Competitive Ability and Fitness Differences between Two Introduced Populations of the Invasive Whitefly Bemisia tabaci Q in China

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Abstract

Background: Our long-term field survey revealed that the Cardinium infection rate in Bemisia tabaci Q (also known as biotype Q) population was low in Shandong, China over the past few years. We hypothesize that (1) the Cardinium-infected (C+) B. tabaci Q population cannot efficiently compete with the Cardinium-uninfected (C-) B. tabaci Q population; (2) no reproductive isolation may have occurred between C+ and C-; and (3) the C- population has higher fitness than the C+ population.

Methodology and Results: To reveal the differences in competitive ability and fitness between the two introduced populations (C+ and C-), competition between C+ and C- was examined over several generations. Subsequently, the reproductive isolation between C+ and C- was studied by crossing C+ with C- individuals, and the fitnesses of C+ and C- populations were compared using a two-sex life table method. Our results demonstrate that the competitive ability of the C+ whitefly was weaker than that of C-. There is that no reproductive isolation occurred between the two populations and the C- population had higher fitness than the C+ population.

Conclusion: The competitive ability and fitness differences of two populations may explain why C- whitefly populations have been dominant during the past few years in Shandong, China. However, the potential role Cardinium plays in whitefly should be further explored.

Introduction

The sweet potato whitefly, Bemisia tabaci (Gennadius) (Hemiptera: Aleyrodidae), is a major crop pest [1,2] and this species complex contains at least 31 cryptic species identified based on mitochondrial cytochrome oxidase I (mtCOI) sequences and crossing experiments [3,4]. The best known species are MEAM1 (commonly known as biotype B, hereafter referred to as B. tabaci B or B) and MED (commonly known as biotype Q, hereafter referred to as B. tabaci Q or Q) because both are extremely invasive and globally distributed [3]. B. tabaci Q was first detected on the ornamental poinsettia (Euphorbia pulcherrima Willd.) in China in 2003 [5]. Since then, it has gradually displaced B. tabaci B which was introduced in the mid-1990s. Since 2008, B. tabaci Q has become the dominant whitefly species in most regions of China [6–9].

The symbiont Cardinium was first found in cell cultures established from the tick Ixodes scapularis Say [10] and was named Cardinium Cardinium hertigi by Zchori-Fein et al. [11]. Cardinium can alter the reproduction of its hosts by feminization [12,13], parthenogenesis [14], or cytoplasmic incompatibility of infected hosts [15–17]. Prior studies showed that infection with this symbiont can improve the fitness of its host [18–20]. Harris et al. [21] reported that Cardinium infection frequency can increase greatly within the wasp Encarsia pergandii population after nine generations. However, our long-term field survey revealed that the Cardinium infection rate in B. tabaci Q populations was low (7.6% to 17.3%) in Shandong, China, during 2006–2009 [22], though the whitefly may have 10–12 generations per year in this area. Pan et al. [23] also reported that the infection frequency of Cardinium in B. tabaci Q was not very high (16%) in 61 localities in 19 provinces of China in 2009. On the basis of these data, we hypothesize that (1) the Cardinium-infected (C+) B. tabaci Q population cannot efficiently compete with the Cardinium-uninfected (C-) B. tabaci Q population; (2) no reproductive isolation may have occurred between C+ and C-, and if there had been reproductive isolation between them, C+ would have been completely displaced by C- after long-term coexistence in the field; and (3) the C- population has higher fitness than the C+ population.

To reveal the differences in competitive ability and fitness between the two introduced populations (C+ and C-), competition...
between C⁺ and C⁻ was examined over several generations. Subsequently, the reproductive isolation between C⁺ and C⁻ was studied by crossing C⁺ with C⁻ individuals, and the fitnesses of C⁺ and C⁻ populations were compared using a two-sex life table method [24].

**Materials and Methods**

**Ethics Statement**

The research complies with all laws of the country (China) in which it was performed and was approved by the Department of Science and Technology of the Qingdao Agricultural University, China (permit number: 20110712).

