

Mate Discrimination in Invasive Whitefly Species

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Abstract Mate discrimination could be critical for invasive species that need to locate rare suitable mates and avoid costs associated with misdirected courtships to establish in new environments. Here, we tested whether individuals of two invasive whitefly species in the *Bemisia tabaci* species complex, commonly known as the B and Q biotypes, could discriminate between potential mates based on their species and sex. Behavioral observations showed that B females were more discriminating than Q females. Males of both species were able to discriminate between mates based on their species and sex, but in general B males discriminated more effectively than Q males. By incorporating these behavioral data into a conceptual model, we show that variation in mating behavior between females of different species was a more significant factor affecting mating than variation between males. These results indicate that mate discrimination could affect interactions between whitefly species and influence a species' ability to colonize novel environments.

Keywords *Bemisia tabaci* complex · mate choice · mating behavior · reproductive interference

Introduction

Mate choice affects fitness in many animal taxa (Andersson 1994; Arnold and Duvall 1994; Bonduriansky 2001). Insects often choose mates based on phenotypic

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indicators of quality such as body size or age (Jennions and Petrie 1997; Bonduriansky 2001). As mate choice is associated with variation in the quality of potential mates, selection should favor males and females that choose mates with indicators of high reproductive capacity to maximize their fitness (Andersson 1994; Bonduriansky 2001; Kokko et al. 2003). Mate choice, however, can also be affected by the availability of suitable mates, and both males and females are expected to be less discriminating when suitable mates are rare (Andersson 1994; Kokko and Mappes 2005).

Negative sexual interactions within or between species (reproductive interference) can limit the availability of suitable mates and therefore could strongly affect mate discrimination (Kokko and Mappes 2005; Liu et al. 2007; Gröning and Hochkirch 2008). Sexual selection is expected to favor individuals that effectively recognize and choose suitable mates despite reproductive interference (Andersson 1994; Gröning and Hochkirch 2008). As courtship and reproduction are energetically expensive activities often associated with life-history costs (Partridge and Farquhar 1981; Cordts and Partridge 1996; Höglund and Sheldon 1998), a complementary hypothesis is that selection could favor individuals that avoid misdirected courtships with unsuitable mates (Harari et al. 2000; Serrano et al. 2000; Gröning and Hochkirch 2008).

Misdirected courtships occur when individuals attempt to reproduce with unsuitable mates from another species, or when individuals engage in homosexual mating behavior (Thornhill and Alcock 1983; Bonduriansky 2001; Switzer et al. 2004). Misdirected interspecific sexual behavior occurs between species with incomplete mate recognition (Gröning and Hochkirch 2008). Homosexual mating behavior could have similar effects if individuals waste energy by attempting to copulate in courtships with no fitness benefit (Harari et al. 2000; Serrano et al. 2000). Although both misdirected interspecific sexual behavior and homosexual mating behavior are commonly observed in insects (Thornhill and Alcock 1983; Bonduriansky 2001; Switzer et al. 2004; Gröning and Hochkirch 2008), the hypothesis that individuals avoid costs associated with misdirected courtships through mate discrimination rarely has been tested.

Mate discrimination could have particular importance for invasive species (Reitz and Trumble 2002; Gröning and Hochkirch 2008). Invaders are typically initially rare in invaded ecosystems, and their ability to locate suitable mates and avoid misdirected courtships can affect their ability to establish (Reitz and Trumble 2002; Gröning and Hochkirch 2008). Additionally, the presence of invasive species can negatively affect closely-related native species if natives waste energy by attempting to mate with the invader (Butler and Stein 1985; Nasci et al. 1989; Liu et al. 2007). Such behaviors can reduce the fitness of native species compared to invaders and facilitate invasions (Butler and Stein 1985; Nasci et al. 1989; Liu et al. 2007).

Invasive whitefly species in the *Bemisia tabaci* complex often compete extensively with native whitefly species in invaded ecosystems, and these interactions are often mediated by mating behavior (Liu et al. 2007; Crowder et al. 2010a, b). Dinsdale et al. (2010) showed that the *B. tabaci* species complex comprises 24 cryptic species that lack distinguishable morphological variation (Rosell et al. 1997). The two species known as Asia Minor—Middle East 1 and Mediterranean contain what are widely known as the B and Q biotypes of *B. tabaci*,

respectively (Dinsdale et al. 2010). Both B and Q are widespread invaders and serious agricultural pests that often co-occur (Denholm et al. 1998; Dalton 2006; Crowder et al. 2010a, b). Reproductive interference between B and Q is common due to incomplete mate recognition, as males attempt to mate with females from the other species (Crowder et al. 2010b; Xu et al. 2010). Available data, however, indicate the existence of high levels of mating incompatibility between these two species, as interspecific copulation seldom occurs, and fertile hybrid offspring are rarely if ever produced (Crowder et al. 2010b; Xu et al. 2010). As both B and Q are haplodiploid, virgin females produce only male offspring while mated females produce a mixture of male and female offspring (Byrne and Devonshire 1996). Thus, a female's ability to mate early in life affects the overall sex ratio of her progeny, where females that are less successful at mating early in life produce a higher proportion of male offspring over their lifespan (Liu et al. 2007; Crowder et al. 2010a, b). In turn, the ability of females of one species to mate more successfully and produce a higher proportion of female offspring than females of another species can lead to displacement of the inferior species (Liu et al. 2007; Crowder et al. 2010a, b). Therefore, selection could be strong for females that reject courtships from males of a different species in order to increase their ability to mate with a suitable male of their own species. Additionally, selection could be strong for males that chose females of their own species and avoid homosexual courtships.

