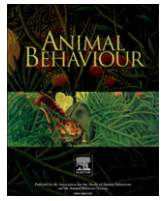




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Plasticity in mating behaviour drives asymmetric reproductive interference in whiteflies

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Reproductive interference between species with incomplete mate recognition can disrupt the process of mate acquisition. Accordingly, reproductive interference can reduce female and male fitness and lead to sexual exclusion. Here, we tested the hypothesis that flexible mating behaviours could mitigate the fitness-reducing effects of reproductive interference between reproductively incompatible biotypes of the haplodiploid whitefly *Bemisia tabaci*. We show that females of the globally distributed and invasive B biotype respond to reproductive interference from the Q biotype by increasing their acceptance of copulation attempts from B males. This behavioural plasticity increases the ability of B females to mate successfully and maintain a constant sex ratio in their offspring despite reproductive interference. In contrast, females of competing biotypes have invariant behaviour and produce fewer female offspring because of reproductive interference from the B biotype. Heuristic simulation models incorporating data on behavioural and life history traits of the B and Q biotypes obtained here, and published data on other biotypes, show that this plasticity in mating behaviour of B females could contribute to sexual exclusion of closely related biotypes. Our results demonstrate a powerful link between mating behaviour, reproductive interference and sexual exclusion, suggesting that variation in mating behaviour could determine the effects of reproductive interference and drive sexual exclusion among closely related species or among biotypes of a species.

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Reproductive interference is any negative sexual interaction between species with incomplete mate recognition, such as misdirected courtships and heterospecific mating (Gröning & Hochkirch 2008). While reproductive interference is similar to resource competition in many regards, it differs in the absence of a shared and limiting resource (Butlin 1989; Gröning & Hochkirch 2008). Reproductive interference can reduce fitness when individuals waste time, energy or gametes in interspecific sexual encounters (Gröning & Hochkirch 2008). Asymmetrical reproductive interference, where the fitness of one species is affected more than another, can lead to the displacement of the inferior species, a process known as sexual exclusion (Ribeiro & Spielman 1986; Kuno 1992; Reitz & Trumble 2002; Gröning et al. 2007; Hochkirch et al. 2007; Liu et al. 2007; Kishi et al. 2009).

As reproductive interference can reduce fitness and drive sexual exclusion, selection could favour species with plastic mating

behaviours that mitigate the fitness-reducing effects of reproductive interference. For example, if local species' composition limits the number of suitable males a female will encounter, selection should favour females that increase acceptance of appropriate mates (Souroukis & Murray 1995; Jirotkul 1999; Kokko & Mappes 2005; Heubel et al. 2008). However, the role of behaviour in determining the consequences of reproductive interference on fitness, and the resulting impact on population dynamics and species coexistence, has received little attention from ecologists (but see Takafuji et al. 1997; Hochkirch et al. 2006, 2007; Gröning et al. 2007; Liu et al. 2007; Kishi et al. 2009).

The whitefly *Bemisia tabaci* is a species complex with morphologically indistinguishable biotypes (also known as races of *B. tabaci*), which are distinguished based on differences in biological, physiological and genetic traits (Brown et al. 1995; De Barro et al. 2005). Although a recent review by De Barro et al. (in press) suggested that many biotypes of *B. tabaci* are cryptic species rather than races of *B. tabaci*, the topic remains controversial. Therefore, we have retained the commonly used term biotype here to link this study with previous literature. Courtship and mating between certain biotypes is common, although postmating barriers to interbreeding occur across many biotypes (De Barro et al. 2005, in

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press). As *B. tabaci* is haplodiploid, mated females produce a mixture of female and male offspring, while unmated females produce only male offspring (Byrne & Bellows 1991; Byrne & Devonshire 1996). Accordingly, if reproductive interference negatively affects the ability of females of a biotype to mate, females from this biotype would produce a high proportion of male offspring over their lifetime. In contrast, females of another biotype that are less affected by reproductive interference would produce a higher proportion of female offspring. Such variability in response to reproductive interference could lead to a faster rate of population growth for the biotype that produces more females, and ultimately sexual exclusion of the inferior biotype (Liu et al. 2007).

Here, we used experiments involving the B and Q biotypes of *B. tabaci* to determine the role of mating behaviour on reproductive interference and sexual exclusion. The B and Q biotypes are invasive biotypes that have been introduced to many of the same regions of the world (Denholm et al. 1998; Dalton 2006; McKenzie et al. 2009). We hypothesized that between-biotype variability in mating behaviour in response to reproductive interference could allow females of one biotype to mate more effectively and produce a higher proportion of female offspring than females of another biotype, which could lead to sexual exclusion. Data on the B and Q biotypes obtained here, published data on reproductive interference between the B and two other biotypes, and heuristic simulation models were also used to more generally explore the role of mating behaviour and life history variation in sexual exclusion of *B. tabaci* biotypes.

METHODS

Insects

The B biotype population was derived from a cotton field in Yuma, Arizona, U.S.A., in 2004 and has since been reared on cotton plants. The Q biotype population was derived from poinsettia plants collected in Tucson, Arizona, in 2006 and has since been reared on cotton plants. Every 6 weeks, 20 individuals from each population were checked for biotype based on established DNA screening protocols (Khasdan et al. 2005), and the results showed that each population contained individuals of only one biotype.