**Bemisia Tabaci Laboratory Population**

The stock population of B. tabaci was obtained from laboratory colonies established from prior field collections. The C⁺ and C⁻ populations were provided cotton plants and maintained in isolated whitefly-proof screen cages in a greenhouse under controlled lighting and constant temperature (27±1°C) for about ten generations. The primary symbiont, Porteria, as well as secondary symbionts belonging to the genera Arsenophonus, Cardinium, Hamiltonella, Rickettsia, and Wolbachia, have been detected in B. tabaci Q [22,25–27]. Using the specific primers of the primary symbiont, Porteria, and the secondary symbionts, Arsenophonus, Cardinium, Frischina, Hamiltonella, Rickettsia, and Wolbachia, we found that the C⁺ and C⁻ populations were also infected with Porteria and Hamiltonella. Both C⁺ and C⁻ populations were maintained in separate cultures on potted cotton plants, Lu-Mian-Yan 21 cultivar. The purity of each of the cultures was monitored every 30 days by sampling 20 adults using PCR. Cotton plants were cultivated in 1 L plastic pots with nutritional soil and enclosed in whitefly-proof screen cages under controlled light and temperature in a screen house. Three pesticide-free, insect-free, young potted whitefly-proof screen cages under controlled light and temperature were cultivated in 1.5 L plastic pots with nutritional soil and enclosed in a screen house. Three pesticide-free, insect-free, young potted cotton plants were used in the large cages (40 cm ×25 cm ×30 cm). Plants were at the five to seven fully expanded true leaf stage. Plants were watered and replaced as necessary. All experiments were conducted in controlled climate chambers (27±1°C, 16:8D, and 60±5% RH).

**Bemisia Tabaci Species Determination and Detection of Cardinium**

Adult whiteflies were collected with a hand-held aspirator, preserved immediately in 95% ethanol, and stored at −20°C until processing. Genomic DNA was extracted from individual adult whiteflies of each collection using the DNAzol kit (Molecular Research Center, Inc., Cincinnati, OH) and stored at −20°C for subsequent use. The cleavage amplified polymorphic sequence (CAPS) of the mtCOI gene was used to determine the species of B. tabaci. The primers used for detection of the species were C1-J-2195 (5'-TTGATTTTTTGTTGTAACGAGAGT-3') [28] and R-BQ-2819 (5'-GTGAATATCGRCGAGGCTC-3') [29]. All PCR reactions were performed in 20 μl buffer containing 2 μl 10× buffer, 1.5 mM MgCl₂, 0.2 μM of each primer, 0.2 μM dNTPs, 1 unit Taq DNA polymerase, and 2 μl template DNA. Reaction conditions were as follows: 1 cycle of 94°C for 5 min, 35 cycles of 94°C for 1 min, 52°C for 1 min, 72°C for 1 min, and final extension at 72°C for 10 min. The presence of mtCOI amplicons was visualized by electrophoresis in 1% agarose gel electrophoresis and ethidium bromide staining. The mtCOI fragment (623 bp) was cleaved using the restriction endonuclease VspI [30]. Aliquots of the PCR products (13 μl) were each digested with 5 U VspI (in 20 μl total reaction volume) at 37°C for 2 h. Specimens whose mtCOI fragments were cut by VspI were identified as B. tabaci Q.

*P*CR detection of Cardinium was performed using the primers Car-sp-F (5'-CGG CTT ATT AAG TCA GTG AAA TCC TAG-3') and Car-sp-R (5'-TCC TTT CTT CCG CTT AGA CG-3'). All PCR reactions were performed in 20 μl of reaction buffer containing 1× buffer, 0.16 mM of each dNTP, 0.5 mM of each primer, 0.5 unit Taq DNA polymerase, and 2 μl template DNA. Reaction conditions were as follows: 1 cycle of 95°C for 1 min, 35 cycles of 95°C for 30s, 57°C for 30 s, 72°C for 1 min, and final extension at 72°C for 5 min. All PCR reactions included a negative control (sterile water instead of DNA) to detect DNA contamination, and a positive control (DNA from previous sequencing) to prevent false negatives. The PCR products (544 bp) were electrophoresed in a 1.0% agarose gel in TAE [31].

