Assessments of Fitness Effects by the Facultative Symbiont \textit{Rickettsia} in the Sweetpotato Whitefly (Hemiptera: Aleyrodidae)

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ABSTRACT The sweet potato whitefly, \textit{Bemisia tabaci} (Gennadius) (Hemiptera: Aleyrodidae), harbors several bacterial symbionts, including the obligate primary symbiont \textit{Portiera aleyrodidarum} and the facultative secondary symbionts \textit{Arsenophonus}, \textit{Cardinium}, \textit{Fritschea}, \textit{Hamiltonella}, \textit{Rickettsia}, and \textit{Wolbachia}. The roles of these symbionts are yet unknown. In this study, we tested for possible effects of one symbiont, \textit{Rickettsia}, on some fitness parameters of \textit{B. tabaci} (biotype B) by comparing whiteflies that carry this symbiont to whiteflies that do not. Preadult development of \textit{Rickettsia}-carrying whiteflies was faster, but all the other parameters that were measured: longevity, total number of progeny, sex ratio, and nymphal survivorship did not differ significantly. Estimates of the intrinsic growth rate ($r$) were almost identical for the two groups. Cross-mating between \textit{Rickettsia}-carrying and \textit{Rickettsia}-free whiteflies provided no evidence for cytoplasmic incompatibility. Vertical transmission of \textit{Rickettsia} was found to be nearly complete. Our results do not clearly identify a selective advantage that would explain the high prevalence of \textit{Rickettsia} in \textit{B. tabaci} populations, thus, other fitness parameters and horizontal transmission routes are suggested and discussed.

KEY WORDS secondary symbionts, fitness, longevity, fecundity, survival

Inherited bacteria living within arthropod host cells are prevalent and are broadly divided into two groups: primary symbionts and secondary symbionts. Primary symbionts are, by definition, obligatory and mutu- alistic to the host, as they are essential to the hosts’ survival and development. Such symbionts are generally confined to specialized cells, bacteriocytes, and are strictly vertically transmitted (Baumann 2005). Secondary symbionts are not necessarily critical for host survival, but they may still play an important role in their host’s ecology and evolution. Although secondary symbionts are routinely transmitted vertically, there is some evidence of limited horizontal transmission routes for this group as well (e.g., Moran and Dunbar 2006).

To be maintained in a host population, theory predicts that a symbiont that is strictly vertically transmitted must either contribute to its host fitness, or manipulate the host’s reproduction in a way that enhances its own transmission (Bull 1983). Indeed, various secondary symbionts have been shown to adopt one of these tactics. Examples of fitness contributions include conferring heat tolerance (Montllor et al. 2002), natural enemy- and pathogen-resistance (Oli- ver et al. 2003; Ferrari et al. 2004; Scarborough et al. 2005; Hedges et al. 2008) and enabling host plant use (Tsuchida et al. 2004).

Some secondary symbionts, most notably \textit{Wolbachia} spp., apply the second tactic, i.e., they alter the hosts’ reproduction in a way that promotes the production of infected female hosts that transmit the symbiont (Werren et al. 2008). The most common reproductive manipulation is cytoplasmic incompatibility (CI), which, at its simplest, occurs when uninfected females produce few or no offspring after mating with infected males. Because of the effective sabotage of the reproduction of uninfected females, infected females gain a selective advantage and the symbiont spreads. In haplodiploid insects, CI is expected to result in a male-biased sex ratio, because fertilized, incipient female eggs will either die, or lose the paternal set of chromosomes and develop into male progeny (Werren et al. 2008).

The sweet potato whitefly, \textit{Bemisia tabaci} (Gennadius) (Hemiptera: Aleyrodidae), is an extremely polyphagous insect capable of developing on hundreds of plant species, including many agricultural crops (Oliveira et al. 2001). This whitefly is actually a species complex composed of >20 biotypes that may differ from each other genetically and biologically (Boykin et al. 2007; for definition of biotype, see Brown et al. 1995).

\textit{B. tabaci} harbors a primary symbiont, \textit{Portiera aleyrodidarum}, that is prevalent in all species of the Aleyrodidae studied so far and most probably produces...
Amino acids lacking in the phloem diet (Thao and Baumann 2004; Baumann 2005). In addition, a variety of secondary symbionts may be hosted by B. tabaci: Arsenophonus, Cardinium, Fritschea, Hamiltonella, Rickettsia, and Wollbachia (reviewed in Baumann 2005; Gottlieb et al. 2006). Some of these secondary symbionts are biotype-specific: in Israel, Hamiltonella has been found only in the B biotype, whereas Arsenophonus and Wollbachia have been found only in the Q biotype. Rickettsia was highly prevalent, but not fixed, in both of those biotypes (Chiel et al. 2007).

