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Host nuclear genotype influences phenotype of a conditional mutualist symbiont

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sex ratio;
sweetpotato whitefly;
uniparental inheritance;
Wolbachia.

Abstract

Arthropods commonly carry maternally inherited intracellular bacterial symbionts that may profoundly influence host biology and evolution. The intracellular symbiont *Rickettsia* sp. nr. *bellii* swept rapidly into populations of the sweetpotato whitefly *Bemisia tabaci* in the south-western USA. Previous laboratory experiments showed female-bias and fitness benefits were associated with *Rickettsia* infection, potentially explaining the high frequencies of infection observed in field populations, but the effects varied with whitefly genetic line. Here, we explored whether host extranuclear or nuclear genes influenced the variation in the *Rickettsia*–host phenotype in two genetic lines of the whitefly host, each with *Rickettsia*-infected and uninfected sublines. Introgression between the *Rickettsia*-infected subline of one genetic line and the *Rickettsia*-uninfected subline of the other was used to create two new sublines, each with the maternally inherited extranuclear genetic lineages of one line (*Rickettsia*, two other symbionts and the mitochondria) and the nuclear genotype of the other. Performance assays comparing the original and new lines showed that in addition to *Rickettsia*, the interaction of *Rickettsia* infection with host nuclear genotype influenced development time and the sex ratio of the progeny, whereas the extranuclear genotype did not. Host nuclear genotype, but not extranuclear genotype, also influenced the titre of *Rickettsia*. Our results support the hypothesis that differences in host nuclear genotype alone may explain considerable within-population variation in host–symbiont phenotype and may contribute to the observed variation in *Rickettsia*–whitefly interactions worldwide.

Introduction

Although nuclear genes comprise most of the hereditary material in a eukaryotic cell, extranuclear genes, including organelle genes and maternally inherited bacterial symbiont genes, may also have large influences on an organism's physiology and fitness (O'Neill *et al.*, 1997; Moran *et al.*, 2008; Ballard & Melvin, 2010; Ballard & Pichaud, 2014). Mitochondrial variants are often thought to be either under purifying selection (Stewart *et al.*, 2008) or neutral (Ballard & Rand, 2005), although several examples show that mitochondrial genotypes may vary in their influence on individual fitness (e.g. Clancy, 2008; Aw *et al.*, 2011; Ballard *et al.*, 2007). In arthropods, the frequent presence of one or more maternally inherited bacterial symbionts adds more genes and capacity to the host's collective extranuclear genome. While the long-term vertical transmission of symbionts and organelles assures evolutionary interests that are generally aligned with each other and benign to the organism/host (Engelstaedter *et al.*, 2007; Vautrin & Vavre, 2009), their maternal inheritance can select for female-bias, relaxed selection for male function, and hitchhiking of mildly deleterious alleles along with selective sweeps of others (Perlman *et al.*, 2015). Further, the high mutation rate of both symbionts and mitochondrial genomes (Moran, 1996; Nachman *et al.*, 1996) coupled with varying selection

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pressures in diverse host environments can lead to maintenance of variants (Moran *et al.*, 2008). For example, a heat-shock mutation in the obligate aphid nutritional symbiont *Buchnera* causes heat sensitivity in pea aphids, but provides fitness advantages in cool environments (Dunbar *et al.*, 2007; Burke *et al.*, 2010).

The entirety of the host genotype, the sum of nuclear and extranuclear genes, has repeatedly been shown to influence the host–symbiont phenotype in insects. For example, the phenotype of the symbiont may change qualitatively or in degree of expression or penetrance when a symbiont is introduced by microinjection to a new host background (Boyle *et al.*, 1993; Fujii *et al.*, 2001; McGraw *et al.*, 2002; McMeniman *et al.*, 2009). However, symbiont transfection studies are not able to parse the relative contributions of nuclear and extranuclear host genes to variation in the host–symbiont interaction. Distinguishing nuclear vs. extranuclear contributions to symbiont phenotype may be especially relevant in a system in which a symbiont may simultaneously increase host fitness and manipulate host sex ratio to gain a transmission advantage. Whereas all genetic lineages within a host benefit from a fitness increase, maternally inherited symbionts and organelles gain a transmission advantage by biasing the host sex ratio towards females, in extreme cases causing parthenogenesis (genetic males convert to genetic females) or feminization (genetic males develop as functional females) (Werren *et al.*, 2008; Perlman *et al.*, 2015). No female-biasing effects align with the interests of biparentally inherited nuclear genes, which are selected for equal investment in males and females (Dusing, 1884; Fisher, 1930; Stouthamer, 1997; Werren, 2011). Symbionts that increase both fitness and manipulate host reproduction may act as conditional mutualists and cause selection for either cooperation or resistance in different genetic lineages within the host, depending on the net benefit to those lineages.

