Competition for food affects the strength of reproductive interference and its consequences for species coexistence

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Abstract

Competition for food and reproductive interference (negative interspecific sexual interactions) have been identified as major drivers of species exclusion. Still, how these biotic interactions jointly determine competitive dominance remains largely unknown. We tackle this by coupling population models and laboratory experiments with two spider mite sibling species. Using experiments specifically designed to measure the single and combined effects of food competition and reproductive interference, we first show that the strength and symmetry of reproductive interference between species changes in presence of food competition. Next, we show that population models incorporating each type of interaction alone or their effects when combined lead to markedly different predictions, from exclusion by one species or the other, to exclusion of the less abundant species. Moreover, accounting for the observed reduction in the strength of reproductive interference in the presence of food competition changes the threshold frequency determining the dominant competitor, favouring the superior competitor for food. Finally, model predictions for short-term population dynamics were corroborated with an independent population cage experiment. Altogether, our results suggest that trophic interactions can modulate sexual interactions, significantly impacting population dynamics and competitive outcomes. Hence, the joint consideration of food competition and reproductive interference is critical to accurately predict and understand species coexistence.

Introduction

How species that compete for common resources coexist is arguably one of the fundamental questions in ecology (Hutchinson 1961, Tilman 1980, Huston 1994, Chesson 2000). Modern coexistence theory posits that two competing species coexist when their niche differences, *i.e.* the degree to which intraspecific competition exceeds interspecific competition, are greater than their fitness differences, *i.e.*, their differences in intrinsic growth rate weighted by their overall sensitivity to competition (Chesson 2000, 2018, Adler et al. 2007, HilleRisLambers et al. 2012). A rich and vast literature also addresses how different abiotic and biotic factors affect the drivers, and ultimately, the outcomes of species competition and coexistence (Dunson and Travis 1991, Chesson 1994, McPeek 2014, Spaak et al. 2021). In particular, competition for food often co-occurs with other types of biotic interactions, but our knowledge of their joint effects is mostly limited to trophic ones, such as predation (Kotler and Holt 1989, Chesson and Kuang 2008, Song et al. 2020, Shoemaker et al. 2020) or parasitism (Terry et al. 2021, Rovenolt and Tate 2022, Hasik et al. 2023), whereas the impact of other types of interactions, within the same trophic level, has received comparatively less attention to date.

Interspecific sexual interactions, owing to imperfect signal recognition, are relatively common between various assemblages of sexually reproducing species that generally also compete for food (Webb et al. 2002, Burns and Strauss 2011, Kyogoku 2015, Weber and Strauss 2016, Servedio and Hermisson 2020). Such interactions are commonly referred to as ‘reproductive interference’ as they generally have negative fitness consequences on at least one of the species involved (Gröning and Hochkirch 2008). Because these interactions encompass a wide range of underlying mechanisms, varying from signal jamming, heterospecific rivalry, misdirected courtship and heterospecific copulations (incl. gamete wastage), up to the production of inviable or infertile hybrids (Gröning and Hochkirch 2008),

they include both direct (interference) and indirect (exploitative) competition for limited shared resources associated to species reproduction (*i.e.*, breeding space, mates and/or gametes). Although historically reproductive interference has mostly been studied in speciation research, due to its central role in reproductive character displacement and reinforcement (Butlin 1987, Servedio and Noor 2003, Yamaguchi and Iwasa 2015), it recently attracted growing interest in ecological research (Cothran 2015, Weber and Strauss 2016, Christie and Strauss 2020, Gómez-Llano et al. 2021, 2023, Grether et al. 2024).

Theoretical studies posit that reproductive interference can be a major driver of competitive outcomes (Kuno 1992, Yoshimura and Clark 1994, Kishi and Nakazawa 2013, Schreiber et al. 2019, Yamamichi et al. 2023). Because this interaction causes species to be more negatively impacted by heterospecifics than by conspecifics, it promotes positive frequency-dependence (*i.e.*, it always disfavours the rarer species, contrarily to food competition, which generally promotes negative-frequency dependence). This phenomenon, whereby the relative abundances of each species determine which one excludes the other and known as priority effects (Grainger et al. 2019), leads to reproductive interference driving exclusion faster than food competition (Kuno 1992, Yoshimura and Clark 1994). These effects can thus preclude long-term coexistence between ecologically equivalent species, as evidenced by an increasing number of laboratory and field studies (Takafuji et al. 1997, Konishi and Takata 2004, Hochkirch et al. 2007, Liu et al. 2007, Kishi et al. 2009, Runquist and Stanton 2013, Kishi 2015, Whitton et al. 2017, Ting and Cutter 2018). Yet, as for other types of interactions (Chesson 2000, Amarasekare 2002), reproductive interference could promote coexistence via a trade-off with food competition, such that the species most affected by food competition is less affected by reproductive interference or vice-versa (Kishi and Nakazawa 2013, Schreiber et al. 2019). A limitation of prior work, however, is the assumption that food competition and reproductive interference operate independently from

one another. In fact, several empirical studies revealed that, within species, food competition can affect the reproductive success of individuals (as better-fed males may be stronger competitors for mates, or even more attractive to females; Sigurjónsdóttir 1984, Fisher and Rosenthal 2006), and reciprocally, that different reproductive strategies may result in different levels of food competition (for instance, males which invest more in securing mates may be weaker competitors for food and vice-versa; Thiel and Dennenmoser 2007). Such interplay between food competition and sexual interactions within species may thus also occur between species, such that food competition and reproductive interference affect the strength of each other when simultaneously at play, as proposed by recent theory (Yamamichi et al. 2023).

