Insect symbiont facilitates vector acquisition, retention, and transmission of plant virus

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Tomato yellow leaf curl virus (TYLCV) was first detected in China in 2006, following the introduction of Bemisia tabaci Q into China in 2003. Since then, the incidence of TYLCV in tomato fields in China has greatly increased as has the abundance and distribution of Q whiteflies containing the bacterial symbiont Hamiltonella with high frequency. This suggested that the symbiont Hamiltonella might associate with the transmission efficiency of TYLCV by the whitefly vector. Here we report the first evidence that the Hamiltonella is closely associated with the acquisition, retention, and transmission efficiency of TYLCV by the whitefly vector. Our findings combined with the outbreaks of TYLCV following the introduction of Q, provided an explanation for why Hamiltonella is being maintained at a relatively high level in Chinese B. tabaci Q and also have implications for disease and vector management.

As a vector of viruses, the whitefly Bemisia tabaci (Gennadius) (Hemiptera: Aleyrodidae) is one of the most economically important pests of vegetables, cotton, and flowers. One of the viruses transmitted by B. tabaci is Tomato yellow leaf curl virus (TYLCV), which originated in the Middle East–Mediterranean region, but has since been introduced into many other regions worldwide and is now among the most devastating viral diseases of tomato. Infected tomato plants have small, curled leaflets and are stunted and bushy. Severe infections can result in complete crop loss. Like other Begomoviruses, TYLCV is transmitted in a circulative and persistent manner by B. tabaci. Outbreaks caused by TYLCV and other whitefly-transmitted viruses have often coincided with invasions by particular biotypes of B. tabaci. Recent studies indicate that B. tabaci is a species complex that contains at least 24 reproductively isolated but morphologically indistinguishable cryptic species. Because discrimination at the species level within the B. tabaci complex has yet to be fully resolved, we have retained the commonly used term ‘biotype’ to refer to the cryptic species. These biotypes differ in host range, life history traits, insecticide resistance, transmission competency for begomoviruses, and the symbionts that they harbor. The two most invasive and destructive biotypes are B. tabaci biotype B (or simply B) and biotype Q (or simply Q). B belongs to the Middle East-Minor Asia 1 (MEAM1) genetic group, and Q belongs to the Mediterranean (MED) genetic group.

Although B. tabaci was first recorded in the late 1940s in China, crop losses caused by this insect did not become serious until the introduction of B in the 1990s. Q was first found in Yunnan Province in 2003 and was considered a new, invasive whitefly in China. Q has now displaced well-established populations of B in many areas and has become the dominant form of B. tabaci in field agricultural systems in most parts of China.

Bemisia tabaci is host to bacterial symbionts. The maternally inherited, endosymbiotic bacteria of insects are prevalent and are broadly divided into two groups: primary symbionts (P-symbionts) and secondary symbionts (S-symbionts). P-symbionts benefit host insects by aiding in digestion of food or by providing nutrients that are limited or lacking in the diet. S-symbionts, in contrast, may not be required for host survival but may play important roles in host biology and evolution. To date, one P-symbiont and six S-symbionts have been reported from B. tabaci. The P-symbiont is Portiera, and the S-symbionts are species of Hamiltonella, Arsenophonus, Cardinium, Wolbachia, Rickettsia, and Fritschea. Recent research has speculated that the S-symbiont Hamiltonella was probably associated with the transmission efficiency of TYLCV by the whitefly vector. Israeli Q lacks Hamiltonella and cannot effectively transmit TYLCV, while Chinese Q frequently contains Hamiltonella and can efficiently transmit TYLCV to tomato.
plants. Hence, we hypothesize that the ability of *B. tabaci* Q to acquire, retain, and transmit TYLCV is affected by *Hamiltonella*. To test this hypothesis, we: 1) established *Hamiltonella*-infected (H+) and uninfected (H−) *B. tabaci* Q strains with the same genetic background via antibiotic treatment and introgression; and 2) compared the ability of H+ and H− strains to acquire, retain, and transmit TYLCV.