Rickettsia sp. nr. *bellii* is a facultative arthropod symbiont that both manipulates sex ratio and increases fitness in its whitefly host, *Bemisia tabaci*. In the south-western USA, *Rickettsia* frequencies in this whitefly rose rapidly from low frequencies to near fixation in a 6-year period (Himler *et al.*, 2011). In laboratory studies that compared *Rickettsia*-infected (='R^+') and uninfected (='R^-') sublines in a homogeneous nuclear background, R^+ whiteflies laid more eggs, survived better to adulthood, developed faster, and produced more female-biased sex ratios than R^- whiteflies (Himler *et al.*, 2011). In field studies, *Rickettsia* infection frequency in whiteflies increased rapidly in population cages (Asiimwe *et al.*, 2014). Although the laboratory and field assays appeared to explain the rapid increase in the south-western USA, *Rickettsia* frequency is heterogeneous between regions in the USA (Cass *et al.*, 2015). The heterogeneous interaction between *Rickettsia* and its whitefly host was further demonstrated at a local scale when *Rickettsia* appeared to confer no fitness benefits in a second genetic line collected from the same location in Arizona, USA (Cass *et al.*, 2016). In population cages in the laboratory, the frequency of *Rickettsia*-infected whiteflies did not increase relative to uninfected whiteflies of the same genetic line, despite female bias caused by *Rickettsia* (Cass *et al.*, 2016). Because the two lines were established independently from the field, these results do not reveal whether differences are caused by *Rickettsia* genotype, the genotype of the other symbionts or mitochondria, or whether the differences lie in the host nuclear genotype. Here, we explore the role of host nuclear genotype vs. the extranuclear genotype in determining the phenotypic expression of *Rickettsia* in *B. tabaci*.

Materials and methods

Whiteflies and symbionts

In the whitefly species complex collectively known as *B. tabaci,* bacterial symbionts are prevalent and vary among whitefly species (Gueguen *et al.*, 2010; Bing *et al.*, 2013). The focal species of this study, known provisionally as *B. tabaci* 'MEAM1', (De Barro *et al.*, 2011) or 'B' invaded in the USA in the late 1980s and rapidly colonized the southern states (Oliveira *et al.*, 2001). In *B. tabaci* B, *Rickettsia* co-occurs with two other vertically transmitted symbionts that are fixed (100%): *Portiera aleyrodidarum* and *Hamiltonella defensa*. *Portiera aleyrodidarum* is an obligate nutritional symbiont found throughout the whitefly family Aleyrodidae (Thao & Baumann, 2004; Sloan & Moran, 2013), whereas *H. defensa* appears to provide additional metabolic capacity for limiting amino acids and cofactors (Luan *et al.*, 2015; Rollat-Farnier *et al.*, 2015).

Two whitefly genetic lines were established from whiteflies collected in 2006 ('MAC1') and 2009 ('MAC2') from cotton at the University of Arizona's Maricopa Agricultural Center, AZ, USA. To establish the original R^+ and R^- lines, ~100 isofemale lines were established and infection status determined. Progeny from these were combined by infection status to create separate R^+ and R^- cultures. An introgression series was then performed to homogenize the nuclear genes of the R^+ and R^- sublines (Fig. 1; details in Himler *et al.*, 2011; Cass *et al.*, 2016). For the current experiment two introgression series of six generations each were performed between MAC1 and MAC2 lines (i.e. R^+ females of MAC1 were backcrossed with R^- males of MAC2 and vice versa; Fig. 1). Each introgression series involved groups of approximately 50 R^- males mated with similar numbers of R^+ females. The hybrid, R^+ offspring were then mated to R^- males again. After six generations of backcrosses to R^- males, an average of > 98% of nuclear genes of the R^+ and R^- sublines will be the same (Himler *et al.*, 2011). When the

introgression series were complete, two new R^+ sublines were produced, one with the MAC1 extranuclear genotype and MAC2 nuclear genotype ('M1eM2n$^+$') and one with the MAC2 extranuclear genotype and the MAC1 nuclear genotype ('M2eM1n$^+$'). Thus, we used a total of six sublines (the original MAC1$^+$, MAC1$^-$, MAC2$^+$, MAC2$^-$ plus the two new lines) to test the relative influence of nuclear genotype and the extranuclear genotype on the *Rickettsia*–host phenotype. The lines were created and the experiments performed within a 6-month period. Whiteflies were maintained in laboratory cultures on cowpea (*Vigna unguiculata*) in a controlled environment chamber at 27 °C with a photoperiod of 16L : 8D and 65% relative humidity.