In sum, both reproductive interference and competition for food have strong impacts on species coexistence, but their joint effect remains largely experimentally unexplored. This knowledge gap may be due to the fact that, unlike other abiotic or biotic factors that can easily be singled out (*e.g.*, temperature, salinity, competitors, parasites or predators), reproductive interference is part of the interaction between species that are simultaneously competing for food. Therefore, designing experiments that measure the impact of each of these interactions separately (which is needed as a control to evaluate their joint effect) is a true challenge. Although a previous study successfully uncoupled the effects of trophic and reproductive interactions on the population growth of two co-occurring *Enallagma* damselfly species (Gómez-Llano et al. 2023), and another assessed their joint effects on two parapatric *Streptanthus* jewelflower species (Christie and Strauss 2020), none have, to our knowledge, directly compared the consequences of such interactions when acting separately *versus* jointly. Herbivorous spider mites represent an ideal system to do so, given their amenability to experimental manipulation of both competition for food (Ferragut et al. 2013, Lu et al.

2018, Fragata et al. 2022) and reproduction (incl. reproductive interference; Smith 1975, Ben-David et al. 2009, Clemente et al. 2018, Sato and Alba 2020).

Here, we studied the single and combined effects of reproductive interference and competition for food between two sibling species of spider mites, *Tetranychus urticae* and *T. cinnabarinus* (Kuang and Cheng 1990, Li et al. 2009), also often referred to as the green and red forms of *T. urticae* (Auger et al. 2013). These two species have overlapping distributions and host plant ranges (Migeon and Dorkeld 2023), sometimes being found on the same host plant (Lu et al. 2017, Zélé et al. 2018), on which they compete for food (Lu et al. 2017, 2018). These mite species also naturally engage in heterospecific sexual interactions (Smith 1975), and suffer from strong reproductive interference due to variable reproductive incompatibilities and unfit hybridization (ranging from partial to complete hybrid sterility and hybrid breakdown; Dupont 1979, de Boer 1982, Sugasawa et al. 2002, Cruz et al. 2021, Xue et al. 2023). With this experimental system, we used recent modelling advances rooted in coexistence theory (Schreiber et al. 2019) and its recent extensions accounting for priority effects (Ke and Letten 2018), and performed a set of experiments manipulating the presence or absence of food competition and reproductive interference. This combination of theory, modelling, and experiments allowed to estimate the strength of both types of interaction, when occurring alone or together, as well as to predict their single and joint contributions to competitive outcomes. Predicted dynamics of the two mite species interacting through both food competition and reproductive interference were then validated using data from an independent population experiment.

Methods

Biological materials

Spider mite source populations

We used one source population of *T. urticae* (‘Tu’) and one of *T. cinnabarinus* (‘Tc’), both free of any known endosymbiont and fully homozygous for the presence (Tu) or absence (Tc) of a recessive nonsynonymous mutation coding for pesticide resistance, which can be used to distinguish the individuals of each species in addition to differences in body colouration (Figure 1; see detailed explanations in Appendix S1: Section S1). Due to incomplete pre-zygotic isolation (Cruz et al. 2024) and strong post-zygotic isolation (Cruz et al. 2021) between these two populations, strong reproductive interference is expected when they share the same environment (Smith 1975). Indeed, females do not display any mate preference (non-assortative mating), whereas males of both species prefer to mate with Tc females (Cruz et al. 2024). Moreover, sex ratio distortion occurs in heterospecific crosses between Tu females and Tc males (with an overproduction of haploid sons instead of hybrid daughters, probably due to fertilization failure; Cruz et al. 2021), and both directions of heterospecific crosses result in fully sterile hybrids (most hybrid females do not lay eggs and the few eggs laid do not hatch; Cruz et al. 2021).

Creation of replicate populations

Prior to the experiments, five independent replicates were derived from each of the Tu and Tc source populations by transferring 200 adult females to a population cage containing two 14 days-old bean plants (*Phaseolus vulgaris*, cv. Contender seedlings obtained from Germisem, Oliveira do Hospital, Portugal). All replicate populations were then maintained under discrete generations at constant population size, by transferring 200 young adult females to new population cages every 14 days, and subsequently used in the experiments. Individuals from a given replicate population were always tested against those from the replicate population with the same label (*i.e.*, Tu or Tc individuals of a given replicate were always tested against Tu or Tc individuals of the same corresponding replicate, and so on).

Creation of age cohorts

All replicates of the two populations were expanded by transferring 100 adult females to a separate population cage containing one fresh bean plant. Eleven to 15 days later, age cohorts were created from each of these additional boxes to obtain a sufficiently large number of individuals of roughly the same age for each experiment. Groups of 75 (for Experiment 1; see below) or 50 (for Experiments 2 and 3; see below) mated females, as well as 50 virgin females (to produce only males for Experiment 2), were collected from each replicate population and installed to lay eggs for 3 days on detached bean leaves placed on water-soaked cotton in petri dishes. For Experiments 1 and 3, adult mated female offspring were collected from each age cohort of each replicate population 14 days later, and directly used on the first day of the experiments. For Experiment 2, virgin female offspring were collected 11-12 days later (two days prior to the start of the experiment), while they were still deutonymphs undergoing their last moulting stage. They were placed for two days in groups of 30 without males on *ca.* 9 cm^2 bean leaf fragments, such that they emerged as adults while remaining virgin. Virgin males were directly collected from the cohorts on the first day of the experiment. Population maintenance and all experiments were performed under the same controlled laboratory conditions (24±2ºC, 70% humidity, 16/8h L/D).

Measuring food competition, reproductive interference, and their combined effects

To uncouple the effects of food competition and reproductive interference on the growth of interacting species, as well as to measure the effect of reproductive interference in the presence of food competition, we performed a set of three different experiments, which are fully detailed below. Briefly, to quantify how food competition affected the growth of both species in absence of reproductive interference (Experiment 1), focal females that had mated

with conspecific males were exposed to increasing densities of conspecific and heterospecific competitors, and the number of their female offspring was counted (Figure 2a). Second, to quantify the strength of reproductive interference while restricting competition for food (Experiment 2), we counted the number of offspring produced by females isolated on individual food patches after they interacted sexually with conspecific males only (control), or with both conspecific and heterospecific males. This was done over 2 generations to encompass the costs resulting from the production of sterile F1 hybrids (Figure 2b). Third, to quantify the combined effect of food competition and reproductive interference on the growth rate of each species (Experiment 3), we assessed the per capita production of daughters by females of each species when they interacted sexually and shared a common resource over 3 generations (Figure 2c). In all three experiments, controls with no competitors for food (nor heterospecific mates) were also used to estimate the individual growth rate of each species in absence of interactions.