**Results**

**Screening for the presence of symbionts.** At the outset of this study, the Q population only harbored the P-symbiont *Portiera* and the S-symbiont *Hamiltonella*, the other S-symbionts *Arsenophonus*, *Cardinium*, *Wolbachia*, *Rickettsia*, and *Fritschea* were not detected. Continuous rearing of newly-emerged adult insects on cottons for 6 generations of introgression, the relative concentration of *Portiera* did not differ in the introgressed H+ vs. H− strains (F1,4 = 0.121, P = 0.746) (Fig. 1). However, *Hamiltonella* almost disappeared from the H− strain, and the relative concentration of *Hamiltonella* was 3600-fold higher in the introgressed H+ strain than in H− strain (F1,4 = 108.36, P < 0.0001) (Fig. 1).

**Acquisition of TYLCV DNA by H+ vs. H− Q whiteflies.** As indicated by conventional PCR, the shortest AAP for acquisition of TYLCV from infected plants was 0.5 h for H+ Q and 1.0 h for H− Q (Fig. 2). The percentage of adults with detectable TYLCV DNA increased with the length of AAP and reached 100% after a 10-h AAP for H+ Q and after a 12-h AAP for H− Q (Fig. 2). The percentage of whiteflies that acquired TYLCV was significantly affected by AAPs (Wald’s χ2 = 10.525, P = 0.001), whereas not by the infection status of *Hamiltonella* (Wald’s χ2 = 2.023, P = 0.155) and the interaction between these two factors (Wald’s χ2 = 0.545, P = 0.46). Also, the relative amount of TYLCV (as indicated by q-PCR) was significantly higher in H+ Q than in H− Q after each of the eight AAPs (Fig. 3). TYLCV DNA peaked after a 12-h AAP in H+ Q and after an 18-h AAP in H− Q (Fig. 3).

**Retention of TYLCV DNA by whiteflies.** As indicated by conventional PCR, a high percentage of viruliferous adults of H+ and H− Q retained TYLCV DNA after feeding for 5 to 35 days on cotton, which is not a host for TYLCV (Fig. 4). According to q-PCR, however, the relative amounts of TYLCV per whitefly were greater in of H+ than in H− Q whiteflies (B. tabaci) after 10 acquisition access periods (AAPs) as determined by q-PCR. Values are means ± standard deviation. Asterisks indicate that gene expression was significantly greater in H+ than in H− Q (*P < 0.05, ** P < 0.01). The black and red arrows indicate the time when viral titer reached a steady-state in H+ and H− Q, respectively.

**Figure 1** | Relative amount of TYLCV (normalized to the host nuclear β-actin gene) in H+ (black bars) and H− Q (red bars) whiteflies (B. tabaci) as determined by q-PCR. Vertical lines indicate standard deviations of the mean. The asterisk indicates that symbiont gene expression differed (P < 0.001) between H+ and H− Q, and n.s. indicates not significant (P > 0.05).

**Figure 2** | Percentage of H+ vs. H− Q whiteflies (B. tabaci) that acquired TYLCV after 13 acquisition access periods (AAPs) as determined by conventional PCR. For each whitefly type and AAP, 20 adults were assessed. The percentage of whiteflies that acquired TYLCV between H+ and H− Q was compared by binary logistic regression.

**Figure 3** | Relative amount of TYLCV in H+ and H− Q whiteflies (B. tabaci) as indicated by conventional PCR. After a 24-AAP on TYLCV-infected tomato, whiteflies were placed on cotton for 5 to 35 days. For each whitefly type and duration of feeding on cotton, 20 adults were assessed. The percentage of whiteflies with TYLCV did not differ between H+ and H− Q for any feeding time on cotton (P > 0.05).
Transmission of TYLCV by whiteflies and viral accumulation in plants. Based on the presence or absence of TYLCV symptoms on tomato plants, transmission frequency was much higher with H\(^+\) Q females than with H\(^-\) Q females, and these differences were statistically significant at each of the three whitefly densities (Fig. 6). For H\(^+\) Q females, transmission frequency was high (60%) with only one female per plant and increased to 100% with 5 and 10 females per plant. For H\(^-\) Q females, transmission frequency was only 0, 10, and 30% with 1, 5, and 10 females per plant, respectively (Fig. 6).