Diagnostic PCR

Rickettsia infection status of experimental whiteflies and the source laboratory lines was regularly checked with diagnostic polymerase chain reaction. DNA was extracted from individual whiteflies with a Chelex protocol and tested using *Rickettsia*-specific primers [16S rDNA primers 529F/1044R as described previously (Chiel *et al.*, 2009)]. Negative and positive controls were used to guard against false-positive and false-negative results. A 'limits of detection' test showed that *Rickettsia* is reliably detected in individual whiteflies by PCR with our PCR and electrophoresis protocols (Cass *et al.*, 2015).

Performance experiments

Whitefly performance and sex ratios were compared among the six sublines on whole plants. For set-up, leaves bearing fourth-instar nymphs of each subline were placed abaxial side up in ventilated 100-mm Petri dishes containing 1% agar. Adult whiteflies that emerged overnight were counted and sexed with a dissecting microscope. These newly emerged adults were then introduced onto 1-week-old cowpea seedlings grown in an 11.5-cm-diameter pot and caged with an inverted one-gallon (3.79 L) clear, ventilated plastic jar with a sleeve to allow introduction of insects. Twenty male and 20 female whiteflies were introduced to each cage containing one cowpea plant and allowed to oviposit until a moderate density of whitefly eggs was reached: 48 h in the first experimental block and 66 h in the second block. All whitefly adults were then removed from the plant. Fourteen days after the parental whitefly adults were removed, leaves bearing the F1 whitefly nymphs were removed from the plant and placed abaxial side up on 1% agar in ventilated (100-mm) Petri dishes. The nymphs were then counted and the dishes incubated to allow adult emergence. Each day the number and sex of adults emerging from each leaf was recorded until no more adults emerged. The experiment was performed in two blocks; in each block, five or six caged plants were assigned to each of the six sublines. After one plant died, the final replication was 9–11 replicates per treatment.

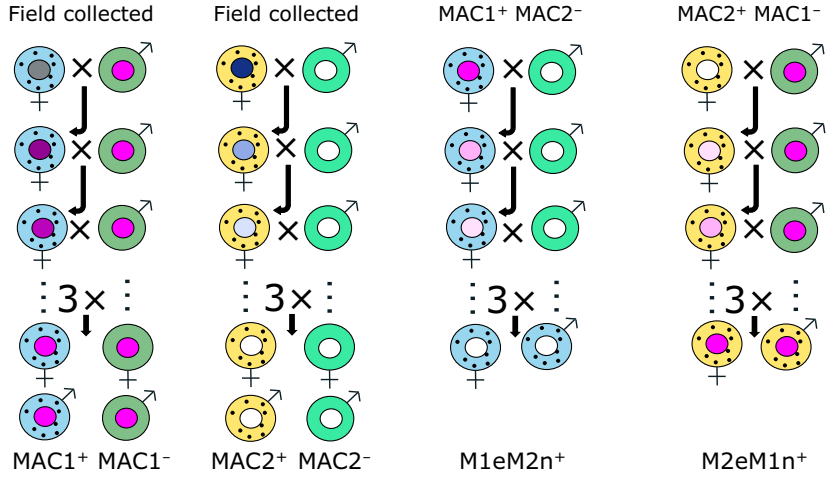

Fig. 1 Diagram of introgression series used for this study. The outer circle colour represents the extranuclear genotype, the spots indicate *Rickettsia* infection, and the inner circle colour represents the nuclear genotype. The arrows point to the female progeny of each cross that were used in the subsequent generation to backcross with the R$^-$ paternal line. In the case of the original MAC1 and MAC2 lines, isofemale lines were started with field-collected females, families were combined into cultures according to *Rickettsia* infection status, and these cultures were used to start the introgression series (Cass *et al.*, 2016; Himler *et al.*, 2011). The change in shade of the 'nucleus' in the daughters of each cross reflects the increasing proportion of paternal line nuclear genes with each generation. The introgression series was finished after six generations when both R^+ and R^- sublines are expected to share > 98.8% of nuclear genes. The bidirectional introgression of MAC1 and MAC2 lines (in which R^+ females of MAC1 were backcrossed with R^- males of MAC2 and vice versa) produced two R^+ sublines with the extranuclear genotype of one and the nuclear genotype of the other parental line. Note that the MAC1$^-$ and MAC2$^-$ sublines have extranuclear genotypes that are potentially unique among the six sublines.