Experiment 1: *Food competition in absence of reproductive interference*

One focal adult mated female of the Tu and/or Tc population was installed on a *ca.* 9 cm^2 bean leaf disc placed on water-soaked cotton contained in a petri dish to lay eggs for 3 days, either alone, or together with 2, 7, or 19 conspecific or heterospecific female competitors (hence seven different 'treatments' were performed; Figure 2a). At the end of the egg-laying period, all females were discarded, and 11 days later (14 days after the experiment onset) the number of surviving adult daughters produced by the focal females on each leaf disc was counted (in the case of intraspecific competition, the total number of daughters per disc was divided by the initial female density, whereas for interspecific competition, offspring of the focal female were identified based on body colouration; see Figure 1). This procedure allowed quantifying the effects of intraspecific and interspecific competition by relating per-

capita offspring production along a density gradient of competitors (Hart et al. 2018). The entire experiment was performed in 10 independent experimental blocks, installed over the course of 2 weeks (5 blocks per week, installed on 5 consecutive days). Each competition treatment and replicate population were tested simultaneously, with one experimental replicate (*i.e.*, leaf disc) of each being carried out per block. Hence, 10 experimental replicates (one per block) were performed per replicate population of each species and per treatment, for a total of 700 replicates (10 experimental replicates*5 replicate populations*2 species*7 treatments) obtained at the end of the experiment.

Experiment 2: *Reproductive interference in absence of food competition*

We installed 5 virgin females and 5 virgin males from each cohort of each replicate population on a *ca*. 9 cm^2 bean leaf disc placed on water-soaked cotton contained in a petri dish (*i.e.*, a 'big' mating patch to prevent individuals from incidentally leaving the patch and drowning), either in the absence of heterospecifics ('control treatments'; one for each species), or with 5 virgin females and 5 virgin males from a cohort of the other species, to allow for reproductive interference between the two (*i.e.*, 'reproductive interference treatment'; Figure 2b). Individuals could freely mate for 2 days before males were discarded and each female was isolated on 2.5 cm^2 bean leaf discs (individual patches) to lay eggs for 1 day. The offspring produced by each female thus developed in the absence of food competition with the offspring of other females. Nine days later, when all female offspring were at (or about to enter) their last moulting stage and most male offspring were already adults, all offspring within a given experimental replicate were merged on a fresh mating patch, where they could emerge as adults and interact (incl. mating) for 3 days. Then, the same number of females as initially used in each treatment (10 for the reproductive interference treatment and 5 for the control treatments) were randomly sampled in each

mating patch and isolated on individual patches to lay eggs for 1 day. After 11 days, the number of adult daughters in each individual patch was registered and the total number of daughters of each species per replicate was computed (*i.e.*, species identification was based on body colouration in this experiment; see Figure 1). Offspring production was registered over two generations to encompass all costs resulting from reproductive interference, including offspring sex ratio distortion and the production of sterile F1 hybrids. The entire experiment was performed in 5 independent experimental blocks installed over the course of 5 weeks, with a maximum of 2 blocks on consecutive days. Two experimental replicates (*i.e.*, mating patch) per treatment and replicate population were carried out simultaneously within each block. Hence, 10 experimental replicates (2 experimental replicates*5 blocks) were performed per replicate population and per treatment, for a total of 150 experimental replicates (10 experimental replicates*5 replicate populations*3 treatments) obtained at the end of the experiment.

Experiment 3: *Combined effects of food competition and reproductive interference*

We installed 5 Tc and 5 Tu adult mated females on a *ca.* 9 cm^2 bean leaf disc for 3 days (*i.e.*, ‘interactions’ discs; Figure 2c). Eleven days later (14 days after the experiment onset), the number of adult daughters of each species was counted and up to 10 adult females were randomly selected and transferred to a fresh bean leaf disc (*ca.* 9 cm^2) to start the next generation. Female offspring was counted 11 days later, as before, and the entire procedure was repeated until the third generation. Because all females used to initiate the experiment were already mated to conspecific males, the first generation was only affected by food competition and no hybrids were formed. Then, because the females that were randomly sampled at the first and second generations were already mated to either conspecific, heterospecific, or both types of males, the second and third generations were affected by both

food competition and reproductive interference, and hybrid daughters were also produced (and counted). Finally, females of either species were also installed individually on *ca.* 9 cm^2 bean leaf discs to estimate their individual growth rate in this experiment (*i.e.*, 'absence of interactions' discs, one for each species). These females could lay eggs for 3 days and their daughters were counted 11 days later. As in the previous experiments, the identification of females of each species (as well as their hybrids) was based on body colouration (Figure 1). The experiment was carried out in 3 independent experimental blocks, installed over the course of 2 weeks (2 blocks installed on consecutive days at the start of the first week and the third block installed at the start of the second week). For each of the five replicate populations, 4 experimental replicates of the 'interactions' discs were performed in the first block and 3 were carried out in the remaining two blocks, for a total of 50 'interactions' discs (10 experimental replicates*5 replicate populations). Single females (*i.e.*, 'absence of interactions' discs) were tested concurrently with the first and third blocks of the experiment, in 15 experimental replicates per block, for a total of 150 'absence of interactions' discs (30 experimental replicates*5 replicate populations).

