

Mating behaviour, life history and adaptation to insecticides determine species exclusion between whiteflies

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Summary

1. Negative interspecific interactions, such as resource competition or reproductive interference, can lead to the displacement of species (species exclusion).
2. Here, we investigated the effect of life history, mating behaviour and adaptation to insecticides on species exclusion between cryptic whitefly species that make up the *Bemisia tabaci* species complex. We conducted population cage experiments independently in China, Australia, the United States and Israel to observe patterns of species exclusion between an invasive species commonly referred to as the B biotype and three other species commonly known as biotypes ZHJ1, AN and Q.
3. Although experimental conditions and species varied between regions, we were able to predict the observed patterns of exclusion in each region using a stochastic model that incorporated data on development time, mating behaviour and resistance to insecticides.
4. Between-species variation in mating behaviour was a more significant factor affecting species exclusion than variation in development time. Specifically, the ability of B to copulate more effectively than other species resulted in a faster rate of population increase for B, as well as a reduced rate of population growth for other species, leading to species exclusion. The greater ability of B to evolve resistance to insecticides also contributed to exclusion of other species in some cases.
5. Results indicate that an integrative analysis of the consequences of variation in life-history traits, mating behaviours and adaptation to insecticides could provide a robust framework for predicting species exclusion following whitefly invasions.

Key-words: community ecology, displacement, invasion ecology

Introduction

Ecologists have long focused on the effects of interspecific interactions on shaping communities (e.g. Gause 1936; Denno, McClure & Ott 1995; Reitz & Trumble 2002; Gröning & Hochkirch 2008). Early studies involving insects indicated that negative interspecific interactions, such as resource competition, are too weak to be a major force affecting insect communities (e.g. Hairston, Smith & Slobodkin 1960; Shorrocks *et al.* 1984). More recent evidence, however, indicates that displacement of inferior species (species exclusion), one

of the most severe outcomes of interspecific interactions, is relatively common (Reitz & Trumble 2002).

Species exclusion is often mediated by a combination of factors (Reitz & Trumble 2002). Between-species variation in life-history traits can lead to exclusion when a superior resource exploiter drives resources below levels necessary to support a competitor (Tilman 1982; Murdoch, Briggs & Nisbet 1996). Behavioural interactions can also contribute to exclusion when a species is asymmetrically aggressive towards a competitor (Holway & Suarez 1999). Negative interspecific interactions between closely related species during the process of mate acquisition (i.e. reproductive interference) can also lead to species exclusion (Ribeiro &

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Spielman 1986; Kuno 1992; Hochkirch, Gröning & Bücker 2007; Liu *et al.* 2007; Gröning & Hochkirch 2008; Kishi, Nishida & Tsubaki 2009; Crowder, Sitvarin & Carrière 2010). Additionally, the ability of a species to adapt to selection pressures can provide an advantage over species that adapt more slowly (Johansson 2008). In particular, in agricultural ecosystems, pesticide use imposes intense selection pressure on populations of many species. In these systems, the ability of species to respond to pesticide pressure can strongly influence population demographics and patterns of species exclusion or coexistence (Reitz & Trumble 2002). While many factors can contribute to species exclusion, an integrative understanding of the role of such factors in producing patterns of exclusion has rarely been achieved (Reitz & Trumble 2002; Crowder & Snyder 2010). For example, Kishi *et al.* (2009) showed that the bean weevil, *Callosobruchus chinensis* (Linnaeus), was able to exclude the cowpea weevil, *Callosobruchus maculatus* (Fabricius), in shared niches, despite being an inferior resource competitor by interfering with mating by *C. maculatus*.

Another insect that has excluded ecologically similar species is the whitefly *Bemisia tabaci* (Gennadius) (Perring 1996; Dalton 2006; Liu *et al.* 2007). Dinsdale *et al.* (2010) concluded that *B. tabaci* comprised 11 major genetic groups that encompass 24 cryptic species, which collectively are referred to as the *B. tabaci* species complex. Available data indicate the existence of high levels of mating incompatibility between these species, which have previously been referred to as 'biotypes' of *B. tabaci* (Xu, De Barro & Liu 2010). Reproductive interference between members of the complex is also common, as males attempt to mate with incompatible females of a different species. Because *B. tabaci* is haplodiploid, and mated females produce a mixture of male and female progeny, whereas unmated females produce only male progeny (Byrne & Bellows 1991), such reproductive interference can delay female mating and affect the sex ratio of *B. tabaci* populations (Liu *et al.* 2007; Crowder *et al.* 2010). Specifically, a species that is superior at locating and copulating with suitable mates compared with another can produce a higher proportion of female offspring, which can lead to a faster rate of population growth and species exclusion (Liu *et al.* 2007; Crowder *et al.* 2010). Furthermore, life-history traits that relate to resource exploitation, and resistance to insecticides often vary widely between members of the *B. tabaci* complex (Pascual & Callejas 2004; Horowitz *et al.* 2005; Zang, Chen & Liu 2006; Crowder *et al.* 2009, 2010), and this variation could contribute to patterns of species exclusion or coexistence between *B. tabaci* species (Pascual & Callejas 2004; Crowder *et al.* 2010). Thus, the *B. tabaci* complex is an ideal system to explore the roles of life history, mating behaviour and adaptation to insecticides on species exclusion.