Quantitative PCR to measure *Rickettsia* titre

The relative *Rickettsia* titre in the four R^+ sublines was measured within a month of the final introgression cross using quantitative PCR. The quantitative PCR was performed on a BIO-Rad CFX Connect Real-Time System with methods as described previously (Himler *et al.*, 2011) using the $2^{-\Delta Ct}$ method (Livak & Schmittgen, 2001). The mean normalized expression values of the *Rickettsia gltA* gene relative to the whitefly *β-actin* for each line were compared by ANOVA as described below. For each whitefly, each reaction was performed in triplicate, and the average of the three values used, unless one value was more than 0.2 greater than the standard deviation away from the mean, in which case that value was excluded and the average of the other two values was used for the expression estimate. For each genetic line, *Rickettsia* titre was measured in 21 individual female whiteflies.

Statistical analysis

Statistical analyses were structured such that the effects of block, *Rickettsia* presence/absence and nuclear genotype on various response variables were analysed across all six sublines, whereas the effects of block and extranuclear genotype were analysed among the four R^+ sublines only. This reduced set of treatments was chosen for analysis of the potential explanatory effect of the extranuclear genotype for two reasons. First, a leading contender for a potential extranuclear genotype effect would be the *Rickettsia* genotype itself, present only in the four R^+ sublines, so analyses containing both variables were likely to be confounded. Secondly, because of the direction of the original introgression series, the two R^- sublines bore potentially unique extranuclear genotypes, not replicated in any of the R^+ lines (Fig. 1).

The effects of *Rickettsia* presence/absence and nuclear genotype on the number of adult progeny in each cage was analysed with ANOVA in R (R Development Core Team 2010). The roles of nuclear and extranuclear genotype on relative *Rickettsia* titre ($2^{-\Delta Ct}$ values as the response variables) were analysed similarly in the four R^+ sublines. Each analysis was performed in a stepwise fashion, with block, *Rickettsia* presence, and nuclear genotype as initial explanatory variables across the six treatments. When block was not significant, it was removed and a reduced model analysed. The effect of the extranuclear genotype (including *Rickettsia* genes) on progeny number and time to adult emergence was analysed among the four R^+ sublines with block and extranuclear genotype as explanatory variables. The time to adult emergence for each whitefly was analysed in linear mixed-effects models in two sets of analyses with lme4 in R (Bates *et al.*, 2015). The full data set was used to analyse the day of emergence, with nuclear genotype, *Rickettsia* presence/absence and block as fixed effects and cage as a random effect. The effects of block and extranuclear genotype were analysed among the four R^+ sublines. Likelihood ratio tests, yielding χ^2 test statistics, were performed to determine statistical significance for main effects and interactions.

The effects of *Rickettsia* presence/absence, nuclear genotype (among six sublines) and extranuclear genotype (among four sublines) on whitefly sex ratio was analysed using logistic regression in R, with quasi-binomial errors and an F-test performed when the residual deviance was greater than the residual degrees of freedom, indicating a model that is overdispersed (Crawley, 2007).

Significant interactions between *Rickettsia* presence and nuclear genotype on response variables were interpreted as support for the hypothesis that host nuclear genes influence *Rickettsia* effects. A significant effect of extranuclear genotype would support the hypothesis that extranuclear genotype variation (including variation in *Rickettsia* genes as well as those of other symbionts or mitochondria) influences performance of the host.

Results

The presence of *Rickettsia*, host nuclear genotype, and the interaction of *Rickettsia* with host nuclear genotype were dominant influences on measures of whitefly performance and on progeny sex ratios, whereas the extranuclear genotype did not influence any of the performance measures. The number of adult progeny produced among lines (Fig. 2) was significantly increased by *Rickettsia* presence ($F_{1,59} = 13.49$, $P = 5.18 \times 10^{-4}$) and the lines bearing the MAC2 nuclear genotype produced more progeny than the MAC1 ($F_{1,59} = 8.64$, $P = 0.0047$), but there was not a significant interaction between *Rickettsia* and host nuclear genotype ($F_{1,59} = 1.51$, $P = 0.22$), nor was extranuclear genotype significant ($F_{1,39} = 0.65$, $P = 0.43$).

Whitefly development time was approximately 1 day faster when *Rickettsia* was present (0.98 days ± 0.07 SE, Fig. 3a, b; $\chi^2_1 = 179.66$, $P < 2.2 \times 10^{-16}$). Both nuclear genotype and extranuclear genotype significantly influenced development time as well (nuclear genotype $\chi^2_1 = 4.51$, $P = 0.034$; extranuclear genotype $\chi^2_1 = 25.25$, $P = 5.03 \times 10^{-7}$), but the effect sizes were small, with whiteflies with MAC2 nuclear genotypes ($MAC2^-$, $MAC2^+$, $M1eM2^+$) developing an average of 0.11 ± 0.05 days faster than those with MAC1 nuclear genotypes, and among the four R^+ treatments, whiteflies with the MAC1 extranuclear genotype developing 0.26 ± 0.05 SE days faster. Development time was more strongly affected by a nuclear genotype by *Rickettsia* presence interaction ($\chi^2_1 = 54.22$, $P = 1.79 \times 10^{-13}$), with whitefly development time reduced more by the presence of *Rickettsia* in the MAC1 sublines