Viral accumulation in tomato plants was significantly affected by the infection status of Hamiltonella (F\(_{3,16}\) = 1132.039, P < 0.0001), the sex of B. tabaci (F\(_{2,16}\) = 112.333, P < 0.0001), and by the interaction between these two factors (F\(_{3,16}\) = 79.434, P < 0.0001) (Fig. 7). Specifically, relative viral accumulation was 1.69-fold higher in plants exposed to one H\(^+\) Q female than to one H\(^-\) Q male (F\(_{1,8}\) = 118.771, P < 0.0001). Relative viral accumulation was 9.0-fold higher in plants exposed to one H\(^+\) Q female than to one H\(^-\) Q female (F\(_{1,8}\) = 1003.696, P < 0.0001) and was 7.6-fold higher in plants exposed to one H\(^+\) Q male than to one H\(^-\) Q male (F\(_{1,8}\) = 278.252, P < 0.0001). Relative viral accumulation did not differ between plants exposed to one H\(^-\) Q female and one H\(^-\) Q male (F\(_{1,8}\) = 3.537, P = 0.097).

**Discussion**

Our studies indicate that the presence of Hamiltonella is involved in acquisition, retention, and transmission of TYLCV by B. tabaci Q and in significant differences for TYLCV accumulation in plants exposed to the whiteflies. On the other hand, the result also indicated that virus transmission efficiency seems to be more related with differences in symbiont composing than with whitefly biotypes, regardless several literature stressing the importance of the biotypes in viral transmission. This result coupled with the outbreaks of TYLCV following the introduction of Q\(^+\), provided a possible explanation for why Hamiltonella is being maintained at a relatively high level in Chinese B. tabaci Q populations\(^1\)

Although the P-symbiont Portiera was maintained in H\(^+\) and H\(^-\) Q strains of the whitefly B. tabaci, the S-symbiont Hamiltonella can’t detected from the H\(^-\) Q strain by diagnostic PCR. After six genera-

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**Figure 5** Relative amount of TYLCV (normalized to the host nuclear \(\beta\)-actin gene) in H\(^+\) (black dots) and H\(^-\) (red dots) Q whiteflies (B. tabaci) after a 24-h AAP on a TYLCV-infected tomato plant and 3 to 12 days of incubation on cotton. Vertical lines on each bar represent standard deviations of the mean. Asterisks indicate significant difference with the rate of viral titer decline per day (B) between H\(^+\) and H\(^-\) Q (P < 0.01).

**Figure 6** Transmission of TYLCV as affected by type (H\(^+\) vs. H\(^-\) Q) and number of B. tabaci females. Transmission was assessed based on the presence or absence of TYLCV symptoms 30 days after tomato plants had been exposed to whiteflies for 24 h. Each combination of whitefly type and density was represented by 20 replicate plants. Asterisks indicate significant differences between H\(^+\) and H\(^-\) Q at each of three insect densities (P < 0.001).

**Figure 7** Relative amount of TYLCV in tomato leaves as affected by type (H\(^+\) vs. H\(^-\) Q) and sex of B. tabaci. H\(^+\) F: H\(^+\) females; H\(^+\) M: H\(^+\) males; H\(^-\) M: H\(^-\) males. The abundance of the TYLCV gene (normalized to the abundance of the Tomato 25S ribosomal RNA gene) was assessed by q-PCR 10 days after tomato plants had been exposed to a single whitefly for 24 h. Each combination of whitefly type and sex was represented by five replicate plants. Vertical lines on each bar represent standard deviations of the mean. Different letters indicate significant differences between the four treatments (P < 0.05).
tions of introgression, the level of *Portiera* did not differ between the introgressed H*-* and H*-* Q strains, however, relative amounts of *Hamiltonella* were 3600-fold higher in the introgressed H*-* Q than in H*-* Q. This indicates that the relative amount of *Portiera* is not affected by changes with *Hamiltonella* abundance in a short-term period, even though these symbionts are confined together in the bacteriocytes in the same host. The current results also confirmed that *Portiera* had an obligatory relationship with *B. tabaci* and could not be removed by antibiotic treatment. *Hamiltonella*, in contrast, almost disappeared from *B. tabaci* by antibiotic treatment, as determined by q-PCR.