The species referred to in Dinsdale *et al.* (2010) as Middle East – Asia Minor 1 contains what is known as the B biotype of *B. tabaci*. This species is the most invasive member of the complex, having spread to more than 50 countries throughout Asia, Africa, Australia, Europe and the Americas (Dalton 2006; Liu *et al.* 2007). Invasions by B have been associated

with declines or extinction of indigenous whitefly species, including A in the United States, AN in Australia and ZHJ1 in China (Perring 1996; Liu *et al.* 2007). Here, we used models incorporating data on life history, mating behaviour and insecticide resistance to predict species exclusion in population cage experiments involving B, ZHJ1, AN and Q from China, Australia, the United States and Israel. We hypothesized that between-species variability in behaviour, life history and insecticide resistance could increase the fitness of one species compared with another and lead to species exclusion.

Materials and methods

We conducted experiments in China, Australia, the United States and Israel to characterize patterns of species exclusion between members of the *B. tabaci* species complex and measure traits influencing exclusion. Results from the experiments in China, Australia and Israel have been published previously (De Barro & Hart 2000; Horowitz *et al.* 2005; De Barro *et al.* 2006; Zang *et al.* 2006; Liu *et al.* 2007; Wilson *et al.* 2007). Here, we used stochastic simulation models and data on life history, mating behaviour, and insecticide resistance from these experiments, as well as data from experiments in the United States, to obtain an integrative understanding of the role of these traits in driving species exclusion between whitefly species.

INSECTS

Description of the species (B and ZHJ1 in China; B and AN in Australia; B and Q in Israel) from previously published experiments is provided in Data S1 (Supporting Information). The B and Q species used in experiments performed in the United States are described below. To avoid confusion, and to ensure a capacity to link this study with previous literature, we have retained the old biotype terminology (i.e. ZHJ1, AN, B, Q), but indicate that these biotypes now equate to the following putative species (species in parentheses), ZHJ1 (Asia II 2), AN (Australia), B (Asia Minor – Middle East 1) and Q (Mediterranean) (Dinsdale *et al.* 2010).

The population of B from the United States (US-B) was collected from a cotton field in Arizona in 2004. The population of Q from the United States (US-Q) was collected on poinsettia plants in a retail store in Arizona in 2006. Both populations were reared on cotton plants, a suitable host for both, under controlled conditions (25 ± 1 °C, $50 \pm 10\%$ RH, 16 : 8 h light : dark). Before initiation of the experiments, 20 individuals from each population were identified to species using the methodology of Khasdan *et al.* (2005), and the results showed that both populations only contained individuals of one species.

SPECIES EXCLUSION EXPERIMENTS

Description of previous published experiments conducted in China, Australia and Israel is provided in Data S1. The conditions used in experiments conducted in the United States are described below.

To initiate the experiments, 65 male adults and 65 female adults (all 2- to 5-day-old virgins) of both B (US-B) and Q (US-Q) were added to a cage ($50 \times 35 \times 75$ cm) containing eight cotton plants at the 5–6 node stage. Every 25 days over a 125-day period, 30 adults were randomly sampled from each cage and identified to species. The experiments were conducted in growth chambers (27 ± 1 °C, $50 \pm 10\%$ RH, 16 : 8 h light : dark).

Two treatments were conducted, in which plants in cages were treated or not with the insecticide pyriproxyfen (Knack™ 0.86 EC; Valent, Walnut Creek, CA, USA). These treatments were designed to explore the effects of adaptation to insecticides on species exclusion. In the two replicated untreated cages (US-U1 and US-U2), all plants added to cages were first sprayed with distilled water. Every 30 days, the oldest four plants in each cage were removed and replaced with four water-sprayed uninfested plants, and the four younger plants were also sprayed with water. In the two replicated treated cages (US-T1 and US-T2), all plants added to cages were first sprayed with a concentration of pyriproxyfen ($0.1 \mu\text{g mL}^{-1}$) that killed 83% and 7% of individuals from the US-B and US-Q populations, respectively (Table S1). Every 30 days, the oldest four plants in each cage were removed and replaced with four pyriproxyfen sprayed uninfested plants, and the four younger plants were also sprayed with $0.1 \mu\text{g mL}^{-1}$ of pyriproxyfen.