Here, we used behavioral experiments to explore whether mate discrimination affects reproductive success in B and Q. We tested whether males and females of each species could discriminate between mates based on their species and sex. To test whether species recognition and sex discrimination affected mating success, we developed a conceptual model to assess whether individuals that avoided misdirected courtships were more successful at mating in various invasion scenarios that reflect field observations of whitefly invasions. Our results show clear differences between B and Q in mate recognition and mating success, which could affect interspecific interactions and drive displacement between whitefly species.

Methods

Study System

The B population used in this study was collected from a cotton field in Yuma, AZ, in 2004 and has since been reared on cotton plants. The Q population was collected on poinsettia plants at a retail store in Tucson, AZ, in 2006 and has since been reared on cotton. Every 6 weeks, approximately 20 individuals from each population were checked for species based on established DNA screening protocols (Khasdan et al. 2005), and results showed that neither culture was contaminated.

Behavioral Observations

From June to September 2007, we compared courtship behaviors in single and mixed-species pairings to determine if males and females of both species differ in their ability to discriminate between potential mates based on their species and sex.

Whitefly behavior was recorded with a digital video camcorder or using a microscope equipped with a camera. The mating arena was a 7 mm diameter plastic washer (2 mm depth) placed on top of a cotton leaf disk. Previous studies have shown that whitefly behavior in these arenas is similar to whitefly behavior on plants (Crowder et al. 2010b), suggesting that the arenas provided sufficient space for insects to interact normally. We applied fluorescent dust to male nymphs to distinguish between species, with adults picking up a few dust particles upon eclosion. In a preliminary set of experiments, we observed the mating behavior of males of both species that were or were not exposed to dust. Results showed that the number of courtships per hour (two sample *t*-test, $P>0.50$ for both species) and courtship duration (two sample *t*-test, $P>0.10$ for both species) did not differ between males that were dusted and those that were not. Furthermore, the steps involved in the courtship process were the same regardless of whether dust had been applied. Thus, the dust had no observable effect on mating behavior. The color of dust was randomly alternated between species. We observed the mating behavior of single B or Q females with two males of the same species (single-species pairings, 25 replicates) and with one male of each species (mixed-species pairings, 30 replicates), with treatments: (1) 1B♀ x 2B♂; (2) 1Q♀ x 2Q♂; (3) 1B♀ x 1B♂ x 1Q♂; (4) 1Q♀ x 1B♂ x 1Q♂. Virgin adults (all 2 d old) were aspirated into the arena, which was covered with a glass cover-slip. Virgin adults were obtained by isolating and sexing late 4th instar nymphs on plants. These nymphs were individually removed with a piece of surrounding leaf and placed in vials containing agar and a cotton leaf disk in a growth chamber (27°C; 50% RH; 16:8 h light:dark) until adult emergence. Emergent adults were sexed for confirmation, and maintained in vials individually until they were 2 d old. For every courtship (male in parallel orientation to another individual) over a 6 h period we recorded the sex of both individuals, the courtship duration, the number of failed copulation attempts, if copulation occurred, and which individual terminated courtships (male or female moved away from partner) that did not end in copulation. Copulation occurred when a male rapidly positioned his abdomen underneath the abdomen of the whitefly he was courting. Successful copulations resulted in the male's abdomen remaining beneath the female's abdomen for approximately two minutes (*Mean*=2.1 min, *SE*=0.089 min). In contrast, failed copulations occurred when a male rapidly positioned his abdomen underneath the abdomen of the whitefly he was courting and immediately withdrew it (failed copulations lasted less than one second). Two randomly selected replicates were conducted per day.

Male Mate Discrimination

We determined if males of B and Q discriminate between females based on their species. We placed a 2 d old virgin male and two 2 d old virgin females (one of each species) into a mating arena, where females were marked with fluorescent dust to distinguish between species. The color of dust applied to females of each species was randomly determined daily. Every 5 min for 3 h, we inspected the arena to determine if the male was courting one of the females. The 5 min period was based on data from the videotaped behavioral observations. From these observations, males only attempted to copulate with females in 1.4% of courtships lasting less than

5 min, while significantly more males (70.2%) attempted copulation in courtships lasting more than 5 min (Fisher's exact test, $P<0.0001$). In addition, a significantly higher proportion of courtships lasting less than 5 min were terminated by males (25.0%) compared to courtships lasting more than 5 min (1.0%) (Fisher's exact test, $P<0.0001$). These data strongly suggest that males remaining in courtships over 5 min were likely to attempt copulation (indicating a choice) unless first rejected by the female, while males in courtships lasting less than 5 min were unlikely to mate. Thus, a male observed courting the same female in two consecutive observational periods was considered to have made a choice. Males that did not court an individual female for two consecutive periods in 3 h were considered to have not made a choice. Each day, we conducted six replicates in each of two 3 h periods, for a total of twelve replicates per day. We performed 100 replicates with males of each species.