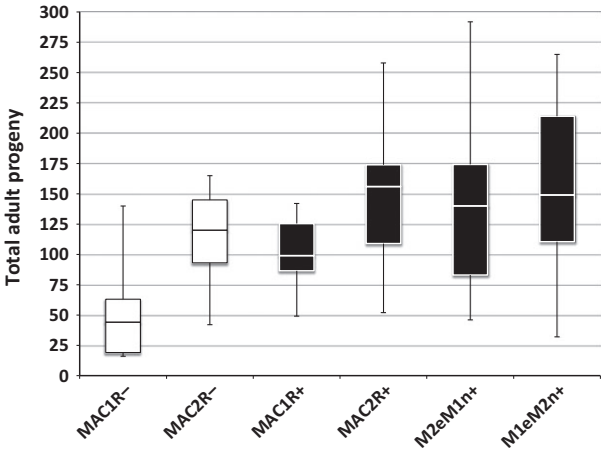

Fig. 2 Number of adult progeny produced by 20 mated pairs of whiteflies on whole cowpea plants for six genetic sublines of *Bemisia tabaci* (both blocks combined). White bars are *Rickettsia*-uninfected MAC1 and MAC2 sublines, the first two black bars are the *Rickettsia*-infected MAC1 and MAC2 sublines and the last two black bars are the *Rickettsia*-infected sublines resulting from introgression of the MAC1 and MAC2 lines in each direction. The centre line in the box is the median, the bottom and top of the box mark the 25th and 75th percentiles of the data, and the lines extend to minimum and maximum data points. *Rickettsia* and host nuclear genotype (MAC1 or MAC2) significantly influenced the number of adult progeny produced across lines; extranuclear genotype was not significant (see text for details). Each treatment was replicated 9–11 times.

(MAC1$^-$ vs. MAC1$^+$ and M2eM1n$^+$) than in the MAC2 sublines. Block was also significant in its influence on development time with block 2 whiteflies taking approximately a day (1.02 ± 0.05 SE) longer to develop ($\chi^2_1 = 360.22$, $P < 2.2 \times 10^{-16}$).

Similarly, *Rickettsia* infection caused progeny sex ratios to be strongly female-biased (Fig. 4; $F_{1,59} = 340.18$, $P = 2.69 \times 10^{-14}$). Nuclear genotype was also highly significant ($F_{1,59} = 208.59$, $P = 1.0 \times 10^{-10}$), with MAC1 genotypes being more female-biased than MAC2, as was a *Rickettsia* by nuclear genotype interaction ($F_{1,57} = 32.98$, $P = 0.0026$), with the greatest increase in female bias associated with *Rickettsia* infection seen in the MAC1 genotypes. The extranuclear genotype did not have an influence on sex ratios ($F_{1,37} = 0.004$, $P = 0.95$) in the four R$^+$ lines.

Nuclear genotype had a significant influence on *Rickettsia* titre (Fig. 5; $F_{1,80} = 10.09$, $P = 0.002$), whereas extranuclear genotype did not ($F_{1,80} = 1.07$, $P = 0.30$). The interaction between nuclear genotype and extranuclear genotype strongly influenced *Rickettsia* titre, however ($F_{1,80} = 27.65$, $P = 1.85 \times 10^{-6}$). This interaction may have reflected the timing of the introgression series. A contrast showed that *Rickettsia* titre was lower in the two lines newly created by introgression than in the original MAC1 and MAC2 lines (Fig. 5; $F_{1,82} = 24.87$, $P = 3.37 \times 10^{-6}$).