INSECTICIDE BIOASSAYS

To understand how insecticide resistance affected species exclusion, bioassays were conducted on US-B and US-Q before initiation of the experiments. Furthermore, bioassays of US-B, US-Q, US-U1, US-U2, US-S1 and US-S2 were conducted after the final experimental sample. Excised cotton seedlings (15–25 cm) were dipped for 20 s in $0.1 \mu\text{g mL}^{-1}$ of pyriproxyfen or in distilled water (control). This diagnostic concentration is useful to monitor resistance, as it only allows for survival of individuals with resistance alleles (Crowder *et al.* 2008, 2009). Twenty mating pairs of a population were aspirated into Petri dishes containing a seedling, with the roots submerged in water. Seedlings thus arranged were held in growth chambers ($27 \pm 1 \text{ }^\circ\text{C}$, $50 \pm 10\%$ RH, 16 : 8 h light : dark) for 48 h. After 48 h, adults were removed, eggs were counted, and seedlings were inserted into 20-mL vials containing tap water. To assess mortality, live nymphs were counted 7 days later. Ten replicates were conducted on treated seedlings and five replicates were conducted on controls. Mortality on treated leaves was corrected for control mortality (Abbott 1925).

Previously published bioassay methods and results (Table S2) for populations of B and Q from Israel (Horowitz *et al.* 2005) are provided in Data S1. Experiments in China and Australia did not investigate the role of insecticides on species exclusion, and thus bioassays were not performed on populations from those regions.

MODELLING SPECIES EXCLUSION

We used stochastic simulation models to predict the outcome of the experiments to determine the roles of life history, mating behaviour and insecticide resistance on exclusion between whitefly species. The full model has been described previously (Crowder *et al.* 2010), and we only summarize it here. The time step of the model was 1 h. Based on behavioural observations of females (Crowder *et al.* 2010), we assumed that each virgin female was courted once per time step until she was mated. The probability of a courtship ending in copulation (P_{success}) was:

$$P_{\text{success}} = P_{\text{intra}} * P_{\text{cop}}, \quad \text{eqn 1}$$

where P_{intra} is the probability of an intra-species courtship, and P_{cop} is the probability of copulation in intra-species courtships. Estimates of P_{intra} and P_{cop} varied based on the proportion of each species present in the overall population, with the values based on published behavioural observations conducted with the experimental populations (Table S3) (Liu *et al.* 2007; Crowder *et al.* 2010). For each courtship, a random number was drawn from a uniform distribution between 0 and 1 and compared with the observed probability values

for P_{intra} and P_{cop} . If either random number was greater than the observed probability, the courtship ended before mating, otherwise the courtship ended in copulation. Mated females laid both male and female eggs (Table S3), whereas unmated females laid only male eggs. Adults survived for 20 days, and females 2- to 20-day-old laid eggs (Crowder *et al.* 2006). The number of eggs laid by females per day depended on female age based on the model of Crowder *et al.* (2006), with females 2–7 days old laying the majority of eggs. The model was written in Visual Basic (Microsoft 2002).

We incorporated insecticide mortality in the model for simulations of the treated cages in the United States and Israel. The timing and concentration of insecticide sprays in the model matched the experiments, and mortality caused by each insecticide lasted for 25 days (Crowder *et al.* 2006). Mortality for B and Q individuals was determined from the bioassays (Tables S1 and S2). In the United States, bioassay results indicated that pyriproxyfen treatments selected for resistance in B during the experiment (Table S2). Thus, we conducted a sensitivity analysis with models that incorporated various rates for the evolution of resistance. The first model assumed that resistance evolved in one generation. In this model, mortality in the first generation was 83%, but mortality in all subsequent generations was equal to mortality after five generations of selection (26%). The second model assumed a linear decline in mortality from the first to the fifth generation. The third model assumed an exponential decline in mortality from the first to the fifth generation. Results obtained with each model were compared with the observed results, and only the best-fit model was used for further simulations. For the IS-BQ population from Israel, we assumed that insecticide treatments did not select for resistance, because data on survival to the insecticides were not collected in the IS-N1 and IS-N2 populations (respectively treated with the neonicotinoid acetamiprid and the neonicotinoid thiamethoxam) throughout the experiment. Thus, mortality caused by each insecticide was constant over model runs.