Bacteria of the genus Rickettsia (α-Proteobacteria) are mostly known as obligate intracellular symbionts of blood-feeding arthropods and as causative agents of many vertebrate diseases. In the past decade, however, Rickettsia has been discovered in beetles, parasitic wasps, aphids, booklice, and leafhoppers, in which it was evidently associated with various phenomena, including reproductive manipulation, heat tolerance, and plant disease (reviewed in Perlman et al. 2006; Perotti et al. 2006). The Rickettsia in B. tabaci is most closely related to R. bellii, is found in all developmental stages of the whitefly, and is maternally transmitted through the egg (Gottlieb et al. 2006).

That 65–74% of B. tabaci are infected with Rickettsia (Chiel et al. 2007) suggests that there might be both fitness advantages of carrying this symbiont, as well as fitness costs that restrain it from fixation. To understand the mechanism by which Rickettsia reaches high prevalence in B. tabaci populations, we tested for fitness effects and reproductive manipulation in this system.

Materials and Methods

Whitefly Rearing. Whiteflies were obtained from the B. tabaci colony (biotype B) in the laboratory of Prof. Dan Gerling, at Tel-Aviv University, and reared on tobacco plants (Nicotiana tabacum ‘Xanthi’). Both Rickettsia-infected and uninfected whitefly colonies (described below) were reared in net cages in separate chambers in a greenhouse. Temperature averages in the rearing chambers were ~30°C during the summer (25–35°C) and 25°C during the winter (20–25°C).

Polymerase Chain Reaction (PCR) Analysis. Individual whiteflies were tested for the presence of Rickettsia and Hamiltonella by means of PCR as described in Chiel et al. (2007).

Establishment of a Rickettsia-Free Colony. To assess the impact of a symbiont, it is important to minimize genetic background differences in its host. Initially, attempts were made to eliminate Rickettsia by feeding adult whiteflies with various antibiotics as described by Ruan et al. (2006), and by injecting antibiotics to adults and nymphs, but none of these procedures resulted in Rickettsia-free whiteflies. Because previous screening revealed that Rickettsia is not fixed in most populations (Chiel et al. 2007), and the B. tabaci colony had been reared on a small scale in the laboratory for a few years, Rickettsia-free individuals were isolated from the inbred laboratory-reared colony described above. Although we acknowledge the possibility of genetic heterogeneity between these cultures, we expect genetic differences to be relatively small.

Twenty single mated females were placed individually on sweet pepper leaf disks (50 mm in diameter) that were kept on 1% water agar inside a transparent plastic cup sealed with a netted lid. The leaf disks were held at 25°C and a photoperiod of 14:10 (L:D) until progeny emergence, at which time five individuals were randomly selected and tested for infection status by PCR. The Rickettsia-free (R−) and Rickettsia-positive (R+) individuals were subsequently pooled together on a new tobacco plant in two separate chambers in the greenhouse. To verify that there was no cross-contamination, the infection status was routinely monitored once a month by sampling 10–20 adults from each colony and testing for the presence of Rickettsia and Hamiltonella with PCR. Both lines carried Hamiltonella, a bacterium that was found in 100% of B biotype individuals tested in Israel (Chiel et al. 2007).

Vertical Transmission of Rickettsia. To test the fidelity of maternal transmission of Rickettsia, 10 leaf disks were prepared as described above, and one R+ male and female pair was placed in each disk. After 7 d, the parent whiteflies were removed, and the disks were kept until progeny emergence. From each disk, 10 males and 10 females were randomly selected to be tested for the presence of Rickettsia by PCR.

Effect of Rickettsia on Longevity, Recruitment, and Sex Ratio of B. tabaci. For each colony, tobacco leaves bearing whitely pupae were placed in a small cage containing a new tobacco seedling for 8 h. The leaves were then removed and the emerged adults were left on the tobacco seedlings for an additional 36 h for mating. Twenty pairs from each cage were then transferred to sweet pepper leaf disks (one male + one female per disk). The disks were checked daily for adult mortality, and, to prevent a sex ratio bias in the progeny, new males were supplied for replicates when males had died. The whitefly pairs were transferred to new leaf disks every 2 wk so that we would not confuse them with their emerging progeny. After the onset of progeny eclosion, whiteflies were collected, sexed, and counted twice a week. Longevity results were analyzed by two-way analysis of variance (ANOVA), with infection status (R+, R−) and sex used as fixed factors. Additionally, a Kaplan–Meier survival analysis was performed.