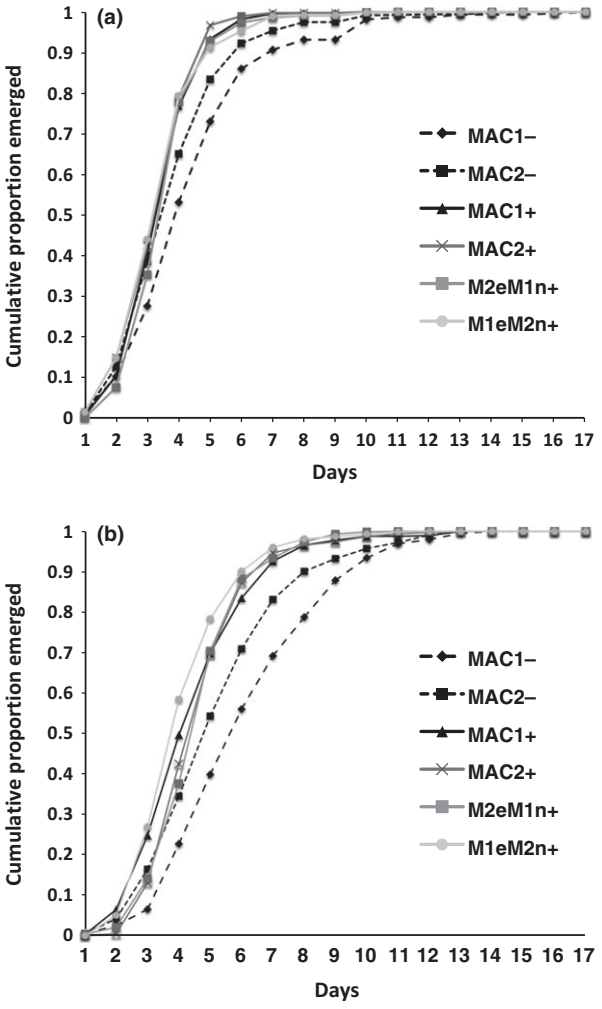

Fig. 3 Cumulative proportion of total adult progeny emerging over time. Each replicate cage was initiated with 20 mated pairs of whiteflies of one of six genetic sublines of *Bemisia tabaci* on whole cowpea plants. Dotted lines represent the two *Rickettsia*-uninfected sublines. *Rickettsia*, nuclear genotype, extranuclear genotype, the interaction between *Rickettsia* and host nuclear genotype, and block all significantly influenced the development time of progeny among lines (see text for details). Each treatment was replicated 4–6 times in each block. (a) Block 1 results. (b) Block 2 results.

Discussion

We examined the relative influence of *Rickettsia* presence, the host nuclear genotype and the extranuclear genotype on select performance parameters of whiteflies. Both *Rickettsia* infection and *Rickettsia* interacting with the nuclear genotype were important effects on whitefly development time and sex ratios, indicating that it is the host nuclear genotype that mediates the distinct influence *Rickettsia* exerts on host phenotype in the two whitefly lines, and not the extranuclear

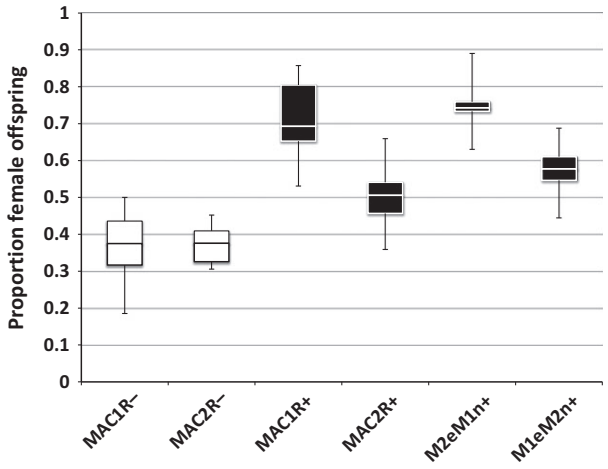

Fig. 4 The offspring sex ratio (proportion of females) for 20 mated pairs of whiteflies on whole cowpea plants (both blocks combined) for six genetic sublines of *Bemisia tabaci*. White bars are *Rickettsia*-uninfected MAC1 and MAC2 sublines, the first two black bars are the *Rickettsia*-infected MAC1 and MAC2 sublines, and the last two black bars are the *Rickettsia*-infected sublines resulting from introgression of the MAC1 and MAC2 lines in each direction. The centre line in each box is the median, the bottom and top of the box mark the 25th and 75th percentiles of the data, and the lines extend to minimum and maximum data points. *Rickettsia,* the host nuclear genotype and the interaction between *Rickettsia* and host nuclear genotype all significantly influenced the proportion of females produced among lines; extranuclear genotype was not significant (see text for details). Each treatment was replicated 9–11 times.