The conditions of model runs for each region and combination of species were set equal to the conditions of the population cage experiments. Furthermore, the number of simulations run for each set of populations matched the number of experimental replicates (except for the Israel populations, where two simulations were run with each set of conditions although there was only one replicate for each experimental treatment). This allowed for unbiased comparisons between simulated and observed data on the proportion of B adults present each day.

We used the model to evaluate whether variation between species in life history, mating behaviour and insecticide resistance could predict patterns of species exclusion observed in the experiments. Each of these factors differed significantly between some of the species (Tables S1–S3). For life history, we considered variation in development time, as this trait differed between pairs of species (De Barro & Hart 2000; Zang *et al.* 2006; Wilson *et al.* 2007; Crowder *et al.* 2010). The mating behaviour parameters included the probability of copulation, the probability of initiating intra-species courtships, and the offspring sex ratio of mated females (Table S3). Resistance to insecticides was based on the bioassays as described previously (Tables S1 and S2).

In control simulations (control model), parameter values for life history and mating behaviour were the same for both species. In simulations with variation in life history (life-history model), the development time (egg to adult) differed between B individuals and individuals of the other species (Table S3). In the life-history model for simulations of B and ZHJ1 from China, we also incorporated a 30% reduction in survival for ZHJ1 based on data showing that survival of ZHJ1 individuals is reduced when they are paired with B compared with when they develop only with individuals of their own

species (S.S.L., unpublished data). In simulations with variation in mating behaviour (behaviour model), the probability of initiating intra-species courtships, the probability of copulation and the offspring sex ratio of mated females differed between species (Table S3). We also ran simulations with variation in both life-history traits and mating behaviour (combined model). For models of Israel B and Q populations, only the behaviour and control models were run, as there were no significant differences in life history between species (Wilson *et al.* 2007). In models where insecticides were used (United States and Israel), we ran simulations with each of these four models after incorporating insecticide mortality (see above). For each set of simulations, all factors not varied were set to the control values.

DATA ANALYSIS

For experiments with B and ZHJ1 from China and B and Q from the United States, we used two-way ANOVA with generation, data type [observed or simulated from each of four models (control, life history, behaviour, combined)], and their interaction as explanatory variables and proportion of B as the response. A separate ANOVA and linear contrasts were used to compare observed data in each generation with simulated data from each model. A lack of significant difference indicated that model predictions did not differ from observed results. We used one-way ANOVA and Tukey's HSD tests (Sokal & Rohlf 1995) to compare the four models across all generations, with model type as the explanatory variable and average per cent difference (absolute value) between observed and simulated data in each generation as the response.

To compare the models from the United States that incorporated evolution of insecticide resistance in B, we compared model results (with the combined model) with the observed data using two-way ANOVA. Generation, data type [observed or simulated from each of three models (resistance in one generation, linear decline in mortality, exponential decline in mortality)], and their interaction were explanatory variables, and proportion of B was the response. We used one-way ANOVA and Tukey's HSD tests to compare models across generations, with model type as the explanatory variable and average per cent difference (absolute value) between observed and simulated data in each generation as the response.

For experiments with B and AN from Australia, we used one-way ANOVA and Tukey's HSD tests, with data type (observed, simulated with four models) as the explanatory variable and proportion B at the end of the experiment (or simulation) as the response. This analysis differed from the other experiments because data were collected at only one time point (after 105 days).

For experiments with the IS-BQ population, there was only one experimental replicate for each treatment, so we used *t*-tests to compare models, with model type (control or behaviour) as the explanatory variable and percent difference between observed and simulated data (absolute value) as the response. In this analysis, the per cent difference between observed and simulated data was calculated for each model every generation. Thus, each generation served as a replicate. Observed and simulated data were pooled for the two insecticide-treated cages (IS-N1 and IS-N2) because of the small sample size in each treatment.

Results

B VS. ZHJ1 (CHINA)

The relative abundance of B increased from 13% to nearly 100% over nine generations (225 days) (Fig. 1). Predictions

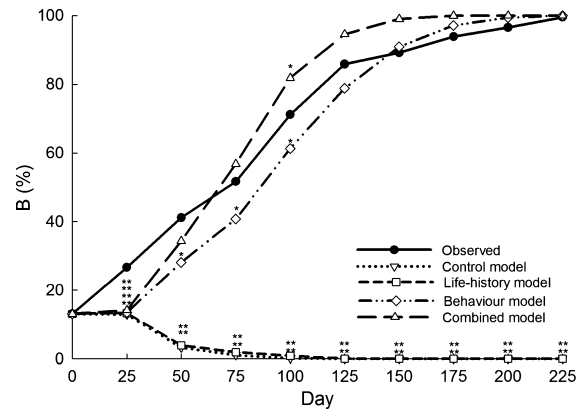


Fig. 1. Observed and simulated data (with four models) of B (%) over time in population cage experiments with B and ZHJ1 populations from China. Two-way ANOVA and Tukey's HSD tests were used to compare observed and simulated data in each generation (* $P < 0.05$; ** $P < 0.0001$).