Data Analysis

Behavioral Observations

We used χ^2 tests to determine if the proportion of heterosexual courtships differed from the proportion of homosexual courtships in each treatment involving combinations of two males and one female. We used 2×2 tests of independence (Sokal and Rohlf 1995) to test if B and Q males differed in their ability to discern males from females in single and mixed-species pairings. A significant effect indicated that males of one species initiated a higher proportion of heterosexual courtships than males of the other species. We also used 2×2 tests of independence to determine if B or Q males were more likely to terminate interspecific homosexual courtships, and if B or Q females were more likely to terminate interspecific heterosexual courtships. A significant effect indicated that males or females of one species were more likely to terminate a misdirected courtship. We determined if the duration of heterosexual and homosexual courtships in each treatment differed using Wilcoxon two-sample tests (Sokal and Rohlf 1995). As many courtships had no failed copulation attempts, we used randomization (permutation) tests (Sokal and Rohlf 1995) to determine if the number of failed copulation attempts differed across treatments. Statistical analyses of copulation data have been reported elsewhere (Crowder et al. 2010b), and results are only summarized here.

Male Mate Discrimination

We used χ^2 tests to determine if the proportion of observations where males of each species chose a female differed from 0.5. We also used χ^2 tests to determine if males of both species were more likely to choose a female of the same species than a female of the other species. We used 2×2 tests of independence to determine if B males were more likely than Q males to select a female of the same species, where a significant effect indicated that males of one species were more likely to choose a female of their own species. We also used 2×2 tests of independence to determine if the proportion of observations where males made a choice differed between species. All analyses were performed in JMP (SAS Institute 2004).

Modeling Effects of Mate Discrimination and Courtship Behavior

For any courtship, the probability of successful mating (P_m) is a function of three factors: (1) the probability of initiating a heterosexual courtship (P_h); (2) the probability that individuals in a heterosexual courtship are of the same species (P_s); (3) the probability of copulation in a heterosexual courtship between individuals of the same species (P_c), where:

$$P_m = P_h * P_s * P_c \quad (1)$$

If males only initiate heterosexual courtships ($P_h = 1$), and only court females of the same species ($P_s = 1$), then $P_m = P_c$. The probability of mating decreases when males engage in a higher proportion of homosexual courtships (P_h decreases) or when males cannot discriminate effectively between females based on their species (P_s decreases). The third parameter, P_c , is the likelihood that a courtship between a male and female of the same species will end in copulation, and is therefore based on both male and female behaviors in a heterosexual intraspecific courtship.

This model determines the probability that a single courtship will end in copulation between a male and a female of the same species. Whitefly females, however, are courted repeatedly throughout their life (Liu et al. 2007), and thus the probability of mating can vary over time. The probability of successfully mating over time (P_t) depends on both P_m and the number of courtships per unit time, $n(t)$, where:

$$P_t = 1 - (1 - P_m)^{n(t)} \quad (2)$$

Using these models, we explored the effects of mate discrimination and courtship behavior on mating success under two scenarios. In the first scenario, hereafter referred to as the refuge scenario, we assumed that both species have incomplete overlap in their habitat distribution. In this case, both species interact in shared habitats, but not in areas occupied by only one species (refuges). In the second scenario, hereafter referred to as the overlap scenario, we assumed that both species completely overlap in their distribution. In this case, there are no refuges. These scenarios are based on observations from Israel, where both B and Q are invasive (Khasdan et al. 2005). The north and south regions of Israel are dominated by a single species, but both species have similar abundances in central Israel (Khasdan et al. 2005). Thus, the overlap scenario is representative of central Israel, while the refuge scenario is representative of Israel as a whole. By analyzing mate discrimination in both scenarios, we were able to test the effects of spatial distribution and habitat use of an invasive species on mating success.

In the refuge scenario, we estimated P_h for males of each species as the proportion of heterosexual courtships initiated (averaged across mixed- and single-species treatments). Averaging across treatments allowed us to estimate behavioral traits assuming that both species compete in some habitats (mixed-species pairings) but not others (single-species pairings). To estimate P_s , we calculated the average proportion of males that chose a female of the same species when only a single species was present ($P_s = 1$), and from the mate choice experiment where both female species were present. For the overlap scenario, P_h for males of each species

was the proportion of heterosexual courtships initiated in mixed-species pairings only, and P_s was the proportion of males that chose a female of the same species in the mate discrimination experiment.