**Identification and Analysis of Orthologous Genes between C⁺ and C⁻ Populations**

To reveal genetic divergence between C⁺ and C⁻ populations, orthologs of *cytochrome P450* genes in the transcriptomes of these populations were analyzed. About 20 μg of total RNA (≥300 ng/μl) from each population (C⁺ or C⁻) was sent to the Shanghai Sangon Institute for library preparation and sequencing on an Illumina HiSeq2000. Raw reads were obtained and *de novo* transcriptome assembly was done with the short-read assembly program Trinity [32]. Pairs of sequences longer than 1000 bp that mapped unambiguously to *cytochrome P450* in the Swissprot database (E value<1e⁻5) were selected as *cytochrome P450* genes. Some *P450* genes such as *P450 4C1* gene has high variation in B. tabaci cryptic species [33].

**Competition between C⁺ and C⁻ Populations**

To compare the competitive abilities of C⁺ and C⁻ populations, we conducted a cage experiment and followed the frequencies of Cardinium infection in whiteflies raised on cotton over ten generations. To observe changes in the relative proportion of C⁺ and C⁻ individuals, 30 pairs of C⁺ and 30 pairs of C⁻ newly emerged adults whitefly cultures were released into a cage and the infection frequency of Cardinium was monitored every 25 days (approximately one generation) by sampling 30 adults using PCR. Infection frequency monitoring of Cardinium started from the 75th day. Three replicates were carried out for this study.

**Crossing Experiments**

To examine the possibility of reproductive isolation between C⁺ and C⁻, we carried out crossing experiments between Cardinium-infected and uninfected populations, QC×SC⁺, QC×SC⁻, QC⁺×QC⁻, and QC⁻×QC⁺, using virgin whiteflies. To obtain newly emerged unmated adults for experiments, adults were allowed to emerge in isolation and were kept individually before crossing. In the evening, cotton leaves with whitely pupae (late 4th instar nymphs with red eyes) were cut from plants, and individual pupae with the attached portion of the leaf were placed into a Petri dish. To maintain humidity, a moist filter paper was put on the bottom of the Petri dish. The next morning (at 7:00 am), the newly emerged adults were collected and sexed using a stereomicroscope [34].

For each cross, newly emerged females were individually transferred onto a cotton seedling as described by Li et al. [35] together with three adult males. Each female was allowed to lay eggs for 72 h. Adults were then collected using a small aspirator and stored at −20°C for later PCR confirmation of identity. To determine the sex ratio, the eggs laid by each female were
observed daily until adult emergence and the newly emerged adults were collected and sexed using a stereomicroscope. Data were analyzed using a one-way analysis of variance (ANOVA) and means were compared using a least significant difference (LSD) test. To normalize the data, an arcsine transformation was used for sex ratio.

**Fitness Assessment of C+ and C− Population using the Two-Sex Life Table Method**

To reveal differences in the fitnesses of C+ and C−, we analyzed the demographic parameters of the population using the two-sex life table method. For the life table study, the rearing containers were made of plastic pots (11.5 cm top diameter, 7.8 cm bottom diameter, and 15.5 cm height), with inverted plastic cups (11.5 cm top diameter, 7.8 cm bottom diameter, and 15.5 cm height) used as covers. The bottom of the plastic cup was cut out and covered with fine mesh cloth for ventilation. A cotton seedling at the two-true-leaves stage was used in this study, only one true leaf was kept on the seedling and the other leaf was removed.

For the life table study, approximately 15 pairs of whiteflies were transferred into rearing containers with a cotton seedling. Eggs laid within 24 h were collected and 15 eggs were used for the life table study. Other eggs were removed. For the C+ or C− populations, 15 replicates were carried out. The developmental stage and survival status of individual eggs were recorded daily starting at day 14, which corresponded to the day before adult emergence. Using this method, the survival rate of eggs was determined.

To determine fecundity, newly emerged whiteflies were collected and paired in egg-laying units [35]. The whiteflies were checked daily for survival and fecundity until the death of all individuals. Cotton seedlings were replaced daily.