Reproductive Interference Experiment

We determined the effects of reproductive interference between and within biotypes on mating success in the B and Q biotypes using offspring sex ratio as a measure of mating success. Unmated females of *B. tabaci* readily lay unfertilized eggs, and females lay most of their eggs in the first week of life (Byrne & Bellows 1991). Accordingly, if reproductive interference prevents females from mating, then females should produce only male offspring. Furthermore, if reproductive interference increases the time for virgin females to become mated, then females should produce a lower proportion of female offspring than females that mate quickly. We therefore conducted an experiment with virgin females and males to determine the effects of reproductive interference on offspring sex ratio.

We placed an excised cotton seedling (15–25 cm tall) with one true leaf in a 20 ml vial filled with tap water. Excised cotton seedlings continue to grow and do not deteriorate in quality for up to 8 weeks in the laboratory, and mortality of *B. tabaci* on excised seedlings in water is similar to that on mature plants or seedlings left in soil (Crowder et al. 2007, 2008). Each vial containing a seedling was placed in a 10 cm diameter pot and covered with soil so that only the stem and leaf extruded. The seedling was then covered with a 10 cm clear plastic cup. Groups of eight virgin

females and eight virgin males (all 2 days old) were transferred to the arena, which was then placed in a growth chamber (27 °C; 50% RH; 16:8 h light:dark cycle). The number of whiteflies transferred to each arena was within the range of densities found in the field (Naranjo & Ellsworth 2005). In each replicate (12 per treatment), all females were of the same biotype (B or Q) and the ratio of B:Q males was (1) 0:8, (2) 2:6, (3) 4:4, (4) 6:2 or (5) 8:0. In this substitutive design, we manipulated the proportion of each biotype while maintaining a constant density, which allowed us to isolate the effects of variation in the proportion of each biotype on reproductive interference and mating success. After 3 days, adults were removed from arenas, eggs were counted, and seedlings were placed back in growth chambers. To determine offspring sex ratio, F₁ adults were collected and sexed daily until all adults had emerged.

Behavioural Observations

We compared mating behaviour of B and Q males and females in the presence and absence of interbiotype reproductive interference. These data were used to test the hypothesis that mating behaviour could explain variation in offspring sex ratio in the presence and absence of reproductive interference. Furthermore, these data were used to determine whether variation in mating behaviour between biotypes could contribute to sexual exclusion. Behavioural observations were recorded with a digital video camcorder or under a microscope equipped with a camera. The mating arena was a 7 mm diameter plastic washer (2 mm depth) covered with a cotton leaf disc. As whiteflies are small insects (≈ 1 mm), the arena was large enough to provide ample space for individuals to interact normally (Perring & Symmes 2006). We applied fluorescent dust (AX fluorescent pigments, Dayglo Color Corp., Cleveland, OH, U.S.A.) to male nymphs to distinguish between males, and the colour of dust applied to each male in an experimental replicate was randomly chosen. Adults picked up the dust upon eclosion.

We observed the mating behaviour of single B or Q females with two males of the same biotype (25 replicates) and with one male of each biotype (30 replicates), with treatments: (1) 1B♀ × 2B♂; (2) 1B♀ × 1B♂ × 1Q♂; (3) 1Q♀ × 2Q♂; (4) 1Q♀ × 1B♂ × 1Q♂. We also observed the mating behaviour of single B or Q females with two males of the other biotype (30 replicates each). However, in these two treatments, copulation was never observed and the data were not analysed further. Adults (all 2 days old) were aspirated into the mating arena, which was covered with a coverslip. For 6 h, we recorded the total number of courtships and the number of intra-biotype courtships. For each courtship, we recorded the courtship duration, the occurrence of female rejection, male interference and copulation, and the number of copulation attempts by males. Courtship duration was the amount of time elapsed after a male initiated a courtship by lining up adjacent and parallel to a female (females never initiated courtships) until either the male or female moved away from its partner. Female rejection occurred when the female terminated a courtship prior to copulation. Male interference occurred when a courting pair was harassed by another male that attempted to court one of the courting individuals, or used its wings to cover or hit a member of the courting pair. Copulation occurred when a male rapidly positioned his abdomen underneath the abdomen of the female he was courting. Successful copulations resulted in the male's abdomen remaining beneath the female's abdomen for approximately 2 min (mean ± SE = 2.1 ± 0.089 min). In contrast, failed copulations occurred when a male rapidly positioned his abdomen underneath the abdomen of the female he was courting and immediately withdrew it (failed copulations lasted

less than 1 s). Two observations were conducted per day, with treatments randomly selected daily.

Life History Traits

We measured several fitness components to compare the biotypes and to parameterize the models. Data on fecundity and mortality were based on treatments in the reproductive interference experiment with females and males all of the same biotype. Fecundity was the number of eggs laid per female per day, and mortality from egg to adult was the number of F₁ emergent adults divided by the number of F₁ eggs. To measure development time, 20–30 mating pairs of a biotype were aspirated onto an excised cotton seedling with two true leaves in a petri dish modified so that the stem extruded and was submerged in water. The seedlings were held in a growth chamber (conditions as above) for 48 h, after which adults were removed, eggs were counted, and seedlings were placed back in the growth chamber. After 14 days, we counted and sexed all late fourth-instar nymphs daily for 2 weeks, after which all nymphs had emerged or died. These nymphs were removed from seedlings and placed in vials containing agar and a leaf disc for emergence. The timing of emergence over the 2-week period was used to calculate developmental time. To measure offspring sex ratio of mated females, 10 virgin females (1 day old) and 15–20 virgin males (2 days old) of the same biotype were placed in a 20 ml vial containing agar and a leaf disc in a growth chamber (conditions as above). After 48 h, we aspirated 10 mating pairs onto an excised cotton seedling in a petri dish. Females in a mating pair were assumed to have mated at least once during the 48 h. Seedlings with adults were placed back in the growth chamber for 48 h. Beginning 2 weeks later, we checked the seedlings daily for late fourth-instar nymphs, which were counted and sexed. All nymphs were removed from seedlings and placed in vials containing a leaf disc for emergence. The sex of emerging adults was determined for confirmation. The sex ratio on each seedling was based on all emergent adults. Twenty replicates were conducted with B and Q biotype females.