Modelling approach to estimate interaction strengths from the experimental data

To estimate key interaction coefficients from data obtained in each of the experiments described above, we adapted a discrete-time population model developed by Schreiber et al. (2019), in which a reproductive interference component was added to the Beverton-Holt function used to predict population dynamics under food competition (Godoy and Levine 2014). In this model, the number of individuals of a species *i* (N_i) is predicted at each generation *t*, accounting for interactions with individuals of a species *j* (N_j), with the following equation:

$$N_{i,\,t+1} = N_{i,t}\,\delta\,\lambda_i \cdot \frac{1}{1 + \alpha_{ii} N_{i,t} + \alpha_{ij} N_{j,t}} \cdot \frac{N_{i,t}}{N_{i,t} + \beta_{ij} N_{j,t}} \qquad \text{(Eq. 1)}$$

The first component of the expression (*i.e.*, $N_{i,t}\ \delta\ \lambda_i$), refers to population growth in absence of limiting interactions for species *i*, with δ being the oviposition period in days and λ_i the per-capita daily intrinsic growth rate of species *i* in absence of any type of interaction. The middle component corresponds to the effect of food competition, with α_{ii} and α_{ij} being, respectively, the per capita effects of intraspecific and interspecific competition for food at generation *t*, hence with $N_{i,t}$ and $N_{j,t}$ competitors of species *i* and *j*, respectively. Finally, the third component of the expression represents the effect of reproductive interference, where β_{ij} is the per capita effect of interspecific sexual interactions with $N_{j,t}$ individuals of species *j* on the growth rate of species *i*.

All parameter estimations were done in the R statistical software (v4.2.2) using custom scripts available at Zenodo (https://doi.org/10.5281/zenodo.10086125). We focused on the production of female offspring (daughters) as we assume that male spider mites, which are much smaller than the females, do not contribute significantly to food competition (as in Bonte and Bafort 2019, and Fragata et al. 2022). Values of λ were computed as the mean (and 95% confidence interval) daily production of daughters ($\lambda_i = N_{i,\ t+1}/\delta$) in the control treatments of each experiment (single females in Experiments 1 and 3, or conspecific females in Experiment 2). For subsequent analyses, parameter estimations and predictions, we either used the average of the λ estimates obtained from Experiments 1 and 2 (in which each type of interaction was measured independently), or used directly the λ estimated from Experiment 3 (in which both types of interactions were measured simultaneously). Values of the α and β interaction coefficients were estimated using a maximum likelihood approach similar to that of Godoy and Levine (2014). Briefly, the method consisted in fitting the log-transformed number of daughters observed experimentally into Eq. 1, along with fixed values of λ obtained from the experiments. Note that, because our estimation of λ already included the effects that a focal individual has on itself, we excluded the focal individual in the food

competition component of Eq. 1 for the estimations of the α and β coefficients (*i.e.*, we considered only the number of neighbour females). Model fitting then allowed identifying the parameter values (randomly sampled within the normal density function *dnorm*) and associated standard deviations (from which the 95% confidence intervals were obtained) that enable the best fit between the model predictions and the observed data (*i.e.*, by minimizing the negative log-likelihoods; function *optim*). This was done with a simulated Nelder-Mead algorithm (Nelder and Mead 1965), instead of the 'L-BFGS-B' method used by Godoy and Levine (2014), to avoid false convergence issues. For all α and β estimations, we used an unrealistic starting value of 4, then repeated the estimations with a more realistic value of 0.1 to verify the consistency in the outcomes.

When only competition for food could occur (Experiment 1), the value of β was set to 0 in Eq. 1 (which is equivalent to removing the reproductive interference component from the model), and both intraspecific (α_{ii} and α_{jj}) and interspecific (α_{ij} and α_{ji}) competition coefficients were estimated using only data from the 'intraspecific' and 'interspecific' competition treatments, respectively (*i.e.*, offspring produced by focal females that had already mated with conspecific males and were exposed to an increasing gradient of conspecific or heterospecific females in the neighbourhood). When only reproductive interference occurred (Experiment 2), the values of all α parameters were set to 0 in Eq. 1 (which is equivalent to removing the food competition component from the model), and the reproductive interference coefficients β_{ij} and β_{ji} were estimated simultaneously using the data obtained after two generations in the treatment in which 5 virgin females and 5 virgin males of both species were initially mixed on a same mating patch, but females were subsequently isolated on individual food patches after they interacted sexually. Finally, when both types of interactions occurred (Experiment 3), we could not estimate both α and β values simultaneously due to mathematical limitations, hence we used a nested approach instead.

Competition coefficients (α) were first estimated using only data obtained from the first generation (during which reproductive interference did not occur), whereas reproductive interference coefficients (β) were estimated using the data obtained from the generations 2 and 3 (hence over 2 generations, exactly as for β_{ij} and β_{ji} estimations in Experiment 2). In this case, the value of the previously estimated α's was fixed in the model. A logistic limitation with this approach is that, by design, we only assessed changes in the β values (reproductive interference) due to the occurrence of food competition (by comparing the values obtained from Experiments 2 and 3), but not changes in the α values (food competition) due to the occurrence of reproductive interference. Also, because in Experiment 3 we could not independently measure the effect of intra- and interspecific competition, as we did in Experiment 1 (*i.e.*, we could not test different absolute densities of intra- and interspecific competitors due to the complexity of the experimental design when subsequently allowing for reproductive interference over 2 additional generations), it was not possible to estimate all α values simultaneously. To mathematically circumvent this issue, we forced the α_{ii}/α_{ij} and α_{jj}/α_{ji} ratios to be equal to the α_{ii}/α_{ij} and α_{jj}/α_{ji} ratios obtained in Experiment 1, respectively. We followed this procedure assuming that, for each species (for each replicate population, as well as for all data independently of the replicate), the relationship between values of intra- and interspecific coefficients would be similar between Experiment 1 and Experiment 3.

Theoretical approach to predict the long-term outcome of species interactions

Key metrics for model predictions

To infer the long-term impact of food competition and reproductive interference between the Tu and Tc populations and predict whether they could coexist, we computed three different key metrics using the estimated parameter values: their average fitness difference, their niche

overlap (Chesson 2000), and their relative strength of reproductive interference (Schreiber et al. 2019).