The percentage of whiteflies that acquired TYLCV was significantly affected by AAPs for H*-* and H*-* Q, and the relative amount of TYLCV acquired was greater in H*-* Q than in H*-* Q when the access acquisition period (AAP) was ≥ 6 h. Prior study has shown that the ability of acquisition and transmission of TYLCV by whiteflies were associated with its feeding behavior36,38, our preliminary feeding behavior study showed that only two of twenty five parameters associated with feeding behavior were a little different between H*-* Q vs. H*-* Q strains (BML, unpublished data). Consequently, this symbiont had little impact on the performance benefits to their feeding behavior of *B. tabaci* Q. Following a 1–2 day AAP, whitefly vectors were previously found to contain TYLCV DNA for several weeks and sometimes for their entire life32,34–36. Following a 24-h AAP on TYLCV-infected plants in our experiments, all of viruliferous adult H*-* and H*-* Q retained TYLCV DNA for their entire adult lifetime. Whether Begomovirus can replicate in its vector whitefly remains controversial, and only one paper has reported that TYLCV can replicate in *B. tabaci*37. In the current study, the rate at which viral titer decreased in *B. tabaci* after the insects were transferred from virus-infected tomato to virus-free cotton was 1.42% per day in H*-* Q and 3.03% per day in H*-* Q. Our results are consistent with previous results obtained by membrane-hybridization of DNA from single *B. tabaci* B, which showed that titers of TYLCV32 and that Tomato yellow leaf curl Sardinia virus (TYLCSV)33 gradually decreased in vectors that fed on plants that were not hosts to the viruses. However, Sinisterra et al. (2005)40 reported that TYLCV titer in *B. tabaci* B remained constant even after 7 days of feeding on cotton plants. These discrepancies might be due to differences in the symbionts harbored by the whiteflies, environmental conditions, virus isolate, and/or the haplotype of *B. tabaci* used in the experiment.

A GroEL homologue produced by endosymbiotic bacteria seems to protect begomoviruses in insect haemolymph and thereby affects the ability of *B. tabaci* to transmit virus43,44. In the current study, transmission efficiency was substantially higher by H*-* Q than by H*-* Q. This result is consistent with Gottlieb et al. (2010)38, who speculated that *Hamiltonella* increased the ability of *B. tabaci* to transmit TYLCV. Prior studies have shown that the efficiency of TYLCV transmission was affected by *B. tabaci* gender and age36,38. We also found that TYLCV accumulation was greater in tomato plants exposed to H*-* Q females than to H*-* Q males. More importantly, we found that TYLCV accumulation was much greater in tomato plants exposed to H*-* Q than to H*-* Q regardless of sex. Lapidot et al. (2001)39 study suggested a positive correlation between TYLCV level in the plant and whitely transmission rate. In that paper, there were two major factors affect the transmission efficiency or virus accumulation in plants: (i) host plant cultivars and (ii) starting virus level in the whitefly. With respect to our results, the factor (i) was the same, however, initial TYLCV inocula (factor (ii)) from H*-* strain were more than H*-* strain (Fig. 3), and indirectly demonstrated H*-* whiteflies are less efficient vector. Therefore, these results indicate that *Hamiltonella* plays an important role in virus transmission by *B. tabaci* Q.

Taken together, our study provides sufficient evidence that the ability of *B. tabaci* Q to acquire, retain, and transmit TYLCV is affected by its S-symbiont *Hamiltonella*. Our findings also have implications for disease and vector management.

