better reflect the outcome of competing phenotypes in nature.

Although fluctuating temperatures are one of the main abiotic mortality factors for whiteflies in the field (Naranjo and Ellsworth 2009), and other insect symbionts provide their hosts with increased heat tolerance (Chen et al. 2000; Montllor et al. 2002; Russell and Moran 2006), our results do not support the hypothesis that *Rickettsia* fitness benefits in whiteflies are due to greater tolerance of temperature stress. In addition, we found no significant effect of *Rickettsia* on whitefly tolerance of heat shock. This is in contrast to the results of Brumin et al. (2011) but consistent with Shan et al. (2014) and Mahadav et al. (2009). Differences in the interaction between *Rickettsia* and host genetic line may underlie these conflicting observations. Differences in experimental approaches among laboratories may also play a role. In this study, for example, heat shock on leaf discs produced more consistent results among experimental replicates than heat shock in glass vials. Further, our introgression of the infected and uninfected lines removes the potentially confounding role of adaptive combinations of symbionts and host genotypes collected from the field (e.g., see Ferrari et al. 2007).

We found that both a cool constant temperature (19 °C) and warm constant temperatures (32, 35 °C) interfere with the development and reproduction of *B. tabaci* on cowpea. The optimum temperature for *B. tabaci* development varies by host plant but has been reported to range from 25 to 33 °C (Wang and Tsai 1996; Drost et al. 1998; Muniz and Nombela 2001), with temperature limits for the successful reproduction and development of 15–35 °C (Wang and Tsai 1996) and 17–35 °C (Muniz and Nombela 2001). There have been mixed reports about whether development is successful at 35 °C (Wagner 1995; Nava-Camberos et al. 2001; Yang and Chi 2006). Guo et al. (2012) observed multigenerational negative effects of high constant temperatures, with populations crashing after two generations at 37 °C and negative effects after three generations at 35 °C. The effects of temperature stress that we observed were greater than we expected given that the MAC1 whiteflies evaluated here performed well in field cages where temperatures ranged from 20 to 42 °C with a mean temperature of 38 °C (Asiimwe et al. 2014), well above the high-temperature treatments of the current study. The nighttime drop in temperature in the field and the microclimate of the leaf surface with temperature buffered by transpiration (Brown 1998) likely provide whiteflies and/or their nutritional symbiont with some essential reprieve from the physiological stress of high temperatures.

In population cage experiments where *Rickettsia* starting frequency varied, *Rickettsia* starting frequency appeared to have only a minor effect on the trajectory of *Rickettsia* spread overall, but these experiments were only one or two generations in duration, and it is likely that small effects measured here may have been more easily discerned after several generations. In the MAC2 line, *Rickettsia* declined in the 10 % starting frequency treatment and increased in the 30 % starting frequency experiment, suggesting positive

frequency dependence for infection in this range, and the possibility of an unstable equilibrium between these two frequencies. In population cages in the field with MAC1 whiteflies, *Rickettsia* frequencies were lower than expected on several dates given subline-specific growth rates, showing an apparent negative frequency dependence at higher ranges of *Rickettsia* frequencies (Asiimwe et al. 2014). In general, frequencies of *Rickettsia* in whiteflies across the United States are high but not fixed; frequency- or density-dependent fitness benefits of *Rickettsia* infection may contribute to this pattern.

In conclusion, these results demonstrate that the effects of *Rickettsia* on whiteflies are not uniform for different whitefly genetic backgrounds collected from the same location. This suggests a potential role of host genes in the heterogeneity in *Rickettsia* frequency observed across the USA (Cass et al. 2015) and the many differences in phenotypic effects associated with *Rickettsia* in *B. tabaci* globally (Kontsedalov et al. 2008; Chiel et al. 2009; Brumin et al. 2011; Himler et al. 2011; Hendry et al. 2014; Shan et al. 2014; Cass et al. 2015). The whitefly host variation in interactions with *Rickettsia* is somewhat puzzling, as host and symbiont evolutionary interests are largely congruent; resistance may be due to cytonuclear genetic conflict, with the *Rickettsia* under selection to increase the fitness of only females (Normark and Ross 2014). Whether these different effects of *Rickettsia* on whiteflies are evidence of genetic constraints in some whitefly lineages or of ongoing host adaptation to the recent symbiont invasion awaits future studies.

The relationship between *Rickettsia* and *Bemisia* described here is just one example of the many facultative symbiont relationships prevalent in natural and agricultural systems. These symbionts can move into insect host populations within an ecological timescale, bringing new sets of genetic capabilities. The new symbiosis may permit host adaptation, with selection acting on the host and bacteria as a functional unit (Zchori-Fein et al. 2014). We are only beginning to understand that the "role" of a symbiont is often context dependent. The geographic mosaic framework for mutualisms (Thompson 1997) developed for interactions between independent-living species may apply here, with symbionts having large influences on the host in some environments and no impacts in others. For invasive species in which genetic heterogeneity has been reduced by the bottleneck of introduction, symbiont-infected hosts may have competitive advantages, with the host–symbiont combinations creating cryptic but important genetic structure and driving host evolution in variable environments.

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Author contribution statement BNC, AGH, and MSH conceived and designed the experiments. BNC, AGH, ECB, JEB, SEK, and SKF performed the experiments. BNC, AGH, ECB, and MSH analyzed the data. BNC, AGH, and MSH wrote the manuscript.

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