The average fitness difference between two species refers to the competitive advantage of one species over the other. Note that the meaning of 'fitness' in the ecological literature of species coexistence differs from that in evolutionary biology (Lankau 2011). This fitness difference is the product of two ratios: the 'demographic ratio' that describes the ratio of intrinsic growth rates between species in the absence of competitors, and a 'competitive response ratio' that describes the degree to which one species is more resistant to competition than the other (Chesson 2012). It is thus computed using the estimated λ and α parameters as follows:

$$\frac{k_j}{k_i} = \frac{\lambda_j - 1}{\lambda_i - 1} \cdot \frac{\sqrt{\alpha_{ij}\alpha_{ii}}}{\sqrt{\alpha_{ji}\alpha_{jj}}} \qquad \text{(Eq. 2)}$$

When the value of this ratio is above 1, species *j* is superior (*i.e.*, it should exclude species *i* in the absence of reproductive interference and under complete niche overlap; see below); when it is below 1, species *i* is superior; and when it is close to 1, the two species are equivalent competitors. Thus, a reduction in fitness difference has an equalising effect on the population dynamics of interacting species (Chesson 2000).

The niche overlap between species, ρ, represents the degree to which each population limits its own growth as compared to how it limits the growth of the competitor species (Chesson 2012). It is computed using the estimated α parameters as follows:

$$\rho = \sqrt{\frac{\alpha_{ij}\alpha_{ji}}{\alpha_{jj}\alpha_{ii}}} \qquad \text{(Eq. 3)}$$

When the value of ρ is below 1, a species limits its own growth more than that of its competitor, favouring coexistence. However, when the value approaches 1 (full niche overlap), species limit each other's growth as much as they limit their own, and coexistence is hampered (one of the species will always exclude the other). Thus, niche difference,

expressed as $1 - \rho$, has a stabilising effect on population interactions (Chesson 2000). Finally, if niche overlap is greater than one, population dynamics may be dominated by priority effects, such that the order of arrival or initial frequencies of individuals from the two species become the determinants for which one will exclude the other (Ke and Letten 2018). Note that the competition coefficients in Eq. 3 (the α parameters) are phenomenological, but we assume in our work that species limit each other mainly through food competition.

The relative strength of reproductive interference, γ, represents the degree to which one species is more sensitive to reproductive interference than the other (Schreiber et al. 2019). It is computed using the estimated β parameters as follows:

$$\gamma = \frac{\beta_{ji}}{\beta_{ij}} \qquad \text{(Eq. 4)}$$

When the value of γ is above 1, species j is superior (*i.e.*, it should exclude species i in absence of food competition, provided that both populations have equal intrinsic growth rate; see below); when γ is below 1, species i is superior; and when it is close to 1, the two populations are equally (symmetrically) affected by reproductive interference. Thus, as for fitness differences, symmetrical impacts of relative reproductive interference have an equalising effect.

Conditions for species coexistence

In absence of reproductive interference, theory predicts that, for species to coexist, they must either be equivalent competitors, or niche differences must be high enough as to overcome fitness differences (Chesson 2012), hence that:

$$\rho < \frac{k_j}{k_i} < \frac{1}{\rho} \qquad \text{(Eq. 5)}$$

In a system only driven by reproductive interference (*i.e.*, in absence of food competition, so that $\alpha_{ii} = \alpha_{jj} = \alpha_{ij} = \alpha_{ji} = 0$), the growth rates of both species reach an equilibrium when:

$$\frac{N_i}{N_j} = \frac{\beta_{ij}}{\lambda_i - 1} = \frac{\lambda_j - 1}{\beta_{ji}} \qquad \text{(Eq. 6)}$$

And coexistence is possible (though unstable, since no stabilizing effects are at play) when:

$$\frac{N_i}{N_j} = \sqrt{\frac{\lambda_j - 1}{\lambda_i - 1} \cdot \frac{1}{\gamma}} \qquad \text{(Eq. 7)}$$

Thus, coexistence is frequency-dependent (Schreiber et al. 2019): it only occurs when the relative frequency of the two species balances the ratio between their 'fecundity' differences (*i.e.*, fitness differences in absence of competition) and relative reproductive interference. In the specific case where both species have the same initial relative density ($N_i = N_j$), the species with the lower fecundity must be proportionally less affected by reproductive interference. If these conditions are not satisfied, alternative stable states can be found (*i.e.*, the system is driven by priority effects), with the species that displays a fitness advantage and/or lower sensitivity to reproductive interference excluding the other, even when outnumbered by its competitor to some extent (*i.e.*, species *i* excludes species *j* when $N_j(\lambda_j-1)/\gamma\, N_i(\lambda_i-1) < 1$).

Finally, in a system in which two species simultaneously engage in food competition and reproductive interference, predictions concerning the conditions allowing for coexistence are not available in the theoretical literature. Because of the complexity of the system, Schreiber et al (2019) provided analytical solutions only for the specific case in which both species are ecologically equivalent ($\alpha_{ii} = \alpha_{jj}$ and $\alpha_{ij} = \alpha_{ji}$). However, because this assumption is violated in our system (see Results) as in other systems (*e.g.*, in annual plant communities; Godoy et al. 2024), and it is beyond the scope of the present study to derive

analytical solutions for the general case, we opted for numerical analyses of population dynamics to predict the long-term outcome of interactions between the Tu and Tc populations studied here. Briefly, we parameterised the population model (Eq. 1) with the parameter values estimated when both types of interaction acted independently (Experiments 1 and 2) or simultaneously (Experiment 3), along with different sets of mock parameter values that are predicted to allow for coexistence (as controls) when the two species only engage in food competition or in reproductive interference, or simultaneously engage in both interactions (see Appendix S1: Figure S1). We simulated population dynamics for a wide range of initial absolute densities and relative frequencies of individuals of the two species (relative frequencies ranging from 1:9 to 9:1 individuals of each species, and total population size of 100 to 1000 individuals in increments of 100, as well as 10 and 10000 individuals). Simulations were run over 20 generations, which was the minimum number of generations necessary for the system to reach stability in all studied scenarios.

Empirical test of predicted population dynamics

To determine whether our estimated interaction coefficients enable accurate predictions of short-term population dynamics, we performed a larger-scale experiment (Experiment 4) in which we followed the relative frequencies of Tu and Tc female mites, as well as that of hybrid females, in cage populations over eight discrete generations (Figure 2). Subsequently, we adapted the mathematical model describing the dynamics of interacting species (Eq. 1) to fit the procedure used in this population cage experiment, then compared the observed proportion of each type of female with those predicted under different scenarios as described below.