with the control and life-history models differed significantly from the observed results in every generation (Fig. 1). The behaviour model under-predicted the rate of increase of B over the first four generations (Fig. 1). Simulations with the combined model did not differ significantly from the observed data except in generations 1 and 4. Across all generations, results with the behaviour and combined models deviated significantly less from the observed results than the control and life-history models (Table S4, Fig. 1).

B VS. AN (AUSTRALIA)

The percentage of individuals of B increased from 9% to 75% during the experiment (Table 1). There was no significant difference between predictions of the combined model and the observed results. Predictions with the other models (control, life history and behaviour) differed significantly from the observed results (Table 1).

B VS. Q (UNITED STATES)

In the untreated cages, the percentage of individuals of B biotype increased from 50% to 100% in four generations (Fig. 2). Predictions with the control model differed signifi-

Table 1. Observed and expected values for the per cent of individuals of B after 105 days in population cage experiments with B and AN in Australia

| Data type | B (%) \pm SE |
|--------------------|------------------|
| Observed | 74.8 \pm 4.0A |
| Control model | 0.0 \pm 0.0C |
| Life-history model | 0.0 \pm 0.0C |
| Behaviour model | 19.6 \pm 0.43B |
| Combined model | 74.6 \pm 1.0A |

Experiments were initiated with 9.1% B individuals and 90.9% AN individuals. Rows not sharing the same letter were significantly different based on one-way ANOVA ($F_{4,5} = 185.7$, $P < 0.0001$) and Tukey's HSD tests ($\alpha = 0.05$).

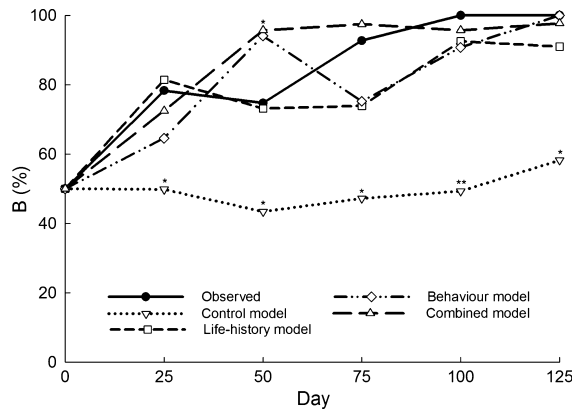


Fig. 2. Observed and simulated data (with four models) of B (%) over time in population cage experiments with B and Q populations from the United States when insecticides were not used. Two-way ANOVA and Tukey's HSD tests were used to compare observed and simulated data in each generation (* $P < 0.05$; ** $P < 0.0001$).

cantly from the observed results in every generation (Fig. 2). Predictions with the life-history, behaviour and combined models did not differ significantly from the observed results in 5, 5 and 4 of the generations sampled, respectively (Fig. 2). Across all generations, results with these three models deviated significantly less from the observed results than the control model (Table S4, Fig. 2).

In the treated cages, the percentage of individuals of B increased from 50% to 100% in five generations (Fig. 3). Models that did not incorporate pyriproxyfen resistance predicted exclusion of B in five or fewer generations, with model predictions deviating significantly from the observed results (Table S5, Fig. 3a). In the sensitivity analysis that incorporated the evolution of resistance, predictions with the model of resistance in one generation differed significantly less from the observed results than the two models that incorporated slower rates for the evolution of resistance (Fig. 4). Using the model with the most rapid adaptation to pyriproxyfen, results with the combined model deviated significantly less from the observed results than the control, life-history and behaviour models (Table S5; Fig. 3b).

B VS. Q (ISRAEL)

In the untreated cage, the percentage of individuals of B increased from 27% to 100% over 12 whitefly generations (Fig. 5a). Predictions with the behaviour model deviated significantly less from the observed results than the control model ($t_6 = 4.9$, $P = 0.0027$) (Fig. 5a). In the cages treated with insecticides, B decreased from 27% to 0% (Fig. 5b,c). The deviation of predictions with the control and behaviour models from the observed results were not significantly different ($t_8 = 1.4$, $P = 0.19$) (Fig. 5b,c).