Effect of Rickettsia on Oviposition Rate of B. tabaci. Newly emerged adults were obtained as described above and placed on sweet pepper leaf disks, one female and one male per disk (22 R+ and 23 R− replicates). After 6 d, whiteflies were removed, and
Table 1. Summary of fitness parameters measured (mean ± SE) for Rickettsia-positive (R⁺) and Rickettsia-negative (R⁻) B. tabaci

| Parameter measured          | Treatment | Statistics* |
|-----------------------------|-----------|-------------|
| Longevity (d)               | ♂️      | 37.6 ± 2 (n = 17) | 32.4 ± 2.3 (n = 13) | NS |
|                             | ♀️      | 27 ± 1.55 (n = 6) | 30.43 ± 2.8 (n = 7) | NS |
| No. progeny                 | ♂️      | 116.3 ± 12.6 (n = 17) | 104.1 ± 8 (n = 13) | NS |
|                             | ♀️      | 51.1 ± 3% (n = 17) | 54 ± 3.3% (n = 13) | NS |
| Eggs/d (6 d)                | ♂️      | 12.35 ± 0.55 (n = 22) | 10.5 ± 0.57 (n = 23) | NS |
|                             | ♀️      | 18.7 ± 0.23 (n = 10) | 19.2 ± 0.12 (n = 10) | Fₜᵣᵢₜ = 6.34, df = 1, P = 0.016 |
| Egg-adult developmental time (d) | ♂️  | 18.6 ± 0.14 (n = 10) | 18.9 ± 0.15 (n = 10) | Fₜᵣᵢₜ = 6.34, df = 1, P = 0.016 |
|                             | ♀️      | 66.4 ± 2.9% (n = 10) | 75.3 ± 3.7% (n = 10) | NS |

*See text for additional statistical details. NS, not significant.

The number of eggs was counted. Results were analyzed by t-test.

Effect of Rickettsia on Egg–Adult Developmental Time and Survival of B. tabaci. Newly emerged adults (obtained as described above) were placed in bulk on a sweet pepper leaf disk for 2 d for physiological adaptation to a new host plant. Whiteflies were then transferred to new leaf disks (10 disks per treatment), eight females per disk for 16 h, after which they were removed and the number of eggs laid on each disk was counted. Disks were monitored daily and, upon emergence, adults were collected, sexed, and counted. The mean egg–adult developmental time was calculated per disk as well as the survival percentage, calculated as the total number of emerging adults divided by the number of eggs on each disk. The average number of adult progeny per replicate, used to calculate these variables was 36.8 ± 5.2 for R⁺ and 39.1 ± 4.3 for R⁻ (t₁₈ = −0.34, P = 0.73). Developmental time was analyzed by two-way ANOVA, with infection status (R⁺, R⁻) and sex used as fixed factors, and the total number of adult whiteflies in each disk as a covariate. The survival experiment was analyzed using the non-parametric Mann–Whitney test because various transformations failed to normalize data.

Mating between R⁺ and R⁻ Whiteflies. To test for the possibility that Rickettsia causes cytoplasmic incompatibility in B. tabaci, reciprocal crosses between R⁺ and R⁻ individuals, and within-line matings were performed. Using a fine needle, pupae from both colonies were removed from leaves and inserted individually into small vials. The emerging adults were sexed and pairs were placed together on a leaf disk for 1 wk and then removed and kept in alcohol to verify their infection status. The leaf disks were incubated until all progeny emerged, and were then sexed and counted. Treatments included: an R⁻ male with an R⁺ female and vice versa, males and females from the same line (either R⁺ or R⁻) and virgin females. Results were analyzed using one-way ANOVA (female proportions data were arcsine transformed before analyzing) and Tukey–Kramer honestly significant difference (HSD) post hoc tests.

Statistical Analysis. All analyses were performed using the JMP software, version 6 (SAS Institute 2002). Data from the experiments was used to construct approximate intrinsic rate of increase (r) values for both whitely lines, using the Populus 5.3 software (http://www.cbs.umn.edu/populus/).

Results

Vertical Transmission of Rickettsia. When the progeny of R⁺ parents were tested for the presence of Rickettsia, in nine of 10 replicates all progeny (10 males + 10 females from each replicate) were infected. In one replicate, all female but no male progeny were tested positive for Rickettsia.