Experiment 4: *Dynamics in cage populations*

Prior to the experiment, the Tu and Tc source populations were expanded by placing 3 to 4 mite-infested bean plants in a large population cage (28 x 39 x 28 cm) with 4 fresh bean plants. The experiment started 14 days later by installing 200 mated Tu females with 200 mated Tc females in a population cage (14 x 20 x 14 cm) containing 4 fresh bean plants (*ca*. 624 cm^2 total leaf area; Figure 3). This amount of food was chosen because it should trigger moderate food competition for a total of 400 females (*i.e.*, it is equivalent to a density of *ca*. 6 individuals on a *ca.* 9 cm^2 bean leaf disc in Experiment 1; see Results). Nine days later, 2 fresh bean plants were added to avoid complete resource depletion, and 5 more days were given to the offspring of both species to become adult and interact sexually. Fourteen days after the onset of the experiment, 200 mated females were randomly sampled from each of the two newest plants (hence a total of 400 females) and transferred to a new population cage to start the next generation. This entire procedure was repeated at each generation for 5 independent replicates (*i.e.*, cage populations), carried out simultaneously until complete exclusion of one of the species and of the hybrids.

The proportions of individuals of each species, as well as their hybrids, were estimated every 2 generations through phenotyping of a subset of female offspring immediately before fresh plants were added on day 9. For this purpose, 80 females undergoing their deutonymph stage were randomly sampled in the experimental cage and installed on a *ca*. 9 cm^2 bean leaf fragment to emerge as virgin adult females. After 3 days, 50 of them were installed on individual 2.5 cm^2 leaf discs placed on cotton soaked with ~0.5% etoxazole solution. Females were given three days to lay eggs before being discarded, and five days later, each disc was checked for eggs and juveniles. As ingestion of etoxazole by susceptible adult females prevents their eggs from hatching (Van Leeuwen et al. 2012), pesticide application in our system was used to identify adult females according to the following criteria: if at least one egg hatched, the female was identified as resistant to

etoxazole, hence as a Tu female; if no egg hatched, the female was identified as susceptible to etoxazole, hence as a Tc female. Finally, because *ca.* 99% of hybrid females in this system are sterile (Cruz et al. 2021), non-ovipositing females were identified as hybrids. This procedure was used to account for the fact that a hypothetical, yet unlikely, very low level of gene flow could hamper the efficiency of phenotyping based on body colouration over multiple generations (conversely to the Experiments 2 and 3, which were performed up to the production of hypothetical F2 hybrids only).

Adjustment of the mathematical model

To predict the proportion of Tu, Tc, and hybrid females in the populations at each generation, we adapted and extended the previously described model (Eq. 1) to fit the procedure used in the 'population cage' experiment.

First, the number of purebred female offspring of species *i* (N_i) produced by a given number of sampled females *i* (S_i), and introduced in a new experimental cage at each generation, is given by:

$$N_{i,\ t+1} = S_{i,t}\lambda_i\delta \cdot \frac{1}{1 + \alpha_{ii}S_{i,t} + \alpha_{ij}S_{j,t}} \cdot \frac{S_{i,t}}{S_{i,t} + \beta_{ij}S_{j,t}} \qquad \text{(Eq. 8)}$$

Note that the only difference between this equation and Eq. 1 is that $N_{i,\ t+1}$ is computed as a function of previously sampled females (S_i and S_j), instead of previously produced females (N_i and N_j).

Second, the number of hybrid female offspring (N_h) produced at each generation by the sampled purebred females of both species (S_i and S_j) as:

$$N_{h,\ t+1} = \frac{S_{i,t}\lambda_i\delta}{1+\alpha_{ii}S_{i,t}+\alpha_{ij}S_{j,t}} \cdot \theta_{ij} \cdot \left(1 - \frac{S_{i,t}}{S_{i,t}+\beta_{ij}S_{j,t}}\right) + \frac{S_{j,t}\lambda_j\delta}{1+\alpha_{jj}S_{j,t}+\alpha_{ji}S_{i,t}} \cdot \theta_{ji} \cdot \left(1 - \frac{S_{j,t}}{S_{j,t}+\beta_{ji}S_{i,t}}\right) \qquad \text{(Eq. 9)}$$

In this equation, the number of hybrid females produced by each species of sampled purebred females is given by the left and right terms of the expression, respectively. In both cases, this

number corresponds to the number of female offspring becoming hybrids instead of purebred females due to reproductive interference. Because, in Eqs. 1 and 8, the 'reproductive interference factor' represents the proportion of female offspring 'escaping' reproductive interference (*i.e.*, successfully developing as purebred female), the opposite of this proportion represents the proportion of female offspring 'affected' by reproductive interference, hence (potentially) developing as hybrid females. However, not all female offspring produced by heterospecifically-mated mothers become hybrids in our system, as some can develop as males instead (likely due to fertilization failure; Cruz et al. 2021). Thus, to account for sex-ratio distortion in heterospecific crosses, we used an additional parameter θ, which corresponds to the proportion of hybrid females produced by a purebred female *i* mated with a male *j* (θ_{ij}), or by a purebred female *j* mated with a male *i* (θ_{ji}). Finally, note that Eq. 8 and Eq. 9 assume that food competition only occurs among purebred females, not with hybrid females. Indeed, as these latter are mostly sterile, they consume very few resources (personal observation). Moreover, the model assumes that purebred females producing hybrids are equally affected by food competition as those whose offspring escape reproductive interference (*i.e.*, the food competition factors are identical in both equations; but see different tested scenarios below).