For each population, the life history raw data of all individuals were analyzed based on the age-stage, two-sex life table theory [24] and the method described by Chi [36]. The software TWOSEX-MSChart [36] (available at http://140.120.197.173/Ecology/download/Twosex-MSChart.rar) was used for raw data analysis. The age-stage specific survival rate (l0j) where x = age and j = stage), age-stage specific fecundity (f0j), age-specific survival rate (lx), age-special fecundity (mx), age-stage life expectancy (exj), reproductive value (vxj), preoviposition period of the adult female (APOP), and total preoviposition period of the female from birth (TPOP) were calculated. Among these parameters, l0j represents the probability that a newborn will survive to age x and stage j, and f0j represents the mean number of offspring produced by a female of age x. In the age-stage, two-sex life table, according to Chi and Liu [37], lx is estimated as lx = ∑k sxj, and mx is estimated as mx = ∑k sxj/fxj, where k is the number of stages. The age-stage life expectancy (exj) is the length of time that an individual of age x and stage j is expected to live. The life expectancy for an individual of age x was calculated as ex = ∑n−k ∑m−n−jxj, where n is the number of age groups, m is the number of stages, and xj is the probability that an individual of age x and stage j will survive to age i and stage j and is calculated by assuming s'xj = 1 [37].

Population parameters, including the intrinsic rate of increase (r), finite rate of increase (λ), net reproductive rate (R0), and mean generation time (T), were calculated as well. A bootstrap method [38] was used to estimate the standard errors of the population parameters. Among these parameters, the intrinsic rate of increase was estimated using the iterative bisection method from the Euler-Lotka equation (∑k=0∞ e−lxT(1+1)xkتكk lxT= 1) with age indexed from 0 [39]. The net reproduction rate (R0) is calculated as $R_0 = \sum_{x=0}^{\infty} l_x m_x$. The mean generation time is defined as the length of time that a population needs to increase R0 fold of its size ($e^{T} = R_0$ or $x^T = R_0$) at a stable age distribution, and the mean generation time was calculated as $T = \ln(R_0)/R_0$. Student's t-test was used to determine differences in developmental times, fecundity, and population parameters between the C+ and C− populations.

**Results**

**Identification and Analysis of Orthologous Genes between C+ and C− Populations**

In the preliminary analysis, 40 cytochrome P450 genes were identified (Table S1). Based on the cytochrome P450 genes identified in this study, the average identity between C+ and C− populations was 99.7% (range, 98.4% to 100.0%) (Table S1), indicating that the genetic backgrounds of these two populations are highly similar.
Competition between \(C^+\) and \(C^-\) Populations

The changes in the relative ratio of \(C^+\) and \(C^-\) individuals within 310 days (approximately 11 generations) are shown in Fig. 1. The mixed cohort began with 50% \(C^+\) and 50% \(C^-\) individuals. The relative ratio of \(C^-\) individuals reached 76% after 75 days and increased steadily over time, reaching 93% after 310 days. By contrast, the relative ratio of \(C^+\) individuals decreased from the initial 50% to 24% after 75 days and remained between 7% and 20% thereafter.

Sex Ratio among Crosses between \(C^+\) and \(C^-\) Populations

No significant differences were observed in the sex ratio of the F1 generation among the four possible crosses between \(C^+\) and \(C^-\) populations (\(P > 0.05\), ANOVA) (Fig. 2). These results suggest that no reproductive isolation or reproductive abnormalities occurred between \(C^+\) and \(C^-\) populations.

\(C^+\) and \(C^-\) Population Parameters based on the Two-sex Life Table Method

The age-stage survival rate \(s_{xj}\) showed the probability that a newly deposited egg of \(B.\ tabaci\ Q\) would survive to age \(x\) and stage \(j\) (Fig. 3). The mean number of offspring produced by a female adult at age \(x\) relative to the age-stage specific fecundity \(f_{xj}\) is shown in Fig. 4. The maximum lifelong fecundity was found to be 152 eggs per female for the \(C^+\) population and 192 eggs per female for the \(C^-\) population.

The age-specific survival rate \(l_x\) curve is the age-specific survival rate, including all individuals of the cohort and ignoring the stage of differentiation, while the age-specific fecundity \(m_x\) is the mean fecundity of all individuals in the total population. The product of \(l_x\) and \(m_x\) is the age-specific maternity \(l_x m_x\) of the \(C^+\) population.