Modelling Effects of Reproductive Interference

We used Monte Carlo simulations to evaluate the contribution of behavioural traits of the B and Q biotypes on the consequences of reproductive interference. These simulations determined whether behaviour could explain the offspring sex ratio produced by B and Q females in the presence and absence of interbiotype interference. Simulations incorporating data from the behavioural observations were conducted to determine the expected sex ratio for each treatment tested in the reproductive interference experiment. The time step of the model was 1 h, based on behavioural observations in which females were courted an average of 1.03 times per hour, with no significant differences across treatments (ANOVA: $F_{3,106} = 1.95$, $P = 0.13$). The model was run for 72 h to mimic the laboratory experiment. Each female was courted once per time step until she was mated. The probability of a courtship ending in copulation (P_{success}) was:

$$P_{\text{success}} = P_{\text{intra}} \times (1 - P_{\text{rej}}) \times (1 - P_{\text{int}}) \times P_{\text{cop}} \quad (1)$$

where P_{intra} is the probability of an intrabiotype courtship, P_{rej} is the probability of female rejection in an intrabiotype courtship, P_{int} is the probability of male interference in an intrabiotype courtship without female rejection, and P_{cop} is the probability of copulation in intrabiotype courtships without female rejection or interference. Interbiotype courtships, female rejection and male interference blocked copulation in behavioural observations (Fig. 1).

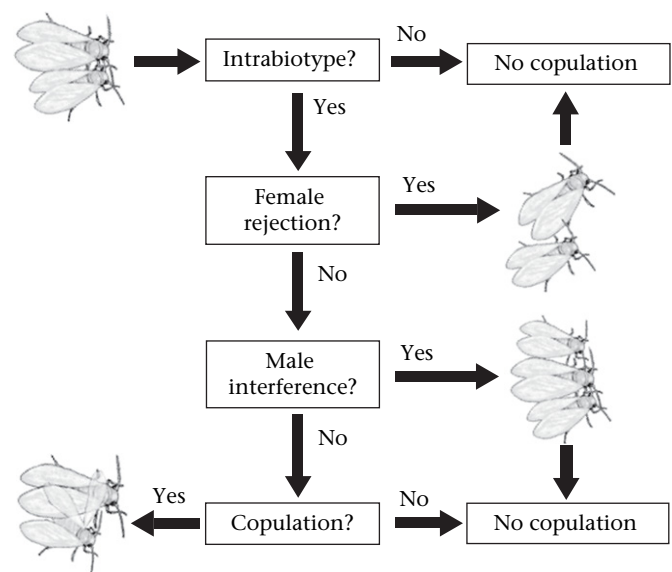


Figure 1. Factors involved in the whitefly courtship process, from initiation of courtships to copulation. Each decision box in the flowchart represents a step in the model.

In the model, the probability of intrabiotype courtships (P_{intra}) was based on the proportion of males of each biotype. From the behavioural observations (see Results), P_{intra} with 50% B and 50% Q males was 0.36 for B females and 0.68 for Q females. For B females, we assumed that P_{intra} increased linearly from 0.36 to 1.0 as the proportion of B males increased from 0.5 to 1.0, and declined linearly from 0.36 to 0.0 as the proportion of B males decreased from 0.5 to 0.0. For Q females, we assumed that P_{intra} increased linearly from 0.68 to 1.0 as the proportion of Q males increased from 0.5 to 1.0, and declined linearly from 0.68 to 0.0 as the proportion of Q males decreased from 0.5 to 0.0. Variation in these assumptions had little effect on model results based on a sensitivity analysis assuming different functions for the change in P_{intra} versus the proportion of B or Q males. The probability values associated with each of the other factors were also based on the behavioural observations (see Results). To test the effect of the observed behavioural plasticity in B females (see Results), we performed simulations with and without plasticity in acceptance of male copulation attempts.

The stochastic model simulated the inherent variability associated with mating behaviour and reproductive interference. For each courtship, a random number was drawn from an even distribution between 0 and 1 and compared to the observed probability values associated with steps in the courtship process (Fig. 1). For each step, if the random number was greater than the observed probability, the courtship ended before mating. Mated females laid 60% female eggs and 40% male eggs based on the observed sex ratio for mated females of the B and Q biotypes (see Results), and we assumed that females began producing female eggs immediately after mating. Unmated females laid 100% male eggs. Thus, the sex ratio of offspring produced by females was based solely on their mating history. Although some females mate multiple times (Liu et al. 2007), repeated mating only affects the offspring sex ratio of older females that lay few eggs, and therefore we did not consider repeated mating in the model. We assumed that facultative sex allocation, where mated females alter the sex ratio of their offspring (Charnov 1982), did not occur, as it has not been observed in *B. tabaci*. Based on results from experiments (see Results), females laid 0.2 eggs/h (5 eggs/day) from hours 12–72 in the model. The model was programmed in Visual Basic for Microsoft® Excel (Microsoft Corp., Seattle, WA, U.S.A.).