For both scenarios, we first focused solely on the effects of mate discrimination on reproductive success by assuming that P_c was the same for both species. In this case, we varied P_c from 0 to 1 to determine how the probability of mating depended on P_c . Second, we varied t from 1 to 72 to determine how the probability of mating depended on mate discrimination and time, with a P_c of 0.1 for both species based on the behavioral data (Crowder et al. 2010b). Finally, we examined the combined effects of mate discrimination and courtship behavior, where values for P_c in the refuge scenario were calculated by averaging the probability of copulation in courtships between a male and female of the same species in single and mixed-species pairings. For the overlap scenario, we estimated P_c for B and Q only from treatments with two males of mixed species and a single female. For all models, we estimated $n(t)$ for both species as the average number of courtships per hour ($n(t)=1$ hr).

Results

Behavioral Observations

Heterosexual vs. Homosexual Courtships

In single-species pairings, both B and Q males initiated significantly more heterosexual than homosexual courtships, although B males initiated a higher proportion of heterosexual courtships (74.8%) than Q males (58.5%) ($\chi_1^2=9.5$, $P=0.0020$) (Fig. 1). In mixed-species pairings, the proportion of heterosexual courtships initiated by B males with females of B (54.2%) or Q (46.0%) did not differ significantly from the proportion of homosexual courtships initiated with Q males (Fig. 1). In contrast, Q males initiated significantly more heterosexual courtships with B (59.6%) and Q (76.1%) females than homosexual courtships with B males (Fig. 1). When B and Q males were paired with a B female, males of both species initiated a similar proportion of heterosexual courtships ($\chi_1^2=0.86$, $P=0.35$), but Q males initiated a significantly higher proportion of heterosexual courtships than B males in mixed-species pairings with a Q female ($\chi_1^2=30.0$, $P<0.0001$). Across treatments, B males attempted to copulate in significantly fewer homosexual courtships (6.9%) than Q males (19.8%) ($N=386$, $P=0.045$).

Courtship Duration

Heterosexual courtships initiated by B males were significantly longer than homosexual courtships regardless of the species of the female or second male (Fig. 2). In contrast, heterosexual courtships initiated by Q males with Q females were not significantly longer than homosexual courtships initiated by Q males with B or Q males (Fig. 2). In single-species pairings, B males had significantly longer heterosexual ($Z=2.3$, $N=243$, $P=0.025$) and homosexual ($Z=1.8$, $N=138$, $P=0.018$) courtships than Q males. Across all treatments, heterosexual courtships by B males

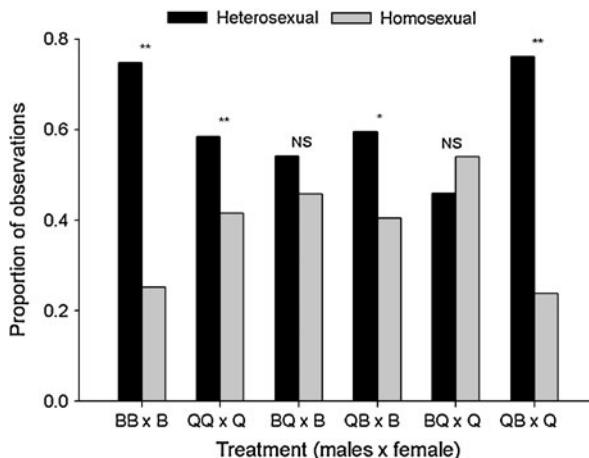


Fig. 1 Proportion of heterosexual and homosexual courtships initiated in six treatments (all combinations of two males and one female). Labels on the x-axis show the species of each male first, followed by the species of the female (i.e., BQ x B indicates a B male, a Q male, and a B female). The total number of courtships observed for BB x B, QQ x Q, BQ x B, QB x B, BQ x Q, and QB x Q were 123, 258, 118, 193, 137, and 176, respectively. In treatments with males of both species, the male listed first initiated the courtship. χ^2 tests were used to determine if the proportion of heterosexual and homosexual courtships differed in each treatment (NS: Not significant; *: $P<0.05$; **: $P<0.0001$).

were significantly longer with B than Q females ($Z=2.3$, $N=327$, $P=0.022$). Heterosexual courtships initiated by Q males were also significantly longer with B than Q females ($Z=2.5$, $N=532$, $P=0.013$). The duration of homosexual courtships initiated by B or Q males did not differ significantly based on the species of the second male ($P>0.16$ for both comparisons).

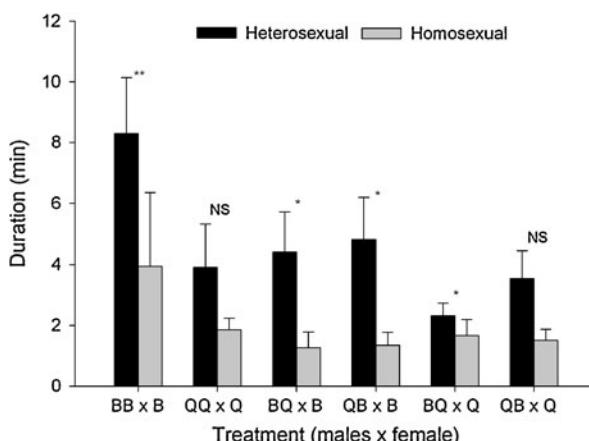


Fig. 2 Duration (min) \pm SE of heterosexual and homosexual courtships in six treatments (all combinations of two males and one female). Labels on the x-axis show the species of each male first, followed by the species of the female (i.e., BQ x B indicates a B male, a Q male, and a B female). In treatments with males of both species, the male listed first initiated the courtship. Wilcoxon signed-rank tests were used to determine if the duration of heterosexual and homosexual courtships differed in each treatment (NS: Not significant; *: $P<0.05$; **: $P<0.0001$).