Discussion

The combined model that incorporated variation in development time and mating behaviour predicted exclusion between

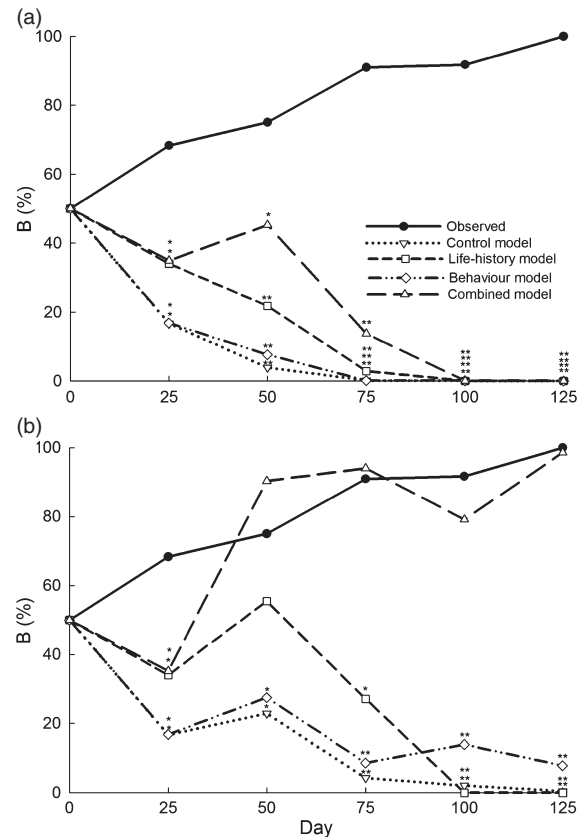


Fig. 3. Observed and simulated data (with four models) of B (%) over time in population cage experiments with populations of B and Q from the United States when pyriproxyfen was used. (a) Models did not incorporate adaptation to pyriproxyfen; (b) Models incorporated adaptation to pyriproxyfen. Two-way ANOVA and Tukey's HSD tests were used to compare observed and simulated data in each generation (* $P < 0.05$; ** $P < 0.0001$).

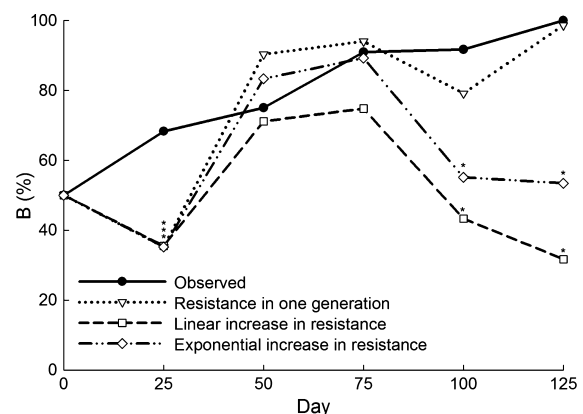


Fig. 4. Observed and simulated data (with three models of the evolution of resistance) of B (%) over time in population cage experiments with B and Q from the United States that were treated with pyriproxyfen. For each model, we assumed variation in both life-history and behavioural traits between species. Two-way ANOVA and Tukey's HSD tests were used to compare observed and simulated data in each generation (* $P < 0.05$).

whitefly species from each region. Although predictions did not match observed results in every generation sampled, the models accurately predicted the trends and ultimate outcome