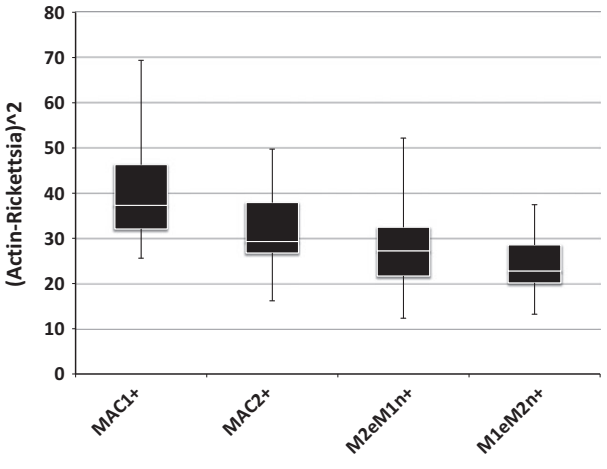

Fig. 5 Relative density of *Rickettsia* in four genetic sublines of *Bemisia tabaci*. *Rickettsia* titre was calculated with the $2^{-\Delta Ct}$ method based on the relative number of copies of the *Rickettsia* gene citrate synthase (*gltA*) to whitefly actin (*β-actin)*. The first two bars are the MAC1 and MAC2 parent sublines, and the last two bars are the new sublines resulting from introgression of the MAC1 and MAC2 lines in each direction. The centre line in each box is the median, the bottom and top of the box mark the 25th and 75th percentiles of the data, and the lines extend to minimum and maximum data points. Host nuclear genotype and the interaction between host nuclear genotype and extranuclear genotype significantly influenced *Rickettsia* titre; extranuclear genotype as a main effect was not significant (see text for details). A contrast between the parent lines (on the left) and new lines (on the right) was also significant, with the two new lines having a significantly lower titre than the original lines. *N* for each subline = 21.

genotype. Whitefly nuclear genotype alone also affected fecundity, development time and sex ratio. In contrast, the extranuclear genotype (the combined genotypes of *Rickettsia*, *Portiera*, *Hamiltonella* and the mitochondria) had only a slight effect on development time among the *Rickettsia*-infected sublines and did not influence any of the other whitefly performance measures.

Rickettsia titre was higher in the MAC1 line, in which the greater sex ratio bias and difference in whitefly performance between R^+ and R^- sublines was seen. The whitefly nuclear genotype interaction with *Rickettsia* had the most significant effect on whitefly sex ratios, the parameter that represents conflict between symbiont and host nuclear evolutionary interests. *Rickettsia* had a higher titre and caused more female bias in the MAC1 nuclear sublines ($MAC1^+$, $M2eM1n^+$) where an average of 74% of whiteflies were female, and lower titre and less female bias in the MAC2 nuclear lines ($MAC2^+$, $M1eM2n^+$; 54% on average), but still a greater proportion of females than in the two male-biased R^- lines (38% female). *Rickettsia* presence also influenced development time more in the MAC1 nuclear sublines, with the R^+ sublines developing faster than their R^- counterparts. It is not clear whether an association between titre and the strength of the *Rickettsia* phenotype is a general pattern for *Rickettsia* in *B. tabaci*, but differential regulation of the symbiont titre by hosts appears to be a common mechanism by which hosts control symbiont phenotype (Perrot-Minnot & Werren, 1999; McGraw *et al.*, 2002; Unckless *et al.*, 2009). As here both greater performance enhancement by *Rickettsia* (in reduced development time) and sex ratio bias (reproductive manipulation) occurred in the MAC1 line, with higher *Rickettsia* titre, it is not clear what the host's best evolutionary interests are with respect to symbiont regulation. If the correlation between titre and effect is general across populations of *B. tabaci* where *Rickettsia* is present, we might expect higher titres in populations where *Rickettsia* is at high frequency, as has been predicted in other systems (Jaenike 2009). Lastly, *Rickettsia* titre was also lower in the two recombinant sublines compared with the parental sublines. The reason for the reduced titre in these new lines may be due to the new association of *Rickettsia* with host nuclear genes from R^- backgrounds. Symbiont titre has been shown to increase or decrease in new host genotypes (Boyle *et al.*, 1993; McGraw *et al.*, 2002), and new host environments (Clancy & Hoffmann, 1998; Correa & Ballard, 2012).

The role of the entire host genetic background (nuclear and extranuclear) on symbiont phenotype has frequently been observed for symbionts that cause reproductive anomalies such as parthenogenesis, male killing, feminization and cytoplasmic incompatibility. Moving a symbiont to a new host genetic background can cause qualitative changes in the phenotype, such as a change from feminization to male killing (Fujii *et al.*, 2001) or from cytoplasmic incompatibility to male killing (Jaenike, 2007). In a new background, bidirectional cytoplasmic incompatibility may become unidirectional (Raychoudhury & Werren, 2012). Further, such a move can change the strength of the effect, for example causing cytoplasmic incompatibility to become stronger or weaker (Bordenstein & Werren, 1998). Notably, in most of these examples and many others in which the role of host genotype was examined, the focal symbiont was held constant as it was moved from one background to another, but the role of the other symbionts and mitochondria, the extranuclear genotype, could not be separated from influences of the nuclear genotype as it was in the current study. Exceptional studies in which the source of the genotypic effects on symbiont phenotype was identified include Jaenike (2007; nuclear) and Weeks *et al.* (2007; extranuclear).