Heuristic Model of Sexual Exclusion

The B biotype has displaced the indigenous AN biotype in parts of Australia and the indigenous ZHJ1 biotype in parts of China (Liu et al. 2007). We therefore expanded our model to explore sexual exclusion of the Q biotype by the B biotype (based on data obtained here), and of the AN and ZHJ1 biotypes by the B biotype (based on data from Liu et al. 2007). For each pair of biotypes, we evaluated potential contributions of plasticity in female acceptance of copulation attempts and variation in male initiation of courtships on sexual exclusion. We also considered variation in development time, but did not evaluate potential differences in fecundity and mortality, as development time was the only life history trait that differed significantly between the pairs of biotypes (see Results; De Barro & Hart 2000; Zang et al. 2006). Values of life history and behavioural traits for simulations with the B and Q biotypes were based on data from the reproductive interference experiment, the behavioural observations and the measured life history traits (see Results). Parameter values for simulations with the B and AN, or the B and ZHJ1 biotypes, were based on published data from three studies (see Results; De Barro & Hart 2000; Zang et al. 2006; Liu et al. 2007).

We analysed the effects of three factors on sexual exclusion: (1) development time; (2) female acceptance of copulation attempts; and (3) male propensity to initiate courtships. Each of these factors differed significantly between some of the biotypes. In control simulations, parameter values were the same for both biotypes. In simulations with variable development time, individuals of the B biotype developed at a different rate than individuals of the other biotype. In simulations with variable female mating behaviour, females of the B biotype increased their frequency of copulation when males of the other biotype were present compared to when only B males were present. In simulations with variable male behaviour, B males initiated courtships at a different frequency than males of the other biotype. We also ran simulations with variation in all of the factors. For each set of simulations, all factors not varied were set to the control values.

To determine whether variation in life history and behavioural traits could drive sexual exclusion by the B biotype when it was initially less abundant than other biotypes, simulations were initialized with 1–50% individuals of the B biotype. For each factor tested, 100 simulations were conducted with each value for the initial proportion of the B biotype. Unless otherwise noted, all parameters and functions were the same as in the previously described model. Simulations were initiated with 200 adults. Females 2–7 days old laid eggs, adults survived for 20 days, and 70% of immatures survived to adulthood. Immature development was incorporated by assuming immatures that reached the development time became adults. Each simulation was run for 750 days (≈ 30 generations). This time frame was appropriate because sexual exclusion of one of the two biotypes, which happened when the population of one of the biotypes went to 0, always occurred in less than 30 generations regardless of the initial model conditions.

Data Analysis

For the reproductive interference experiment, we used two-way ANOVA to determine whether female biotype, proportion males of the same biotype as the female (PMSB), or their interaction affected offspring sex ratio. We used linear contrasts to compare the offspring sex ratio of B and Q females at each level of PMSB. A significant effect would demonstrate that one biotype laid a higher proportion of female eggs for a given level of PMSB, indicating that females of that biotype were more successful at mating under those conditions than females of the other biotype. We also conducted an analysis for females of each biotype using one-way ANOVA and

Tukey HSD tests to determine how offspring sex ratio varied with PMSB. If reproductive interference negatively affects mating of virgin females of one biotype, the proportion of female progeny should decrease significantly as PMSB decreases.

For the behavioural observations, we used chi-square goodness-of-fit tests (Sokal & Rohlf 1995) to determine whether the proportion of courtships initiated by B and Q males differed from a 1:1 ratio in observations with B or Q females and one male of each biotype. We used randomization tests to determine whether the number of failed copulation attempts differed for males paired with a second male of the same biotype versus a male of the other biotype. We used logistic regression for binary data (Sokal & Rohlf 1995) to determine whether the probability of female rejection, male interference and copulation in intrabiotype courtships depended on male biotype (two males of the same biotype or mixed), courtship duration and the interaction. Courtship duration was included as a covariate because the odds of female rejection, male interference and copulation increased with longer courtships. A significant effect of male biotype would indicate that the odds of a response differed when all males were of the same biotype compared to when males were mixed. A significant interaction term would indicate that the association between courtship duration and odds of each response differed when all males were of the same biotype compared to when males were mixed. If either male biotype or the interaction were significant, probability values for the response were not pooled in the model. Otherwise, probability values were pooled.

We used two-sample *t* tests to determine whether life history traits (fecundity, mortality, development time and sex ratio of mated females) differed between the B and Q biotypes. We compared observed data with model results using two-way ANOVA to determine whether offspring sex ratio for B and Q females was affected by data type (observed or simulated), PMSB, and their interaction. We used linear contrasts to determine whether model results matched observed data at each level of PMSB. A lack of a significant difference would show that the model accurately predicted the observed patterns. For models of sexual exclusion, we used paired *t* tests to compare each model that varied one or more factors (development time, female behaviour, male behaviour, all) to the control model, where results with each value for the initial proportion of the B biotype served as a replicate. For any factor, a significant result would indicate that sexual exclusion was more or less likely to occur than when there was no such variation. All statistical analyses were performed in JMP (SAS Institute, Cary, NC, U.S.A.).