Courtship Termination by Males and Females

B males terminated significantly more courtships from Q males (71% of courtships) than Q males terminated courtships from B males (61% of courtships) when B females were present ($\chi_1^2=13, P=0.0003$). B males were marginally more likely to terminate courtships initiated by Q males (62% of courtships) than vice versa (55% of courtships) when Q females were present ($\chi_1^2=3.2, P=0.073$). B females terminated 86% of courtships from Q males, significantly more than Q females terminated courtships from B males (66%) ($\chi_1^2=9.3, P=0.0023$).

Male Mate Discrimination

B males did not make a choice (72% of observations) significantly more often than they made a choice (28% of observations) ($\chi_1^2=19, P<0.001$). The likelihood of Q males making a choice (41% of observations) was marginally lower than the likelihood of Q males not making a choice (59% of observations) ($\chi_1^2=3.2, P=0.072$). The proportion of observations, however, where males made a choice did not differ significantly between B and Q males ($\chi_1^2=3.7, P=0.053$) (Fig. 3). Both B ($\chi_1^2=17, P<0.001$) and Q ($\chi_1^2=5.5, P=0.019$) males were more likely to choose a female of the same species than of a different species, although B males were more likely to choose a female of the same species than Q males ($\chi_1^2=4.1, P=0.043$) (Fig. 3).

Modeling Effects of Mate Discrimination and Courtship Behavior

The values of P_h , P_s , and P_c from the behavioral observations that were used in the model are shown in Table 1. In the refuge scenario, the relative difference in the probability of a single courtship resulting in mating between a male and a female of the same species was 10% greater for B compared to Q males with any value for the probability of copulation (Fig. 4a). However, when the number of courtships initiated per hour (Table 1) was factored into the model, the probability of mating

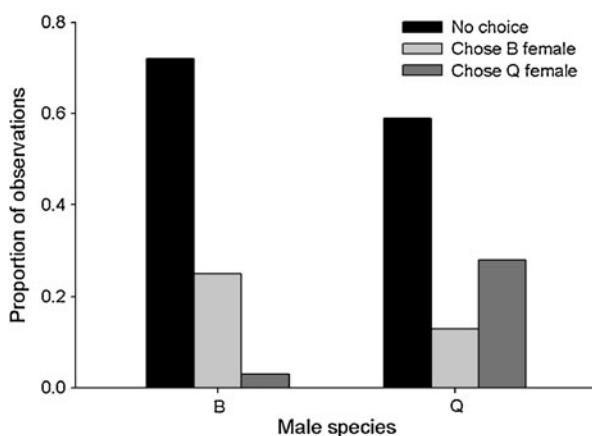


Fig. 3 Proportion of observations (out of 100) where B and Q males chose to court a B or Q female and the proportion of observations where no choice was made in a 3 h time period.

Table 1 Behavioral Traits for B and Q Used to Estimate Parameters in the Refuge and Overlap Model Scenarios. Traits Examined Were the Probability that Males Initiated Heterosexual Courtships (P_h), the Probability that Males Initiated Courtships with a Female of the Same Species (P_s), the Probability of Copulation in a Courtship Between a Male and Female of the Same Species (P_c), and the Number of Courtships Initiated Per Hour

Trait and species	Refuge scenario			Overlap scenario
	Single-species pairing	Mixed-species pairing	Average	
P_h				
B	0.75	0.50	0.62	0.50
Q	0.59	0.68	0.63	0.68
P_s				
B	1.00	0.89	0.95	0.89
Q	1.00	0.68	0.84	0.68
P_c^a				
B	0.10	0.33	0.22	0.33
Q	0.10	0.10	0.10	0.10
Courtships per hour				
B	0.90	0.90	0.90	0.90
Q	1.07	1.07	1.07	1.07

^a Data on frequency of copulation in both species from Crowder et al. (2010b)

over time was slightly higher for Q compared to B when P_c was the same for both species, because Q males initiated more courtships per hour than B males (Fig. 4b). Yet, when P_c differed between species based on the behavioral data (Table 1), the probability of successfully mating over time was greater for B compared to Q, particularly in the first 36 h (Fig. 4c). This result was obtained because the superior ability of B to copulate in the refuge scenario overrode their slight deficiency in initiating courtships with a female of the same species.