### Methods

#### Insect source, plant cultures, and TYLCV agroinoculation.

The laboratory Q population was collected from the poinsettia, *Euphorbia pulcherrima* Willd. ex Klotz., in Beijing, China in 200936. Since then, it was maintained in isolated whitefly-proof screen cages in a greenhouse under natural lighting and controlled temperature (26 ± 2 °C). The purity of the Q population was monitored by sampling 15 adults per generation using a molecular diagnostic technique, CAPS (cleavage amplified polymorphic sequence), and a molecular marker, mitochondrial cytochrome oxidase I genes (mtCOI)41, Tomato (*Solanum lycopersicum* Mill. cv. zhongza 9) and cotton (*Gossypium hirsutum* L. cv. DP99BB) were used. Tomato is a host of TYLCV but cotton is not. Healthy tomato and cotton plants were grown in a potting mix in 1.5-L pots (one plant/pot) under natural light and with controlled temperature (26 ± 2 °C) in a glasshouse. When plants grew to the 6–7 true-leaf stage, they were used in the experiments. TYLCV-infected tomato plants were obtained by agro-inoculation using a cloned TYLCV genome (GenBank accession number: AM288784) that was originally isolated from tomato plants in Shanghai, China40. Tomato plants were inoculated when they had three true leaves and were assumed to be infected with virus when they developed characteristic leaf-curl symptoms.

#### Detection of symbionts and TYLCV.

Nucleic acids from individual whiteflies and plants were extracted using the methods of White et al. (2009)46 and Xie et al. (2002)47. The PCR procedure described by Pan et al. (2012)48 was used to detect the P-symbiont and S-symbionts in whiteflies. As indicated in the Results, the only symbionts detected in the Q population were the P-symbiont *Portiera* and the S-symbiont *Hamiltonella*. A TYLCV DNA fragment (~410 bp) was amplified using the primer pairs C473 (5'-AGTCACGGGGCCCCTTACA-3') and V61 (5'-ATACTGGAAACCTAAATGGC-3')49. The resultant PCR products were electrophoresed on a 2.0% agarose gel in a 0.5× TBE buffer and visualized by Gelview staining.

#### Quantitative real-time polymerase chain reaction (q-PCR).

Amplifications for symbionts and TYLCV were performed with 2.5 × Real Master Mix (SYBR Green) (TIANGEN Biotech (Beijing), Co., Ltd) and 5 pmol of each primer. The cycling conditions for symbionts were: 5 min activation at 94 °C, followed by 40 cycles of 30 s at 94 °C, 30 s at 60 °C, and 60 s at 72 °C. For each amplification run, standards were loaded in the same plate to build the appropriate standard curve. PCR reactions were carried out in 96-well optical plates in the Applied Biosystems 7500 real-time PCR instrument and the

| Gene          | Primer set | Expected size (bp) | Primer sequence (5’ to 3’) | Reference |
|---------------|------------|--------------------|---------------------------|-----------|
| *Portiera* 16S rDNA | Port-F     | 229                | TAGTCACCGCTGTAACGG       | 56        |
|               | Port-R     | 243                | AGGCCACCCCTCCATCT         |           |
|               | Ham-F      | 243                | GCATCGAGTGACACAGTT        | 57        |
|               | Ham-R      | 243                | TATCTTCTGACCCGGCTAA       |           |
| TYLCV         | C473       | 410                | AGTCACGGGGCCCCTTACA       | 48        |
|               | V61        | 144                | ATACCTGGAAACCTAAATGGC     | 16        |
| TYLCV (q-PCR) | TY-F       | 144                | GTCTACACGCTTACGCC         |           |
|               | TY-R       | 144                | GCAAATCTCTGCTACC          |           |
| /-actin       | /-actin F  | 130                | TCTCAGCGCCATCCTCTTG       | 40        |
|               | /-actin R  |                    | CGGTGATITCCCTTGTGAT       |           |
| 25S Tomato    | 25S rRNA F | 113                | AAACCGATCATCACGTCTCCA     | 39        |
|               | 25S rRNA R |                    | CCGAAGTITCCGATCCTTA       |           |