RESULTS

Reproductive Interference between Biotypes

The proportion of female offspring produced was highly correlated with the number of females produced over the course of the experiment for both B (Pearson correlation: $r_{58} = 0.75$, $N = 60$, $P < 0.0001$) and Q ($r_{58} = 0.88$, $N = 60$, $P < 0.0001$) biotype females. This indicates that offspring sex ratio was an appropriate measure for testing the effects of reproductive interference on mating success. B females produced a higher proportion of female offspring than did Q females for all conditions tested, except when all males were of a different biotype than the female (Fig. 2, Table 1). Although hybrid female progeny were occasionally produced, they were always sterile. The proportion of female offspring produced by B females did not differ significantly in treatments with 100–50% B males, but declined significantly when 25 or 0% of males were B (Fig. 2, Table 1). In contrast, the proportion of female offspring produced by Q females declined linearly as the proportion of B

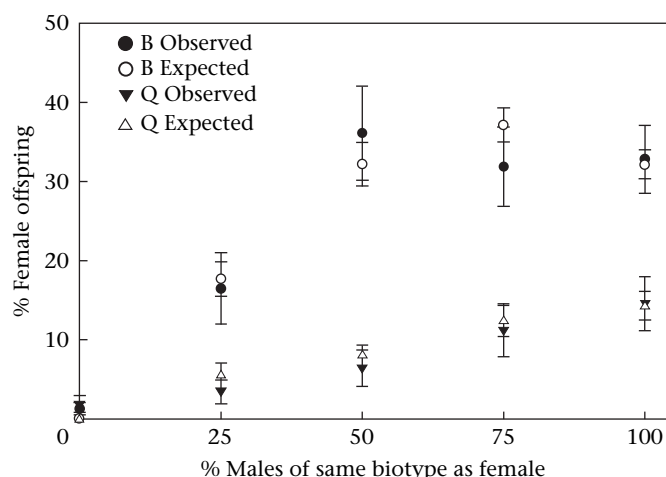


Figure 2. Observed and expected sex ratio of progeny of B and Q females. The observed values (\pm SE) are data from the reproductive interference experiment. The simulated values (\pm SE) are predictions based on frequencies of behavioural traits involved in courtship and copulation.

males increased and the proportion of Q males decreased (Fig. 2, Table 1), indicating that variation in the proportion of each biotype affected Q females more than B females.

Behavioural Observations

Q males initiated more courtships than B males with either B ($\chi^2 = 14.5$, $P = 0.0001$) or Q ($\chi^2 = 26.5$, $P < 0.0001$) females (Table 2). In intrabiotype courtships, the frequency of female rejection and male interference did not depend on the biotype of males present in courtships with B or Q females (Tables 2, 3). The number of male copulation attempts was also not affected by biotype assemblage (randomization tests: $P > 0.5$ for each biotype). However, B females were more likely to accept copulation attempts from a B male when a Q male was present compared to when only B males were present (Tables 2, 3). Furthermore, longer courtships were more likely to end in copulation between a B male and a B female when a Q male was present compared to when only B males were present (Table 3). In contrast, Q females were as likely to accept the copulation attempts of a Q male when a B male was present compared to when only Q males were present, and the copulation frequency did not depend on courtship duration (Tables 2, 3).

Life History Traits

Development time data were pooled for males and females, as no significant differences were detected between sexes ($P > 0.50$ for both biotypes). Development time from egg to adult was shorter

for the B (19.2 ± 0.13 days) than for the Q (23.3 ± 0.13 days) biotype ($t_{108} = 24$, $P < 0.0001$). Fecundity (eggs per female per day) did not differ significantly between B (3.77 ± 0.50) and Q (4.77 ± 0.50) females ($t_{22} = 1.4$, $P = 0.17$). Mortality from egg to adult also did not differ significantly between B ($38.2 \pm 4.8\%$) and Q ($25.7 \pm 4.8\%$) females ($t_{22} = -1.8$, $P = 0.078$).

Offspring sex ratio (% female offspring) of mated B females (58.2 ± 2.9) and Q females (61.8 ± 2.9) did not differ significantly ($t_{38} = 0.89$, $P = 0.38$), indicating that the higher offspring sex ratio observed in B compared to Q females in the reproductive interference experiment (Fig. 2, Table 1) was due to B females mating more rapidly than Q females in the presence and absence of interbiotype interference. Furthermore, mated females produced a higher proportion of female offspring than B ($t_{30} = 5.1$, $P < 0.0001$) and Q ($t_{30} = 10.0$, $P < 0.0001$) females in the reproductive interference experiment with 100% males of the same biotype, showing that unmated females laid a significant number of eggs before they mated in the reproductive interference experiment.

Modelling Effects of Reproductive Interference

Parameter values based on the observed behavioural traits that were used in the model are shown in Table 4. Simulation results did not differ significantly from observed data across all conditions tested ($P > 0.24$ for all comparisons; Fig. 2), indicating that behavioural interactions determined the consequences of reproductive interference on mating success and resulting effects on sex ratio. When we removed plasticity in acceptance of copulation attempts by B females from the model, the production of female offspring declined linearly with an increase in the proportion of Q males, as in Q females when the proportion of B males increased (Fig. 2). This finding suggests that plasticity in mating behaviour allowed B females to maintain a constant sex ratio in their offspring when at least 50% of males were of the B biotype.