In the overlap scenario, the relative difference in the probability of a single courtship resulting in mating between a male and a female of the same species was 3.8% greater for Q compared to B males with any value for the probability of copulation (Fig. 5a). Similar to the refuge scenario, the probability of mating over time was higher for Q compared to B individuals when P_c was equal for both species (Fig. 5b). As in the refuge scenario, however, when P_c differed between species (Table 1) this relationship was inverted, and the probability of mating over time was greater for B compared to Q (Fig. 5c).

Discussion

Our results show that differences in species recognition and sex discrimination can affect mating in the B and Q species of the *B. tabaci* complex. Although this study was conducted in small arenas, individuals had ample space to interact or avoid courtships by moving to an unoccupied portion of the leaf. The behaviors observed

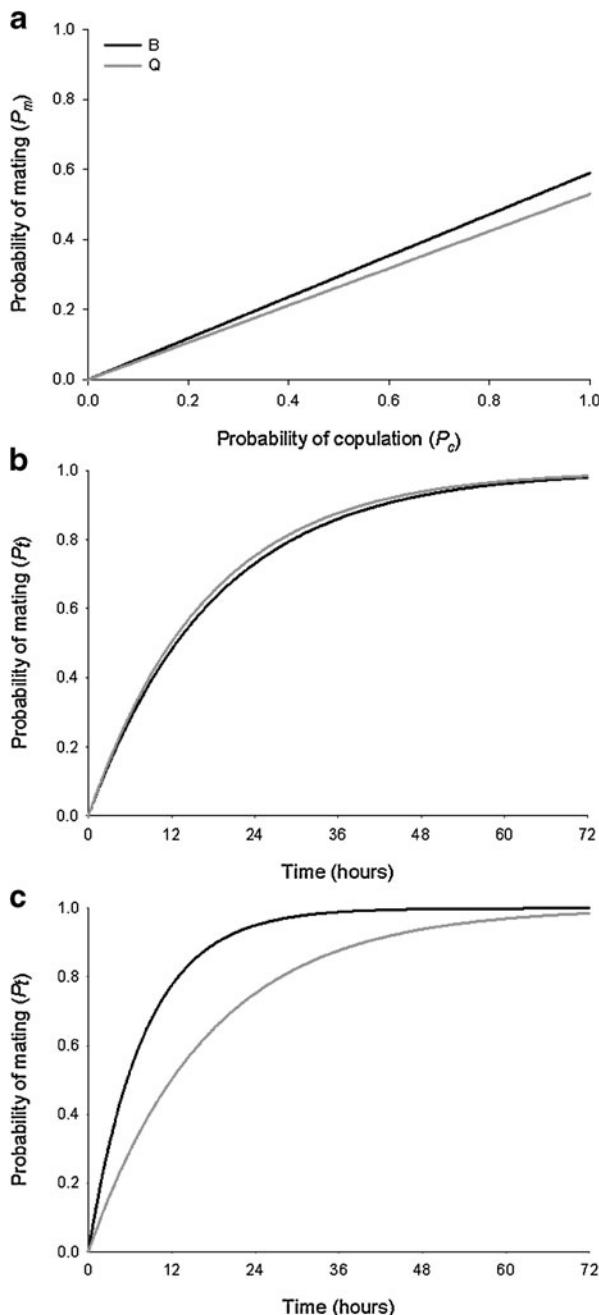


Fig. 4 Probability of mating in the refuge scenario (values in Table 1). **a** The expected probability of mating in a single courtship (P_m) for individuals of B and Q based on the probability of copulation in a courtship between two individuals of the same species; **b** the expected probability of mating over time (P_t) for individuals of B and Q based on the number of courtships (n) with a probability of copulation (P_c) of 0.1; **c** the expected probability of mating over time (P_t) for individuals of B and Q based on the number of courtships (n) with a probability of copulation (P_c) that differed between species.