Finally, the proportion of Tu, Tc, and hybrid female offspring (P_i, P_j, and P_h, respectively) in the population at each generation was computed as:

$$P_{i,\,t} = \frac{N_{i,t}}{N_{i,t}+N_{j,t}+N_{h,t}} \qquad \text{(Eq. 10)}$$

And the number of Tu, Tc, or hybrid females randomly sampled (S_i, S_j, and S_h, respectively) in the population to start the next generation was computed as:

$$S_{i,\,t} = P_{i,t} \cdot S_{tot} \qquad \text{(Eq. 11)}$$

Where S_{tot} corresponds to the total number of female offspring sampled in each experimental cage to start the next generation ($S_{tot} = 400$ in our experiment).

Parameterisation of the model and comparison of predicted vs. observed dynamics

To determine the mechanisms that could best explain the observed dynamics in our population cage experiment, we parameterised the model, in which food competition and reproductive interference are always simultaneously at play, following different scenarios. The first two scenarios aimed at determining whether using parameter values estimated when food competition and reproductive interference acted separately (Experiments 1 and 2) *v*s. simultaneously (Experiment 3) significantly affected the ability of the model to accurately predict population dynamics. Then, building upon the second scenario (estimates from Experiment 3), we considered a third scenario, where hybrid females were less sensitive to food competition than purebred females. The model so far assumed the same levels of food competition between the offspring of purebred females that suffered from, or escaped, reproductive interference. However, this assumption may be unrealistic if hybrid females require fewer resources than purebred females during their development. Note that although not evaluated, we repeatedly observed very low levels of leaf damage in patches hosting developing and adult hybrid females relative to those hosting only purebred females, even at similar female densities. Thus, to determine whether a lower sensitivity of hybrid females to food competition would improve the predictions of the model, we divided all α's in Eq. 9 by a scale factor, testing every integer ranging from 2 (*i.e.*, hybrids suffering half as much from food competition as purebred females) to 30. To determine which scale factor of α enables the best model predictions as compared to the observed data, we used a binomial GLM (generalized linear model with a binomial error structure; package *lme4* in R), in which we fitted the observed proportions of individuals of each species and of the hybrids as the response variable, and the predicted proportions of offspring as fixed explanatory variable. We then compared the fit of each set of predictions based on the R-squared values, intercepts

and regression coefficients of the linear regressions. For all tested scenarios, the model was adapted to account for the fact that no reproductive interference occurred, thus no hybrids were produced, during the first generation of the experiment. Values taken for θ_{ij} and θ_{ji} were the inverse of the MD_{corr} indexes in Cruz et al (2021), which are estimates of the proportion of offspring developing as a haploid male instead of a female in interspecific crosses between our source populations. Upper and lower confidence intervals for the observed proportions of Tu, Tc, and hybrid females were calculated from the data, whereas those for the predicted proportions were calculated as the upper and lower limits that could be obtained given all possible combinations between the confidence intervals of each parameter of the model.

Results

Food competition affects the strength and symmetry of reproductive interference. Overall, all three experiments, in which females of each species were exposed to competition for food only (Experiment 1), reproductive interference only (Experiment 2), or both (Experiment 3), revealed subtle differences in population growth (Appendix S1: Section S2), but large asymmetries in species intrinsic growth rates and interaction coefficients. First, intrinsic growth rates consistently differed between the two species in all 3 experiments: in the absence of competitors for food and heterospecific males, Tc females produced, on average, more offspring than Tu females (estimated coefficients $\lambda_{Tc} > \lambda_{Tu}$ by *ca.* 25%; Figures 4 and 5a; Appendix S1: Table S2). Second, when species competed only for food (Experiment 1), both species were more sensitive to heterospecific than to conspecific competitors (estimated competition coefficients $\alpha_{TcTu} > \alpha_{TcTc}$ by *ca.* 8%, and $\alpha_{TuTc} > \alpha_{TuTu}$ by *ca.* 40%), but Tc females were generally more sensitive to competition than Tu females, regardless of competitor identity ($\alpha_{TcTc} > \alpha_{TuTu}$ by *ca.* 140% and $\alpha_{TcTu} > \alpha_{TuTc}$ by *ca.* 86%;

Figures 4a and 5b; Appendix S1: Table S2). Conversely, when the two species were only exposed to reproductive interference (Experiment 2), Tu females suffered more from the presence of heterospecifics than Tc females (estimated reproductive interference coefficients $\beta_{TuTc} > \beta_{TcTu}$ by *ca*. 276%; Figures 4b and 5c; Appendix S1: Table S2), suggesting that a trade-off between the two types of interaction could occur when both interactions are at play.

Finally, when both interactions occurred simultaneously (Experiment 3), we found lower intrinsic growth rates (λ) and strength of food competition (α) than in the previous experiments, but both species were similarly affected by these changes: Tc females still produced more offspring ($\lambda_{Tc} > \lambda_{Tu}$ by *ca*. 29%) and were more affected by food competition ($\alpha_{TcTc} > \alpha_{\mathrm{TuTu}}$ by *ca*. 200% and $\alpha_{\mathrm{TcTu}} > \alpha_{TuTc}$ by *ca*. 167%) than Tu females (Figure 5a,b; see also Appendix S1: Table S3 *vs.* S2). However, we found a drastic change in the strength and symmetry of reproductive interference (β): the sensitivity of Tc females to the presence of heterospecifics slightly increased and that of Tu females strongly decreased, such that Tu females switched from suffering more to suffering less than Tc females from reproductive interference ($\beta_{TuTc} < \beta_{TcTu}$ by *ca*. 40%; Figure 5c). Hence, when simultaneously engaged in food competition and reproductive interference, Tu suffered less than Tc from both these interactions, and its population growth overall became higher than that of Tc from the second generation onwards (Figure 4c).

The change in strength of reproductive interference due to food competition impacts theoretical predictions for coexistence

When we parameterised the population model with coefficients estimated from the small-scale experiment in which food competition acted alone (Experiment 1), we found negative niche differences in two replicate populations, as both species limited their competitor's growth more than their own (*i.e.*, each species was more affected by heterospecifics than by

conspecifics). This should promote positive density-dependent effects (*i.e.*, priority effects), whereby the species with higher relative abundance excludes the one with lower abundance. However, for three of the replicates and on average, we also observed large fitness differences between the two species, which rather lead to the prediction of Tu excluding Tc regardless of the relative abundance of the two species (Figure 6a).