**Table 1 | List of primers developed and used in this study**

**References:**

1. Lapidot et al. (2001)
2. Hamiltonella abundance in a short-term period.
3. TYLCV (q-PCR) TY-F 144 GTCTACACGCTTACGCC 16
4. TYLCV (q-PCR) TY-R GCAATCTTCGTCACCC 57
5. TYLCV accumulation was greater in tomato plants exposed to H*-* Q females than to H*-* Q males.
6. TYLCV accumulation was much greater in tomato plants exposed to H*-* Q than to H*-* Q regardless of sex.
7. TYLCV can replicate in its vector whitefly remains controversial.
8. TYLCV can replicate in *B. tabaci* B, which showed that titers of TYLCV and that Tomato yellow leaf curl Sardinia virus (TYLCSV) gradually decreased in vectors that fed on plants that were not hosts to the viruses.
9. TYLCV titer in *B. tabaci* B remained constant even after 7 days of feeding on cotton plants.
10. Quantitative real-time polymerase chain reaction (q-PCR).
accompanying software were used for qPCR data normalization and quantification. For each whitefly sample, three replicates were amplified in each of three biologically independent experiments. For each plant sample, three replicates were amplified in each of five biologically independent experiments. The relative expression levels of the TYLCV or symbiont gene in B. tabaci (normalized to β-actin from B. tabaci) and the TYLCV gene in plants (normalized to Tomato 25S rRNA) were calculated based on the $$2^{-\Delta\Delta C_t}$$ method. The specific q-PCR primers used for quantification of Portiera, Hamiltonella, and TYLCV are listed in Table 1.

Antibiotic treatment. Antibiotic treatments were administered using Parafilm membrane sachets for direct feeding by adults in a feeding chamber. The control diet solution was 5 mM phosphate buffer (pH 7.0) with 25% sucrose, whereas the antibiotic diet was the same solution with the addition of 50 μg/ml rifampicin (Amsercos, no. 0146). Approximately 50 newly emerged Q adults (females and males mixed) were introduced into each feeding chamber at room temperature; a 0.4-ml drop of diet was placed on the outer surface of the stretched Parafilm and covered with another layer of stretched Parafilm to enclose the solution without air bubbles between the Parafilm layers. After they had fed for 48 h, 20 of the adults were collected and subjected to PCR detection for symbionts, and the others were placed in cages containing cotton plants. Whiteflies were cycled of their S-symbiont Hamiltonella after they had received rifampicin-infused sucrose (50 μg/ml) for 48 h. 30 adults from a caged strain per generation were subjected to specific PCR detection to confirm elimination of the symbiont. Thereby, they were continuously reared on cotton under the conditions described above.

Introgression. In order to minimize genetic differences among individuals, we carried out an introgression series to homogenize the nuclear background of both H- and H’ strains. We introgressed the H’ Q nuclear background into the H- Q cytophore for 6 generations to yield infected and uninfected lines that shared ~ 98% of their nuclear alleles. Newly emerged virgin whitefly adults from each strain were obtained according to the method of Luan et al. (2008). Both introgressed H- and H’-whitefly strains have been maintained in separate net cages containing cotton plants under natural light and ambient temperature (26 ± 2°C) in a glasshouse. The introgressed H-’ line and H- line were used for all experiments.

Acquisition of TYLCV by whiteflies. We randomly collected twenty newly emerged adults and transferred to a clip-cage attached to a leaf (the second to fifth leaf from the top of TYLCV-infected tomato plants; four clip-cages, all with H- or H’ Q, were attached to each of six plants for each whitefly strain. Thereafter, two clip-cages of H- and H’ Q were randomly collected at the end of 13 acquisition access periods (AAPs: 0, 0.25, 0.5, 1, 2, 3, 4, 6, 8, 10, 12, 24, and 48 h). The collected adults were stored at ~20°C and later assayed individually to detect TYLCV DNA by conventional PCR; the results were expressed as the percentage of individuals with TYLCV DNA. For quantification of TYLCV in whiteflies, 20 adults (females and males in approximately equal numbers) of either H- or H’ Q were randomly collected at the end of each of 10 AAPs (1, 4, 6, 8, 10, 12, 18, 24, 36, and 48 h) on the TYLCV-infected tomato plants. These were stored at ~20°C, and after their total DNA was extracted, they were assayed for TYLCV by q-PCR.