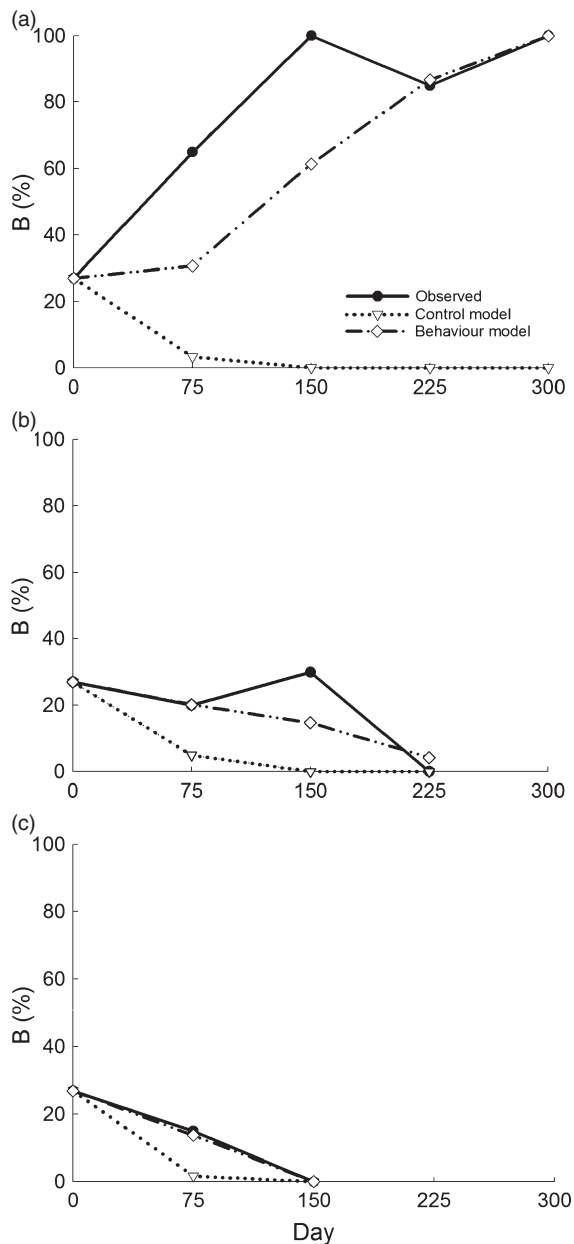


Fig. 5. Observed and simulated data (with two models) of B (%) over time in population cage experiments with the mixed B and Q population from Israel. (a) No exposure to insecticides; (b) treated with acetamiprid; (c) treated with thiamethoxam.

of species exclusion in each experiment. These results suggest that whitefly species with superior life-history and behavioural traits will exclude inferior species, and such exclusion can be predicted in environments that are treated with insecticides or not. To the best of our knowledge, no other study to date has linked observed data on life history and behaviour with models to predict exclusion between species.

In experiments conducted in each region, B excluded or appeared to be excluding other species. In general, variation between species in mating behaviour was a more significant factor driving this exclusion than variation in life history when B was initially rare. In China, B develops slower than

ZHJ1 (Zang *et al.* 2006), but is more successful at mating in mixed species assemblages (Liu *et al.* 2007). In this system, predictions with the combined model were similar to the model that incorporated variation in behaviour alone, and both models predicted the trend as well as the eventual outcome of sexual exclusion of ZHJ1 that matched the observed data. In contrast, predictions with the life-history model did not predict species exclusion by B (Fig. 1). Thus, the behavioural advantage for B due to more effective mate recognition appeared to override costs associated with slower development. Even in the best-fit model, however, simulated data did not match observed data in every generation (Fig. 1), suggesting that factors other than those considered in our model may have also played an important role in mediating the interactions between species. Results from the experiments in Australia further support the role of behaviour as an important factor driving species exclusion. In this system, the behaviour model predicted an increase in the frequency of B over time, although at a slower rate than was observed (Table 1). In contrast, the life-history model predicted exclusion of B, indicating that only variation in mating behaviour that favoured B was sufficient to override the low initial frequency of the B. Only models that incorporated variation in both life history and behaviour produced robust predictions across all experiments, showing that interactions between behaviour and life history affected outcomes. In a similar set of cage experiments, De Barro *et al.* (2006) showed that B could exclude the AN biotype when as few as 2–5% of individuals were of B initially. Along with results reported here, these data strongly suggest that superior behavioural traits allow B populations to mate more effectively than other species, leading to a faster rate of population growth and exclusion of other whitefly species.

Several factors may cause mating behaviour to be a more significant factor affecting species exclusion than life history. Behavioural differences between species not only result in B being more effective at recognizing and copulating with suitable mates, but also allow individuals of B to interfere with mating in other species (Liu *et al.* 2007; Crowder *et al.* 2010). These factors allow B females to produce a higher proportion of females in their offspring than other females, which can result in a faster rate of population increase and ultimately species exclusion. In contrast, although developing faster can benefit one species over another, this advantage may be too weak to overcome difficulty associated with finding suitable and rare mates when reproductive interference is present. These results support theoretical predictions that reproductive interference between species is more likely to drive species exclusion than exploitative competition because interference has a more significant impact on fitness and population growth (Kuno 1992), which has been observed in weevils (Kishi *et al.* 2009).

Results with the B and Q species from the United States showed that variation between species in either life history or mating behaviour were good predictors of species exclusion. In this system, B developed faster than Q, and was more effective at locating and copulating with suitable mates.

Because both species were equally abundant at the onset of experiments, variation in life history and behaviour provided an advantage for B. When compared with results from Australia and China, these results suggest that the effects of life-history traits on species exclusion depend on the initial frequency of species. A species with superior life-history traits is expected to displace an inferior species if both species occur initially at similar frequencies. The species with superior life-history traits can be displaced by an inferior species when the superior competitor is initially rare, if interference between species limits the ability of the superior species to reproduce (a priority effect) (Amarasekare 2002; Munday 2004). Thus, the relative abundances of species across habitats and regions could strongly influence species exclusion and the ability of indigenous whitefly species to withstand invasions (Simberloff 2009).