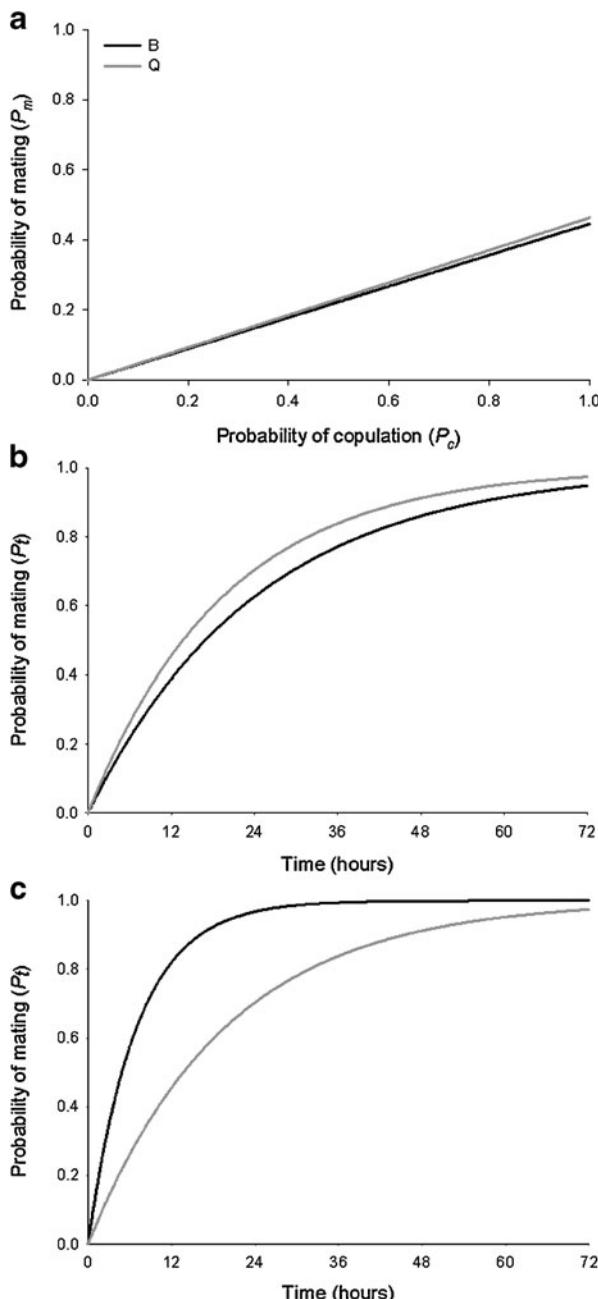


Fig. 5 Probability of mating in the overlap scenario (values in Table 1). **a** The expected probability of mating in a single courtship (P_m) for individuals of B and Q based on the probability of copulation in a courtship between two individuals of the same species; **b** the expected probability of mating over time (P_t) for individuals of B and Q based on the number of courtships (n) with a probability of copulation (P_c) of 0.1; **c** the expected probability of mating over time (P_t) for individuals of B and Q based on the number of courtships (n) with a probability of copulation (P_c) that differed between species.

here were similar to other studies of whiteflies on plants (Liu et al. 2007), suggesting that the results were not an artifact of the experimental conditions. Observing behavioral interactions between whitefly species in the field, however, has proven difficult due to the lack of morphological variation between species, so it is unclear how the observed behavioral differences between B and Q might scale up in larger landscapes. However, as both virgin and mated B and Q females actively produce eggs (Crowder et al. 2010b), and females produce most of their eggs early in life (Byrne and Bellows 1991), the ability of females to mate early in life likely affects the sex ratio of their progeny in the laboratory or field. Accordingly, the ability of whiteflies to effectively discriminate between potential mates based on their species and sex could increase their likelihood of mating early in life, which could allow females to produce proportionally more female offspring over their lifespan. In turn, lower numbers of female offspring can result in a slower rate of population increase for inferior species and displacement by superior species (Liu et al. 2007; Crowder et al. 2010a, b).

By incorporating behavioral traits into a conceptual model, we estimated how species recognition and sex discrimination affected mating success under various scenarios. In one scenario where reproductive interference between species occurred in some habitats but not in others (refuges), B males were more effective at discriminating among potential mates than Q males. Q males, however, initiated more courtships per hour than B males, and this actually increased the likelihood of mating over time for Q compared to B. In a second scenario where reproductive interference between species occurred in all habitats (overlap), Q males were more effective at discriminating among potential mates than B males, and initiated more courtships per hour than B males, making Q males more successful at courting suitable mates than B males. Yet, in both scenarios, B was much more successful at copulating with suitable mates, and the probability of mating between a male and a female of the same species was much greater for B compared with Q individuals.

These results suggest that while mate discrimination can affect mating success in both B and Q, the differences between species were small. Thus, the large difference between the species in their ability to copulate once courtships were initiated was the most significant factor affecting mating success. One factor examined here that may increase the likelihood of mating for B compared to Q individuals is courtship duration. Overall, the duration of courtships observed here were similar to other studies on whitefly behavior (Perring and Symmes 2006; Zang and Liu 2007). Longer courtships lead to an increased frequency of copulation (Crowder et al. 2010b), and B males had longer heterosexual courtships than Q males. Additionally, B females often increase their acceptance of copulation attempts from B males when other species are present (Liu et al. 2007; Crowder et al. 2010b). Both of these factors, which may increase the ability of B individuals to copulate, had an overriding influence on mating success between a male and a female of the same species in both invasion scenarios.

Our models do not take into consideration several additional factors that may affect fitness, such as life-history costs associated with misdirected courtships (Andersson 1994; Switzer et al. 2004; Grönig and Hochkirch 2008). Such costs are expected to mainly affect males when males must locate females, although females also can be affected if they expend a significant amount of energy in courtships

(Arnqvist and Rowe 2005; Gröning and Hochkirch 2008). B males were more effective at discriminating between females than Q males and rejected courtship attempts from Q males more often than Q females rejected B males. Thus, assuming that misdirected courtships entail costs for males and females, B may mitigate the fitness-reducing effects of reproductive interference more effectively than Q by minimizing such courtships. Costs associated with misdirected courtships also can affect both males and females when copulation occurs but does not lead to hybridization (Ribeiro and Spielman 1986; Liou and Price 1994). As individuals of B and Q rarely hybridize (Crowder et al. 2010b; Xu et al. 2010), the effectiveness of B females in rejecting Q males could result in fewer hybrid matings than between Q females and B males and provide a further advantage for B.