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{Figure3.png}
\caption{Age-stage specific survival rate \(s_{xj}\) of Cardinium-infected \((C^+)\) and uninfected \((C^-)\) \(B.\ tabaci\ Q\).}
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\end{figure}
and \( C^- \) populations. Higher peaks of \( m_x \) and \( l_x m_x \) were observed in the \( C^- \) population. The age-stage specific life expectancy (\( e_j \)) of \( B. \) tabaci is shown in Fig. 5. The maximum life expectancy was 45.75 days for the \( C^+ \) population and 50.48 days for the \( C^- \) population. The age-stage life expectancy of the \( C^- \) population was significantly shorter than the \( C^+ \) population.

The age-stage reproductive value (\( v_j \)) (Fig. 6) delineates the contribution of an individual to age \( x \) and stage \( j \) of the future population [40]. In our study, the reproductive values increased sharply to 47.23 in the \( C^+ \) population when females started to emerge at day 19. The corresponding reproductive values increased sharply to 33.96 in the \( C^- \) population when females started to emerge at day 16, 3 days earlier than the \( C^+ \) population.

The fecundity and mean developmental time of each life stage, including pre-adult duration, adult (female, male) longevity, APOP (adult preoviposition period), TPOP (total preoviposition period), and oviposition period, are given in Table 1. The pre-adult duration of the \( C^- \) population was significantly shorter than that of the \( C^+ \) population (\( P<0.05 \)). However, differences in the other parameters between the two populations were not significant (\( P>0.05 \)).

Based on the two-sex life table method (Table 2), the intrinsic rate of increase (\( \lambda \)), net reproductive rate (\( R_0 \)), and finite rate of increase (\( \lambda_0 \)) of the \( C^- \) population (0.09192 d\(^{-1}\), 17.26 offspring, and 2.096 d\(^{-1}\), respectively) were significantly higher than that of the \( C^+ \) population (0.07161 d\(^{-1}\), 10.82 offspring, and 1.078 d\(^{-1}\), respectively). The mean generation time (\( T \)) of the \( C^- \) population (38.09 d) was significantly longer than that of \( C^+ \) population (36.67 d).

**Discussion**

Our results showed that the percentage of \( C^- \) whitefly decreased after three generations, indicating that the \( C^+ \) whitefly has weak competitive ability. The results proved the initial hypothesis that the competitive ability of the infected host whiteflies was weaker than that of uninfected ones, which is in agreement with results of previous field surveys in China [8,9]. Our results also revealed that no reproductive isolation occurred between the two populations.
and the $C^-$ population had higher fitnesses than the $C^+$ population. The $C^-$ whiteflies had higher $r$ values (because they developed faster), higher survivorship of immature stages, and a higher net reproductive rate than those of $C^+$ whiteflies at 27°C. These differences may explain why $C^-$ whitefly populations have been dominant during the past few years in Shandong province, China.

For whiteflies, they possess a haplo-diploid sex determination system, in which unfertilized eggs developed into males and fertilized eggs develop into females [41]. In our study, we did not observe parthenogenesis or feminization in the experimental crosses. Although an analysis of cytochrome $P450$ genes in the two whitefly populations revealed that they were highly similar, the effect of Cardinium on whitefly reproduction should be examined in more detail in future studies. In recent decades, multiple roles of bacterial symbionts in arthropods have been revealed [42, 43]. Bacterial symbionts can manipulate the reproductive biology of hosts or affect the host fitness to increase transmission of the symbiont [18, 21, 43–54]. Most studies have shown that Cardinium can alter the reproduction of its hosts, which, in turn, is helpful to the spread of the symbiont within the population [12–20, 41]. For instance, embryonic mortality resulting from cytoplasmic incompatibility is the most common effect associated with endosymbiont infection [50]; consequently, the symbionts can maximize their spread. Cytoplasmic incompatibility induced by Cardinium has been widely reported in arthropods, such as the parasitoid wasp Encarsia pergandiella [15], spider mite Eotetranychus suganensis [16], sexual spider mite Bryobia sarothamni [55], and whitebacked planthopper Sogatella furcifera [17]. If Cardinium does not alter the reproduction of the whitefly host, then this symbiont may play a