Effect of Rickettsia on Longevity. The mean longevity of R⁺ females was 5 d longer than R⁻ females, whereas an opposite pattern was noted in males, with R⁺ males living 3 d less than R⁻ males (Table 1). The differences, however, were not statistically significant (Fᵣᵡᵢᵣᵢᵢᵣᵢᵣᵡₒₜ = 0.11, df = 1, P = 0.74; Fₛₑₓᵣᵣᵯₑᵣ = 6, df = 1, P = 0.019; Fᵣᵡᵢᵣᵢᵣᵢᵣᵡₒₜ × Sₑₓᵣᵣᵯₑᵣ = 2.14, df = 1, P = 0.15). Similarly, a Kaplan–Meier survival analysis showed a trend toward greater survivorship of infected female whiteflies, but that difference was not statistically significant (Wilcoxon X²ᵣᵣᵯₑᵣ = 3.69; P = 0.055) (Fig. 1).

Effect of Rickettsia on Recruitment and Sex Ratio. Lifetime progeny amount and sex ratio were similar in both treatments (Table 1) and did not differ signifi-
Table 2. Progeny amount and sex ratio (mean ± SE) of cross-mating between R+ and R− whiteflies

| Cross          | N  | Avg no. F1 | Sex ratio (% females) |
|----------------|----|------------|----------------------|
| Unmated ♀+     | 18 | 30.5 ± 4.9A| 0                    |
| Unmated ♂      | 20 | 24.3 ± 2.7A| 0                    |
| ♀+ ♂−          | 17 | 26.3 ± 3.7A| 45.6 ± 3.4           |
| ♀+ ♂+          | 14 | 32.5 ± 4.0A| 44.3 ± 5.3           |
| ♀− ♂−          | 23 | 24.4 ± 2.4A| 45.9 ± 3.3           |
| ♀− ♂+          | 14 | 34.6 ± 5.5A| 36.1 ± 5.6           |
| ANOVA          |    |            | F1,105 = 1.29; F3,67 = 1.63; P = 0.27; P = 0.19** |

*For % females, the unmated female treatments were excluded from the analysis.

cantly (progeny amount: t28 = 0.75; P = 0.45; progeny sex ratio: t28 = 0.63; P = 0.53). The range in recruitment was 32–215 progeny per female among the R+ whiteflies and 51–190 among the R− whiteflies. Sex ratio in the R+ treatment varied between 27.5 and 72.4% females compared with 31.2 and 73.5% in the R− treatment.

Effect of Rickettsia on Oviposition. Over a 6-d period, R+ females laid more eggs than R− females, but the difference was not statistically significant (t28 = 1.51, P = 0.14) (Table 1).

Effect of Rickettsia on Egg-Adult Developmental Time and Survival. Female B. tabaci developed 1.5 d faster, on average, when they carried Rickettsia, whereas developmental time for males was nearly identical for males (Table 1). The overall two-way ANOVA was not significant (F4,35 = 2.54; P = 0.057), but the Rickettsia infection status main effect was statistically significant (F1,35 = 6.34, df = 1, P = 0.016; Fsex = 0.02, df = 1, P = 0.88; Finteraction × sex = 1.5, df = 1, P = 0.22).

Survivorship during development was higher in the R+ whiteflies, but the difference was not statistically significant (Mann–Whitney U test: U = 27; P = 0.09) (Table 1). The intrinsic rate of increase (r) values calculated from all the data detailed above were 0.3794 d−1 for R+ and 0.3893 d−1 for R−.

Effect of Rickettsia on Mating between R+ and R− Whiteflies. The total number of progeny and their sex ratio did not differ between the four crosses, suggesting that Rickettsia does not cause cytoplasmic incompatibility in this colony of B. tabaci (Table 2).

Discussion

Symbionts may promote their spread and transmission in a host population by either conferring fitness advantages to their host, manipulating the host's reproduction or by horizontal transfer routes. Here, as a first step toward explaining the mechanisms behind the high prevalence of Rickettsia in B. tabaci field collections in Israel (65–74%, Chiel et al. 2007), we investigated the fitness consequences of Rickettsia infection in the laboratory, as well as the possibility that Rickettsia causes cytoplasmic incompatibility.