Modelling Sexual Exclusion

Parameter values for simulating sexual exclusion were based on observed behavioural and life history traits of each biotype (Supplementary Material Table S1). Regardless of the initial proportion of the B biotype, sexual exclusion of the Q, AN and ZHJ1 biotypes occurred significantly more often when B females expressed plasticity in acceptance of copulation attempts than when they did not (Fig. 3, Supplementary Material Table S2). The frequency of courtship initiation by B males also affected sexual exclusion, although the effects were not always positive (Fig. 3). When paired with the Q biotype, B males initiated fewer courtships than Q males, and variation in male behaviour benefited the Q biotype (Fig. 3a, Supplementary Material Table S2). In contrast, B

Table 1
Comparison of offspring sex ratio for B and Q females

% Males of the same biotype as females	% Female offspring (\pm SE)			
	B♀	Q♀	t_{110}	P
0	1.27 \pm 0.77a	1.85 \pm 1.1a	-0.11	0.91
25	16.5 \pm 4.6b	3.38 \pm 1.5a	2.6	0.012
50	36.1 \pm 6.0c	6.37 \pm 2.3ab	5.8	<0.0001
75	31.8 \pm 5.0c	11.2 \pm 3.3bc	4.0	0.0001
100	32.8 \pm 4.3c	14.5 \pm 3.4c	3.5	0.0006

Linear contrasts were used to compare the offspring sex ratio of B and Q females in each experimental treatment (t statistics and P values are shown for each comparison). For within biotype comparisons, values in the same column not sharing the same letter were significantly different (Tukey HSD: $\alpha = 0.05$).

Table 2
Behavioural traits affecting reproductive success

Trait	Treatment			
	1B♀+2B♂	1B♀+1B♂+1Q♂	1Q♀+1B♂+1Q♂	1Q♀+2Q♂
Total courtships	113	179	197	189
Intrabiotype (%)	100	36	68	100
Female rejection (%) [*]	73	81	85	80
Male interference (%) [†]	43	50	45	59
Copulation (%) [‡]	12	33	9.1	6.7

^{*} Intrabiotype courtships where female rejected male.

[†] Intrabiotype courtships without female rejection with interference from the second male.

[‡] Intrabiotype courtships without rejection or interference that ended in copulation.

Table 3
Logistic regression analysis of behavioural data

Factor	Female biotype					
	B			Q		
	N*	χ^2	P	N*	χ^2	P
Female rejection†						
Male biotype (same as female or mixed)	177	0.84	0.36	323	0.83	0.36
Courtship duration		9.2	0.0024		8.9	0.0028
Courtship duration × male biotype		0.20	0.66		0.033	0.86
Male interference‡						
Male biotype (same as female or mixed)	42	0.12	0.73	57	1.0	0.31
Courtship duration		0.47	0.49		2.8	0.094
Courtship duration × male biotype		0.56	0.45		0.65	0.42
Copulation§						
Male biotype (same as female or mixed)	23	5.6	0.018	26	0.049	0.82
Courtship duration		9.3	0.0023		0.81	0.055
Courtship duration × male biotype		4.7	0.029		0.94	0.42

* N is the number of each behavioural interaction shown in Table 2.
 † Intra-biotype courtships where female rejected male.
 ‡ Intra-biotype courtships without female rejection with interference from the second male.
 § Intra-biotype courtships without rejection or interference that ended in copulation.

males initiated more courtships than AN or ZHJ1 males, and variation in male behaviour benefited the B biotype when paired with these biotypes (Fig. 3b, c, Supplementary Material Table S2). Variation in development time had little effect on sexual exclusion (Fig. 3, Supplementary Material Table S2). Sexual exclusion of any biotype was most likely in simulations that included plasticity in female copulation acceptance (Fig. 3), suggesting that plasticity in this female behaviour played a dominant role in sexual exclusion.

When we simulated variation in life history and behavioural traits, sexual exclusion of the AN and ZHJ1 biotypes by the B biotype occurred when as few as 5–10% of individuals were of the B biotype initially (Fig. 3b, c). In contrast, sexual exclusion of the Q biotype in models did not occur unless the initial proportion of the B biotype was over 20%, suggesting that the Q biotype would be more resistant to invasions by B than would either the AN or the ZHJ1 biotype (Fig. 3a).

DISCUSSION

The precise fit between model results and observed data in the reproductive interference experiment (Fig. 2) strongly suggests that behavioural traits of the B and Q biotypes (Tables 2, 3) determined the consequences of reproductive interference on mating success. Specifically, B females with the ability to modify their acceptance of

Table 4
Parameters values in Monte Carlo simulation model

Parameter	% Female biotype	
	B	Q
Female rejection (P_{rej})*	76	82
Male interference (P_{int})†	45	54
Copulation (P_{cop})‡		
Males same biotype	12	7.7
Males mixed	33	7.7

* Probability of female rejection in an intra-biotype courtship.
 † Probability of male interference in an intra-biotype courtship without female rejection.
 ‡ Probability of copulation in an intra-biotype courtship without rejection or interference.