Retention of TYLCV DNA by whiteflies. Newly emerged non-viruliferous adult whiteflies of either H- or H’ Q were collected and caged with TYLCV-infected tomato plants to obtain viruliferous adults. After a 24-h AAP, the adults were removed from the TYLCV-infected tomato plants and placed on cotton plants; cotton is not a host for TYLCV. After 5, 10, 15, 20, 25, 30, and 35 days on cotton, 20 live adults were collected following their natural death between 30 and 40 days. During the collection process, the adults remaining on the plant were transferred into a new cotton plant once two weeks to avoid emergence of new adults among the progeny on the same plants. The whiteflies collected were stored at ~20°C and later assayed individually for TYLCV DNA by conventional PCR, and the results were expressed as the percentage of individuals with TYLCV DNA. For quantification of TYLCV in whiteflies that first fed on TYLCV-infected tomato (24-h AAP) and then on cotton, 20 adults (females and males in approximately equal numbers) of either H- or H’ Q were randomly collected after 3, 6, 9, and 12 days on cotton. The collected insects were stored at ~20°C and were later assayed for TYLCV by q-PCR as described earlier.

Transmission of TYLCV to tomato plants and viral accumulation in plants. Newly emerged females of either H- or H’ Q were collected and transferred to a TYLCV-infected tomato plant where they were caged for a 24-h AAP. The whiteflies were then removed from the infected plant and confined in a clip-cage (1.5 or 10 females per cage) on a virus-free tomato seedling (with three true leaves) for a 24-h period of inoculation feeding. The females were removed, and the plants were kept for symptom development in insect-proof cages. Twenty plants were used for each of the six treatments (two whitefly types × three whitefly densities). After 30 days, plants were assessed for virus infection based on the presence or absence of TYLCV symptoms.

For quantification of viral accumulation in plants, a single viruliferous female or male of H- Q or H’ Q (all adults were of similar age) was placed in a clip-cage on the second leaf from the top of a healthy tomato plant (one clip-cage per plant) with three true leaves. Since the results of virus acquisition have demonstrated that viral DNA was present in 100% of the whiteflies for both H- and H’ Q strains after a 12-h AAP on virus-infected tomato plants (Fig. 2), we only allowed the whiteflies to feed on tomato plants for 12 h to acquire the viruses, ensure that each individual should acquire the virus. After that, the adults were removed, and the plants reared in insect-proof cages. In order to compare virus load of different plants, it is advisable to use the first two youngest leaves of an infected plant and TYLCV reached its peak viral load at 10 or 20 days post-inoculation. So, we collected from the first two youngest leaf of each plant at 10 days post-inoculation and pooled them to monitor viral load. The results were expressed as the percentage of whiteflies that retained TYLCV after each of seven periods of feeding on cotton, and the percentage of tomato plants with TYLCV symptoms after exposure to viruliferous whiteflies at each of three densities. The relative quantities of symbiont and virus in H- and H’ Q after each of 10 AAPs were compared by one-way analysis of variance (ANOVA). TYLCV accumulation in plants was compared by two-factor ANOVAs, with infection status and sex of B. tabaci as fixed factors. Means for significant ANOVAs were compared by the honestly significant difference (HSD) test at P < 0.05. All statistical analyses were performed with SPSS 17.0 (SPSS Inc., Chicago, IL, USA).

Data analysis. The percentage of whiteflies that acquired TYLCV between H- and H’ Q was compared by binary logistic regression, with infection status and AAPs as model variables. Chi-square tests were used to compare H- and H’ Q for: the percentage of whiteflies that retained TYLCV after each of seven periods of feeding on cotton, and the percentage of tomato plants with TYLCV symptoms after exposure to viruliferous whiteflies at each of three densities. The relative quantities of symbiont and virus in H- and H’ Q after each of 10 AAPs were compared by one-way analysis of variance (ANOVA). TYLCV accumulation in plants was compared by two-factor ANOVAs, with infection status and sex of B. tabaci as fixed factors. Means for significant ANOVAs were compared by the honestly significant difference (HSD) test at P < 0.05. All statistical analyses were performed with SPSS 17.0 (SPSS Inc., Chicago, IL, USA).
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