Males of both species also avoided misdirected courtships by initiating fewer homosexual than heterosexual courtships. In treatments with individuals of a single species, B males initiated a lower proportion of homosexual courtships than Q males. In contrast, Q males were more effective at avoiding homosexual courtships when males of both species were present. Homosexual courtships, like misdirected interspecific courtships, can entail fitness costs (Harari et al. 2000; Serrano et al. 2000; Switzer et al. 2004). Thus, Q males may gain a slight advantage over B males by initiating fewer homosexual courtships. Q males, however, attempted to copulate more often in homosexual courtships than B males, and B males were more effective than Q males at terminating homosexual courtships. Consequently, if the costs associated with copulation attempts and prolonging homosexual courtships exceed the costs associated with initiating such courtships, B males may gain an additional advantage over Q males.

As costs associated with misdirected courtships result from wasting energy on unsuitable mates (Andersson 1994; Gröning and Hochkirch 2008), reducing the duration of unsuitable courtships could mitigate such costs. Our results suggest that heterosexual courtships between B males and females were longer than homosexual courtships involving B males. In contrast, the duration of heterosexual and homosexual courtships between Q individuals did not differ significantly. These results suggest that B males wasted less energy on misdirected homosexual courtships than Q males. Additionally, both B and Q males had longer courtships with B than with Q females, and therefore B males likely wasted less energy on courtships with unsuitable females than Q males. These results also suggest that B males were better able to discriminate between females after courtships had been initiated, and terminated courtships with unsuitable Q females. In contrast, Q males may have not been able to discriminate between females once courtships had begun, and spent longer attempting to mate with B females.

Although our results suggest that males and females of both species had the ability to recognize individuals based on their species and sex, the mechanisms underlying these recognition abilities remain unknown. Homosexual courtships were initiated by males of both species, and both males in a replicate were often observed to initiate homosexual courtships. This suggests that males initiating homosexual courtships were not dominant or attempting to mimic females (e.g., Harari et al. 2000). Thus, homosexual behavior in B and Q appears to be the result of mistakes rather than an adaptive behavior. Selection could therefore be strong for B and Q males to reduce the likelihood of initiating homosexual courtships.

Although the mechanisms are unknown, studies of interactions between B and other whitefly species suggest that B has a superior ability to locate suitable mates and copulate in mixed-species assemblages (De Barro et al. 2006; Liu et al. 2007; Crowder et al. 2010a, b). B populations were able to displace the AN species on mutually acceptable hosts in population cages at a starting ratio of 1B:20AN (De Barro et al. 2006). In population cage studies in China, populations of B were able to displace the indigenous ZHJ1 species in cages initiated with 13% B (Liu et al. 2007). These studies show that B males can locate females even when they are initially rare, suggesting that a powerful mechanism exists for mate recognition in this species. Our results suggest that the effective mating behaviors of B males and females may contribute to such displacement.

It remains unclear to what extent species recognition and sex discrimination has benefited B and Q as invaders. As mentioned previously, both the refuge and overlap scenarios modeled here may be relevant to invasions in countries such as Israel, where both species occur. Additionally, both species co-occur in other regions of the world including China, Japan, Mexico, Spain, and Italy (Moya et al. 2001; Zhang et al. 2005; Ueda and Brown 2006; Martinez-Carillo and Brown 2007). Our model results suggest that when all factors were considered, B was more successful at mating than Q in both the refuge and overlap scenarios. Both species, however, differed in their mate discrimination ability in each scenario. B males were more effective at discriminating among mates in single-species pairings compared to mixed-species pairings, and would therefore presumably fare better when some habitats provided refuges from reproductive interference. Thus, B may invade more successfully in areas where indigenous species have a narrower niche breadth, and some populations of B are allowed to mate without interference from another species. In support of this, De Barro and Bourne (2010) showed that the rate of displacement of the AN species by B in Australia was accelerated when there were host plants that were suitable only for B compared with a scenario where the only host plants were equally suitable for both species. In contrast, Q males were more effective at discriminating among mates in mixed-species pairings compared to single-species pairings. Thus, Q may be more successful as an invader through direct interference in shared habitats.

Males and females that effectively discriminate among potential mates not only can mate quickly and more often in their lifetime, but they also reduce costs associated with misdirected courtships (Andersson 1994; Gröning and Hochkirch 2008). Here, by incorporating data on observed behavioral traits into a model, we showed that costs associated with misdirected courtships could reduce the likelihood of mating. For haplodiploid species, particularly species where individuals mate multiple times in their lifetime, the ability to discriminate between potential mates could significantly affect fitness. Such fitness differences can strongly affect interspecific interactions and drive displacement among closely-related species (Reitz and Trumble 2002). Thus, the ability to avoid misdirected courtships and locate rare suitable mates could be a factor affecting species invasions in systems where invaders compete with natives for mates. Furthermore, mechanisms involved in mate discrimination could affect competition among species, community ecology, and biological invasions.

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