The presence of Rickettsia provided some fitness benefits to B. tabaci, although only one of them, faster
development from egg to adult, was statistically significant. Together, these data suggest no clear benefits to Rickettsia infection. If anything, Rickettsia seemed to compromise fitness: whereas both lines produced very similar numbers of progeny throughout their life span, the approximate intrinsic rate of increase (r) of R− whiteflies was slightly higher than the R+ colony, probably owing to the higher preadult survival. Other evidence of costs of carrying Rickettsia was shown by Kontsedalov et al. (2008), in which the R+ colony was more susceptible to some insecticides than the R− colony (the colonies used in that study were the same as those used in this study). Similar indications of possible costs of carrying secondary symbionts were demonstrated in the study of Ruan et al. (2006), in which B biotype B. tabaci from China had higher survival and shorter developmental time when the secondary symbiont Hamiltonella was eliminated by certain antibiotics. Rickettsia, however, was not surveyed in that study. The closely related Rickettsia in pea aphids, Acyrthosiphon pisum (Harris)), also showed negative effects on its host such as reduced fecundity, longevity, and body weight (Chen et al. 2000; Montllor et al. 2002; Sakurai et al. 2005), although in Chen et al. (2000), the negative effects were plant- and temperature-dependent.

Rickettsia is involved in reproductive manipulation in several insect hosts (reviewed in Perlman et al. 2006), but in the current study crosses between R+ and R− whiteflies revealed no evidence of cytoplasmic incompatibility, because the number and sex ratio of progeny were similar in the predicted CI cross (R+ males and R− females) and the control crosses. Further, R+ females did not produce significantly more females than R− females, indicating no sex ratio distortion by this symbiont.

What, then, might be the reasons for the high prevalence of Rickettsia in B. tabaci populations? We suggest two possible explanations:

1. Rickettsia may affect other fitness components that were not measured in the current study. For example, Rickettsia might be advantageous for B. tabaci under certain conditions, such as heat shock or heat stress. B. tabaci develops well in hot climates and in high-temperature habitats, such as greenhouses. Moreover, one of the physiological mechanisms activated in B. tabaci in response to elevated temperatures is the synthesis of heat shock proteins, some of which have been speculated to be produced by symbiotic bacteria (Salvucci et al. 2000). Rickettsia-induced heat stress tolerance was demonstrated for one of three pea aphid clones, indicating that this phenotype is determined also by the host genotype (Chen et al. 2000). Symbiont-induced heat tolerance in pea aphids was also reported for Serratia and Hamiltonella, both for heat stress (Chen et al. 2000) and heat shock (Montllor et al. 2002; Russell and Moran 2006). Another possibility is that fitness effects of Rickettsia are density-dependent and expressed only under more crowded conditions (the exper-
iments presented herein compared single, isolated females). For polyphagous insects, such as *B. tabaci*, fitness effects may also be host plant-dependent, as demonstrated for pea aphid *Rickettsia* (Chen et al. 2000) and for other pea aphid symbionts (Tsuchida and Fukatsu 2004; Ferrari et al. 2007). Fitness parameters of *B. tabaci* vary greatly among different host plants (see review of Drost et al. 1998), and *Rickettsia* infection may interact with those differences. Other factors that could be important include resistance to natural enemies or pathogens, as has been demonstrated with the pea aphid and some of its symbionts (Oliver et al. 2003; Scarborough et al. 2005), and interactions with plant viruses.

2. Horizontal transmission. In cases where fitness benefits and reproductive manipulations are not evident, a symbiont may be spreading in its host population by means of horizontal transmission. Possible mechanisms of horizontal transmission include mating, as has been recently found in aphids (Moran and Dunbar 2006), or via a shared plant host. Horizontal transmission of *Rickettsia* in plants was shown by Davis et al. (1998), where the symbiont was perfectly associated with papaya bunchy top disease, transmitted by a leafhopper. *B. tabaci* populations often reach very high densities that may create ample opportunities for horizontal transmission via the plant phloem, or merely by contact of adjacent probosci, contaminated whiteflies or plant surfaces.

Our results demonstrate that *Rickettsia*’s vertical transmission is close to complete and that may supply an explanation for how *Rickettsia* is being maintained at a relatively high level in *B. tabaci*. This explanation does not address how the symbiont reached high levels initially, however, and whether it is able to spread further. Interestingly, the one female that did not show perfect transmission produced completely infected daughters and uninfected males, but several attempts to reproduce these results failed. Very high rates of vertical transmission seem to be the rule for secondary symbionts, and *Rickettsia* lineages that have been tested are no exception (Perlman et al. 2006).

In conclusion, our evidence suggests *Rickettsia* may be a commensal, i.e., neutral with respect to the currently tested life history parameters that contribute to population growth. However, *Rickettsia* may be advantageous to *B. tabaci* under certain selective pressures that were not tested here, or instead may be maintained by horizontal or paternal transmission. In this system, as in the pea aphid–*Rickettsia* system, the mechanism by which *Rickettsia* is maintained in its hemipteran hosts is elusive and awaits further research.

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