![Figure 5. Age-stage specific life expectancies ($e_{x}$) of Cardinium-infected ($C^+$) and uninfected ($C^-$) B. tabaci Q.](doi:10.1371/journal.pone.0100423.g005)
Figure 6. Age-stage specific reproductive value (v_{xj}) of Cardinium-infected (C^+) and uninfected (C^-) B. tabaci Q.
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Table 1. Basic statistics of the life history of Cardinium-infected and uninfected Bemisia tabaci Q.

| Statistic            | Stage or sex | C^+      | Mean ± SE  | C^-      | Mean ± SE  | p    |
|----------------------|--------------|----------|------------|----------|------------|------|
| Pre-adult duration (d) | Pre-adult    | 155      | 28.97 ± 0.43* | 152      | 26.30 ± 0.53 | 0.017|
| Adult longevity (d)  | Female       | 75       | 29.79 ± 1.06 | 87       | 33.37 ± 1.51 | 0.05 |
|                      | Male         | 80       | 29.94 ± 1.24 | 65       | 30.37 ± 1.29 | 0.81 |
| TPOP (d)              | Female       | 75       | 29.23 ± 0.65 | 87       | 28.06 ± 0.69 | 0.23 |
| APOP (d)              | Female       | 75       | 0.68 ± 0.10  | 87       | 0.56 ± 0.10  | 0.44 |
| Oviposition (d)       | Female       | 75       | 18.25 ± 0.85 | 87       | 20.19 ± 0.98 | 0.18 |
| Fecundity (eggs per female) | Female    | 75       | 60.36 ± 3.37 | 87       | 68.12 ± 4.43 | 0.15 |

All P values are calculated using Student's t-test. APOP (adult preoviposition period) and TPOP (total preoviposition period) were calculated using females that produced fertile eggs.

*Significant difference (Student’s t-test, P<0.05).
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different role in the whitely than observed in previous studies that showed it induces feminization [12,13], parthenogenesis [14], and cytoplasmic incompatibility of infected hosts [15–17]. Our results are similar to the observations of White et al. [56,57] and Stefanini & Duron [58], which showed there was no cytoplasmic incompatibility or progeny sex ratio distortion in Cardinium-infected individuals of the parasitic wasp Encarsia inaron [56,57] or the spider Holocnemus pluchei [58].

Based on the two-sex life table method in this study, we revealed that the $C^+$ population had higher fitness than the $C^-$ population (Tables 1 and 2), which might indicate that Cardinium may have cryptic negative effects on the fitness of the host. The role Cardinium plays in changing the fitness cost of the whitely should be examined further in future studies. Many studies have suggested that Cardinium can increase the fitness of the hosts [13,18–20]. For example, Weeks & Stouthamer [18] found that the fecundity advantage of infected females was approximately 1.6 fold higher than that of uninfected females over a 6-day egg-laying period in the predatory mite, Metaseiulus occidentalis, and Giorgini et al. [13] showed that the antibiotic removal of Cardinium reduced offspring production by adult Encarsia luteoviridula females. If it is the case that Cardinium has cryptic negative effects on the fitness of the whitely host, then this would be in disagreement with previous studies that showed that Cardinium has no detectable effect on either reproduction or development of the host [56–59]. Similar negative effects of symbiont on host insect have been revealed for Wolbachia in Drosophila, in which the infection drastically reduces life span [60], and causes widespread degeneration of tissues, culminating in early death of Drosophila melanogaster [61]. On the other hand, because Cardinium still exists in the field, there might be a benefit to the whitely. The evolution of this symbiont strain needs to be further explored.

Finally, the two-sex life table gives the most comprehensive description and analysis of the survival and reproduction of a population and, thus, this method may be highly beneficial in revealing the difference in the two populations. This method takes into account the male population and the variable developmental rate occurring among individuals and can overcome the shortcoming of the traditional female-based, age-specific life table method, which ignores the male individuals, the stage of differentiation, and variable developmental rates among individuals.

### Supporting Information

#### Table S1

The cytochrome P450 genes identified in this study.

| XLS |

### Author Contributions

Conceived and designed the experiments: DC. Performed the experiments: YFW. Analyzed the data: YFW. Contributed reagents/materials/analysis tools: DFJ. Wrote the paper: LLY HLZ DFJ.

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