Why did we see little influence of the extranuclear genotype on whitefly performance in the current study? We cannot distinguish between a lack of standing genetic variation in the extranuclear genotype and the absence of other factors maintaining variation, for example disruptive or balancing selection. The extranuclear genomes could be relatively invariant; the *Rickettsia* sweep into the south-western USA whitefly population from very low frequency between 2000 and 2006 would have swept with it the entire maternally transmitted haplotype, presumably resulting in reduced diversity in the population. If we assume *B. tabaci* in Arizona can complete about 10–13 generations per year (Palumbo *et al.*, 2001), MAC1 and MAC2 lines of the current study were derived from field collections approximately 48 and 72 generations after *Rickettsia* arrived. However, evidence suggests bacterial symbiont evolution can occur rapidly. After just 200 generations of *Drosophila simulans,* Weeks *et al.* (2007) documented a significant reduction in fecundity costs associated with cytoplasmic incompatibility-inducing *Wolbachia,* 20 years after the arrival of the symbiont in the host population. In that study, introgression among lines with different fecundity costs showed the change over time was due to evolution of the extranuclear genotype, most likely *Wolbachia* genes, not the nuclear genotype. Even more surprisingly, Newton & Sheehan (2015) found evidence for *Wolbachia* adaptation to a new host genotype in only three generations. Given these examples, the generally high mutation rates of symbionts, the mixed effects of *Rickettsia* on whiteflies, and the large population sizes of the polyphagous *B. tabaci* in the agricultural south-western USA (Ellsworth & Martinez-Carrillo, 2001; Oliveira *et al.*, 2001), we cannot exclude the possibility of genotypic variation of whitefly symbionts in this population. The absence of evidence of symbiont genes involved in whitefly performance differences found here does not allow us to predict the pattern in other populations or for larger sets of lines.

The large degree of variation in the phenotype of *Rickettsia* in two introgressed whitefly lines originally collected from the same field in Arizona, USA, raises the question of how the average whitefly genotype from the field interacts with *Rickettsia.* All R^+ sublines in the current study ($MAC1^+$, $MAC2^+$, $M1eM2n^+$ and $M2eM1n^+$) produced relatively more females than the R^- sublines, but in the MAC2 nuclear background the *Rickettsia* interaction was less pronounced with respect to the number of progeny produced and whitefly development time. In previous one or two-generation population cage experiments in which R^+ and R^- whiteflies of either MAC1 or MAC2 lines competed, *Rickettsia* climbed in frequency in the MAC1 line, recapitulating the increase seen in the field, although much more rapidly than in the ~70 generations that were needed to approach fixation across the Arizona landscape (Himler *et al.*, 2011). In the MAC2 line, the frequency of *Rickettsia* remained relatively constant after one generation in the laboratory, suggesting a neutral relationship (Cass *et al.*, 2016). In nature, the average genotype might be intermediate to the two in the laboratory, in which *Rickettsia* causes female bias and/or slight to moderate performance benefits such that the frequency of *Rickettsia* climbs steadily over dozens of generations. Both female bias and fitness benefits will tend to increase population sizes and make whiteflies more serious pests.

In summary, this study suggests that host nuclear genotype interacts with *Rickettsia* to influence whitefly sex ratios and performance. The extranuclear genotype, consisting of *Rickettsia,* two other bacterial endosymbionts and the mitochondria, had only a slight influence on whitefly development time, and no effect on the variation in whitefly sex ratios or progeny production. In the south-western USA, where a recent whitefly invader was colonized even more recently by an endosymbiont, our data suggest little role of *Rickettsia* genotypic variants in the variation in phenotype we observed. At broader spatial scales, more factors are likely to be important, including, perhaps, *Rickettsia* genotype. *Rickettsia* frequencies have remained close to fixed since 2006 in the field in Arizona where whiteflies in the current study originated (Cass *et al.*, 2015), whereas *Rickettsia* frequencies vary widely in this species of whitefly at larger spatial scales, for example across the USA, in Israel, and in China, where the whitefly is also invasive (Bing *et al.*, 2013; Cass *et al.*, 2015). In particular, in Israel, where the frequency of

Rickettsia has recently declined to low levels (Cass *et al.*, 2015), fitness assays showed little effect of *Rickettsia* infection on whitefly fitness or sex ratio (Chiel *et al.* 2007) suggesting an even greater range of relationships between symbiont and host are possible than were found in the current study. Climatic and biotic differences among locations (e.g. host plant availability, temperature and humidity, other whiteflies) are obvious likely additional influences in the interaction between *Rickettsia* and whitefly hosts at larger geographic scales as well, and the relative contribution of host genotype vs. other factors to variation in the interaction worldwide remains to be determined.

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