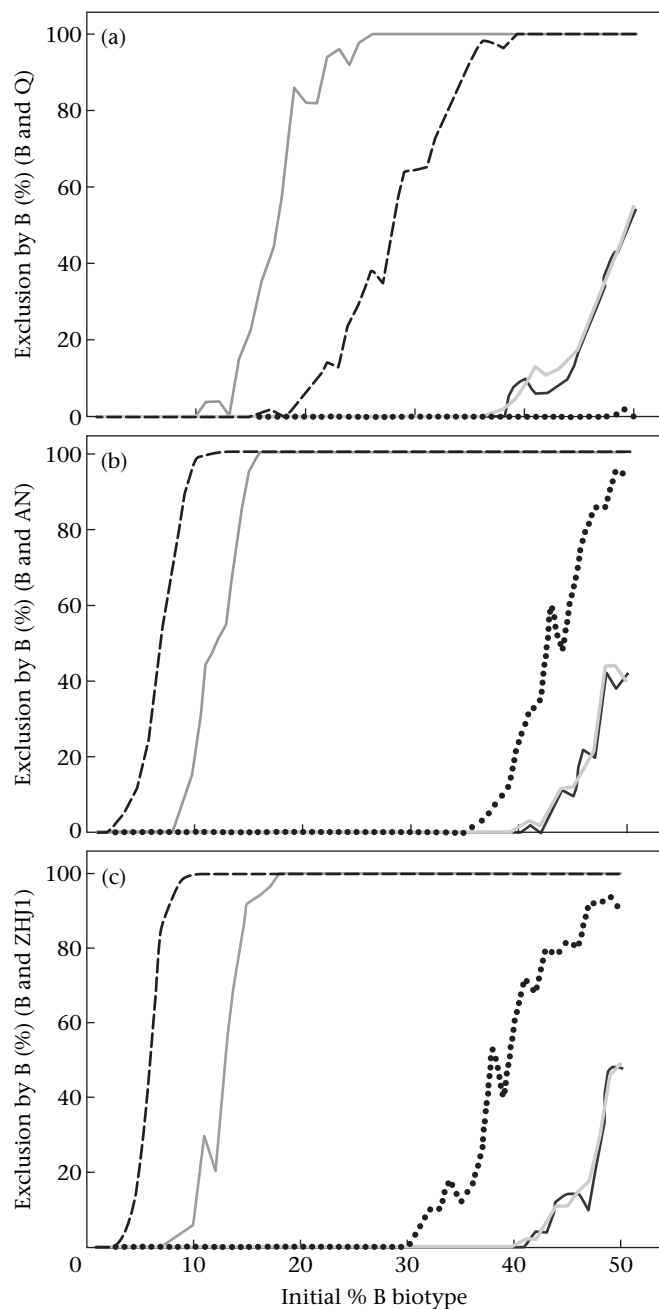


Figure 3. Factors affecting sexual exclusion of whitefly biotypes. The Y axis shows the percentage of simulations in which the B biotype excluded the (a) Q, (b) AN or (c) ZHJ1 biotype in less than 30 generations, with varying initial abundance of the B biotype. Factors tested were development time (light grey line), female acceptance of copulation attempts (dark grey line), courtship initiation by males (dotted line) and variation in all factors (dashed line) (Supplementary Material Table S1). In control simulations (black line), all factors were equal between biotypes. Paired *t* tests were used to compare model results with variation in each factor to results with the control model (Supplementary Material Table S2).

copulation attempts in response to reproductive interference (Tables 2, 3) were more successful at mating early in life, and therefore produced a higher proportion of female offspring than did females of the Q biotype. Increased acceptance of copulation attempts from B males by B females has also been observed in mixed-species assemblages with indigenous Australian (AN) and Chinese (ZHJ1) whitefly biotypes in similar behavioural experiments to those reported here (Liu et al. 2007). These results suggest

that the plasticity in mating behaviour of B females occurs in populations worldwide, and may be a behavioural trait that has benefited this biotype as an invasive species (Fig. 3).

In many species, females exert more influence over mating than males, and thus female behaviour is linked more directly with the effects of reproductive interference (Andersson 1994; Gröning & Hochkirch 2008). The ability of females to recognize conspecific males, or to reject misdirected courtships from unsuitable males, provides an advantage in mixed-species assemblages (Andersson 1994; Gröning & Hochkirch 2008). Selection should also favour females that become less selective when local species composition limits the frequency at which females encounter suitable males (e.g. Jirotkul 1999; Wirtz 1999; Kokko & Mappes 2005; Safi et al. 2006; Heubel et al. 2008). We observed behaviour consistent with these expectations in B but not in Q biotype females (Tables 2, 3). The sex ratio of offspring did not differ between mated females of both biotypes, suggesting that the more stable offspring sex ratio in B compared to Q females (Fig. 2) resulted from a greater propensity of B females to copulate when exposed to interbiotype reproductive interference. Additionally, unmated females laid a significant number of eggs before they mated, suggesting that the higher sex ratio observed in B compared to Q females across all treatments was also due to an increased ability of virgin B females to mate successfully without interbiotype interference. Although the mechanism(s) resulting in the asymmetrical behaviour of B and Q females are currently unknown, such large differences in behaviour are often caused by differences in the recognition ability of females (Wirtz 1999).

An alternative hypothesis for the more stable sex ratio in B compared to Q females is that B females were less affected by a decrease in the abundance of males of the same biotype than were Q females. However, Liu et al. (2007) showed that the offspring sex ratio of B females was constant in the absence of interbiotype reproductive interference despite four-fold variation in male density. Similarly, Pascual & Callejas (2004) found that reproductive interference between Spanish populations of the B and Q biotypes was not affected by density. Such constant offspring sex ratio of B females with varying density of B males suggests that male density alone did not affect mating success in our experiments, but rather the response of females to variation in the proportion of each biotype determined the outcome of reproductive interference. However, we cannot rule out completely some possible effect of variation in density on reproductive interference.

As variation in behavioural traits between the B and Q biotypes drove asymmetrical reproductive interference in the laboratory, similar factors could operate in the field. However, as whitefly biotypes are morphologically identical (Brown et al. 1995; De Barro et al. 2005, *in press*), field observations of mating interactions between biotypes are impractical. Thus, laboratory observations that reflect mating interactions in the field are essential. Although this study was conducted in small arenas, females had ample space to interact with males or avoid courtships by moving to an unoccupied portion of the leaf. While experimental conditions can strongly affect mating interactions (Gröning et al. 2007; Gröning & Hochkirch 2008), the behaviours observed here were similar to those of other studies of whiteflies on plants (Liu et al. 2007), suggesting that the results were not an artefact of the experimental conditions.