The use of insecticides could affect the distribution and frequency of whitefly species across regions (Horowitz *et al.* 2005; Khasdan *et al.* 2005). In experiments with B and Q from Israel, but not the United States, Q excluded B when insecticides were used. These results may help explain why Q has been excluded from the field in the United States (McKenzie *et al.* 2009), but coexists with B in Israel (Horowitz *et al.* 2005; Khasdan *et al.* 2005). These results appear to be linked with the ability of B to rapidly evolve resistance to insecticides in the United States but not Israel. In both regions, Q is more resistant to common insecticides compared with B (Horowitz *et al.* 2005; Crowder *et al.* 2008). In Israel, B populations have relatively little genetic variation for resistance to insecticides, suggesting that individuals with resistance alleles are exceedingly rare (Horowitz *et al.* 2005), and therefore are unable to adapt rapidly when treated with insecticides. Thus, the greater resistance of Q populations can contribute to exclusion of B when insecticides are used. In contrast, in the United States, populations of B have relatively high genetic variability for resistance to insecticides, suggesting that many individuals in B populations have resistance alleles (Dennehy *et al.* 2004; Crowder *et al.* 2008), and therefore often rapidly adapt to insecticide selection. In addition to results reported here, Dennehy *et al.* (2004) showed that populations of B can evolve resistance to pyriproxyfen after one or two generations of selection. Thus, the rapid evolution of insecticide resistance that was included in the model is realistic. When we incorporated the evolution of resistance into models, predictions matched the observed results. This suggests that the ability of B from the United States to evolve resistance to insecticides provided an advantage above and beyond its superior life-history traits and mating behaviours. Furthermore, the models only matched the observed patterns when resistance evolved in one generation. Thus, it was not only the ability of B to evolve resistance that led to species exclusion, but also the ability to evolve resistance rapidly. As invasive species often undergo rapid evolutionary changes in introduced environments (Hänfling & Kollmann 2002; Whitney & Gabler 2008), understanding the evolutionary potential of such species may be critical for predicting their impact on closely related competitors.

Our results show that an integrative approach is needed to understand the factors underlying species exclusion and to predict the effects of interspecific interactions. While the effect of life history on species exclusion has received significant attention (e.g. Holway *et al.* 2002; Reitz & Trumble 2002), the role of behaviour and of the adaptive potential of species has received less attention (Holway & Suarez 1999; Liu *et al.* 2007; Gröning & Hochkirch 2008; Johansson 2008; Kishi *et al.* 2009; Crowder *et al.* 2010). Here, we showed that an integrative analysis of the consequence of variation in life-history traits, mating behaviours and the capacity to respond to selection can lead to a robust framework for predicting whitefly species exclusion in laboratory and field experiments. Although the key traits affecting exclusion or coexistence varied between regions, reproductive interference leading to a decline in the production of fertile females in one whitefly species compared with another is a powerful mechanism that could foster rapid species exclusion across a wide range of conditions. We are currently using stochastic models considering variation in the important traits identified here, and other ecological factors such as landscape diversity, climate and the spatial distribution of populations (Gröning *et al.* 2007), to predict whitefly species exclusion under field conditions. Success in this endeavour could be useful in mitigating the harmful effects of whitefly invasions world-wide.

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Supporting Information

Additional Supporting Information may be found in the online version of this article.

Data S1. Mating behaviour, life history and adaptation to insecticides determine species exclusion between whiteflies

Table S1. Mortality caused by a diagnostic concentration of pyriproxyfen (0.1 µg mL⁻¹) for six populations from the United States prior to the initiation of cage experiments and after the final generation had been sampled

Table S2. Results of probit analysis for the LC₅₀ (concentration that killed 50% of individuals on average) and slope of IS-BQ to two insecticides prior to the initiation of the cage experiments and after B had excluded Q

Table S3. Parameter values for modelling species exclusion. In control simulations, we assumed there were no differences between species in life-history or behavioural traits (values listed in control column). In other simulations, parameter values for one or both species were set to the alternative values

Table S4. Comparison of model predictions with observed results (across all generations) for experiments with B and ZHJ1 from China and B and Q from the US where populations were not exposed to insecticides

Table S5. Comparison of model predictions with observed results (across all generations) for experiments with B and Q from the United States where populations were exposed to insecticides with models that did or did not incorporate the evolution of insecticide resistance

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