Available data suggest a high degree of niche overlap between the B and other biotypes, a prerequisite for reproductive interference to occur in the field (Gröning & Hochkirch 2008). Following introduction of the B biotype to the U.S.A. in the late 1980s, the indigenous A biotype was rapidly displaced throughout the country by the early 1990s (Perring 1996; Reitz & Trumble 2002). Similarly, invasion of the B biotype in Australia and China resulted in sexual

exclusion of the AN and ZHJ1 biotypes in several regions in 3–5 years (Liu et al. 2007). These results suggest a high degree of niche overlap between biotypes in the field, as exclusion of one biotype would not occur otherwise. Similarly, laboratory studies conducted on tomato and pepper, two common hosts in the field, showed that both the B and Q biotypes occurred on similar parts of the plants (Muniz et al. 2002). In Israel, extensive field sampling of 23 host plants from 2002 to 2007 showed that regardless of the time of year, both the B and Q biotypes were found on 19 hosts, the B biotype being the only biotype found on three of the hosts, and the Q biotype the only biotype found on one host (Khasdan et al. 2005; A. R. Horowitz, personal communication). All of these data suggest a high degree of spatial and temporal niche overlap between the B and other biotypes in the field. Thus, our laboratory results that examined interbiotype interactions may reflect a field situation where reproductive interference is common.

Given this high degree of niche overlap between biotypes, our heuristic model of sexual exclusion that assumed complete niche overlap could be useful for predicting the effects of reproductive interference on sexual exclusion in the field. Consistent with observations of Liu et al. (2007), our simulations showed that reproductive interference from the B biotype could drive sexual exclusion of the AN and ZHJ1 biotypes in less than 3 years when as little as 5–10% of individuals were of the B biotype initially (Fig. 3). These results are consistent with observations of Liu et al. (2007), who showed that the B biotype was initially rare in both Australia and China but still sexually excluded both indigenous biotypes.

The situation with the B and Q biotypes in the field is somewhat more complex. Populations of the B and Q biotypes have been introduced to many of the same regions of the world, including the U.S.A., Mexico, China, Japan, Spain and Israel (Denholm et al. 1998; Dalton 2006; McKenzie et al. 2009). In the U.S.A., the B biotype was well established before the introduction of the Q biotype in 2004 (Dennehy et al. 2005). However, despite evidence that the Q biotype has been repeatedly introduced and has been found on ornamental plants in greenhouses from 25 states (MREC 2008; McKenzie et al. 2009), the Q biotype has not been found on field crops to date despite extensive sampling (McKenzie et al. 2009). These data strongly suggest complete exclusion of the Q biotype by the B biotype in the field in the U.S.A. However, we cannot rule out the possibility that the Q biotype has not had sufficient opportunity to invade field crops in the U.S.A., although whiteflies commonly move between greenhouses and field crops (Denholm et al. 1998; Dennehy et al. 2005; Dalton 2006; Liu et al. 2007), making this unlikely.

In contrast, the Q biotype has coexisted with the B biotype in Israel and northern Spain for many years (Moya et al. 2001; Khasdan et al. 2005), while the Q biotype resisted invasion and excluded the B biotype in southern Spain (Moya et al. 2001). In Israel, population cage experiments have shown that the B biotype can sexually exclude the Q biotype when insecticides are not used, but that the reverse occurs when insecticides are used (Horowitz et al. 2005). Thus, variation in response to insecticides, with the Q biotype typically more resistant than the B biotype, may have contributed to the coexistence of both biotypes in Israel (Horowitz et al. 2005; Khasdan et al. 2005). In Spain, population cage experiments have also shown that the B biotype sexually excludes the Q biotype when both are initially equally abundant (Pascual 2006), consistent with data reported here. However, the Q biotype coexists with the B biotype in northern Spain, and has excluded the B biotype in southern Spain (Moya et al. 2001). Similar to Israel, Spanish populations of the Q biotype are typically more resistant to insecticides than are those of the B biotype, which may have contributed to these observed distributions. Furthermore, the Q biotype was present in Spain before the B biotype (Moya et al.

2001), and the introduced B biotype may never have reached thresholds needed to establish and sexually exclude Q biotype populations, as our simulations showed that at least 20% of individuals have to be of the B biotype to avoid sexual exclusion by the Q biotype when insecticides are not used (Fig. 3).

Several factors besides variation in resistance to insecticides could also explain the lack of sexual exclusion of the Q biotype by the B biotype in some regions of Spain and Israel. First, data used in our models were collected from experiments on a single host plant (cotton), and behavioural and life history traits could differ on other hosts. Although cotton is a significant host of whiteflies (Denholm et al. 1998; Liu et al. 2007), the effects of reproductive interference could differ in more complex environments, where variation in host use might affect coexistence between biotypes. Additionally, genetic variation between whitefly populations across regions is often considerable (Moya et al. 2001; Perumal et al. 2009), and could result in strong variation in life history and behavioural traits across populations.

Many haplodiploid species are composed of biotypes, including some mites, thrips, whiteflies, scale insects and parasitoids (Diehl & Bush 1984; Dres & Mallet 2002). Global climate change and increased human commerce has increased the rate at which previously allopatric biotypes of these species are introduced to the same environment (Mooney & Cleland 2001; Hänfling & Kollmann 2002; Dalton 2006). Reproductive interference between biotypes could therefore have dramatic ecological and evolutionary consequences ranging from sexual exclusion and extinction to diversification and speciation. Our results suggest that a detailed understanding of behavioural interactions can be used to predict the effects of such interference on species coexistence, which could therefore lead to an improved predictive framework of sexual selection and sexual exclusion.

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Supplementary Material

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