In absence of food competition, reproductive interference is expected to lead to priority effects (Schreiber et al. 2019), such that the outcome should depend not only on the combination of the relative strength of reproductive interference between the two species and their relative 'fecundity difference', but also on their initial relative frequency (*i.e.*, the ratio between abundances of the two species). Indeed, different combinations of relative reproductive interference and fecundity differences determine different threshold frequencies above which one species is favoured over the other (Eq. 7; Figure 6b). In our system (*i.e.*, with the parameter values we measured experimentally), we predicted that Tc should be favoured not only when both species are initially at equal relative frequency as in our experimental conditions, but also when Tc is less abundant than Tu (*i.e.*, for all data and 3 replicate populations, it should be excluded only when its frequency drops below 33%; and below 50% for another replicate; Figure 6b). Thus, food competition and reproductive interference are predicted to lead to opposite outcomes when acting separately in our system.

Then, to determine how both interactions would jointly affect long-term population dynamics, we performed simulations with interaction strengths measured either when food competition and reproductive interference acted separately (using parameters estimated from Experiment 1 and 2), or when they acted jointly, hence when the strength and symmetry of reproductive interference changed in the presence of food competition (using parameters estimated from Experiment 3). In both cases, and similarly to our predictions for when reproductive interference acted alone, we found priority effects, with the identity of the

species that excludes the other depending on initial conditions (both relative frequency and absolute density in this case; Figure 6c,d). However, accounting for changes in the strength and symmetry of reproductive interference under food competition drastically altered the threshold frequency determining which of the two species will be dominant (see Figure 6d *vs.* Figure 6c). When considering independent effects of the two interactions, we found that reproductive interference should counterbalance the asymmetries in food competition, with the weakest competitor for food (Tc) excluding the strongest competitor (Tu) when it is more abundant (threshold close to 50% on average, Figure 6c; see also Appendix S1: Figures S2 and S3 for variation among replicate populations). In contrast, when the strength of reproductive interference is modulated by food competition, its buffering effect strongly decreases. Under this scenario, we found that Tu should exclude Tc except if its frequency drops below 20% on average (Figures 6d; see Appendix S1: Figures S2 and S4 for variation among replicate populations).

The model accurately predicts dynamics in cage populations

To validate our hypothesis that accounting for changes in the strength of reproductive interference under food competition improves the accuracy of model predictions, we compared the dynamics observed in the population cage experiment (Experiment 4) with those predicted by different fittings of the population model. In the experiment, all replicate populations showed consistent dynamics, with all Tc and hybrid females being excluded by the 6th and 8th generations, respectively (Figure 7a; Appendix S1: Table S4). A similar pattern was also predicted by the model, both when accounting for independent effects of food competition and reproductive interference and when accounting for an asymmetrical reduction in reproductive interference under food competition. However, simulated population dynamics considering interaction strengths measured independently showed

extremely large variance, indicating a strong uncertainty concerning which species should exclude the other, whereas predictions showed much lower variance when based on interaction strengths measured when food competition and reproductive interference acted jointly, with Tu always excluding Tc (R^2 = 0.89; Appendix S1: Figure S5). Thus, these results highlight the importance of incorporating the interplay between the effects of the two interactions to better predict population dynamics. Nevertheless, even when food competition and reproductive interference are acting simultaneously, the model predicted lower proportions of hybrids (from generations 2 to 6), and a faster exclusion of Tc as compared to experimental observations (Appendix S1: Figure S5b *vs.* Figure 7a). Additional simulations in which we varied the sensitivity of hybrid females to food competition as compared to purebred females (Appendix S1: Table S5) revealed that hybrid females should be at least 15 times less sensitive to food competition than purebred ones (Figure 7b) to obtain the best fit between observed and predicted dynamics (R^2 = 0.97; Figure 7c).

Discussion

This study provides the first empirical evidence that the strength of reproductive interference can be affected by competition for food, such that the combined effects of the two types of interaction on population dynamics and competitive outcomes cannot be predicted by their independent action. Specifically, we show that, in absence of reproductive interference, *T. urticae* (Tu) is the stronger competitor for food and it is therefore predicted to invariably exclude *T. cinnabarinus* (Tc). Conversely, in absence of competition for food, Tu suffers more than Tc from reproductive interference and is thus predicted to be excluded, except if it is at least twice as abundant initially (due to positive-frequency dependence). Whereas a trade-off between competition for food and reproductive interference is, in the literature, predicted to enable stable coexistence (Kishi and Nakazawa 2013, Schreiber et al. 2019), our

simulations instead show that positive-frequency dependence driven by reproductive interference should remain when both types of interaction are simultaneously at play (the identity of the species that persists hinges upon their frequencies), be it with independent or interacting effects. When both types of interactions are assumed to have independent effects, food competition should balance out the advantage conferred to Tc by reproductive interference, with both species having the same likelihood to persist at an even initial frequency. However, when the strength of reproductive interference can be modulated by food competition, the superior competitor for food, Tu, becomes the least sensitive to reproductive interference. Our predictions when accounting for such a change indicate the exclusion of Tc in all tested scenarios, except when it is initially extremely abundant relative to its competitor, Tu. The results obtained from an independent population cage experiment were largely compatible with this prediction, with Tc being systematically excluded when both species started at an even initial frequency. Our results therefore provide two straightforward lessons: first, food competition modulates reproductive interference, suggesting non-independent effects of these interactions on species coexistence, and second, modern coexistence theory (Schreiber et al. 2019, Yamamichi et al. 2023) is a suitable framework to predict their effects on the population dynamics and competitive outcomes of closely-related species.

Many studies have investigated the strength of reproductive isolation between Tu and Tc (Murtaugh and Wrensch 1978, Sugasawa et al. 2002, Cruz et al. 2021, 2024, Xue et al. 2023). Still, reproductive interference and its consequences for population dynamics have barely been considered (but see Murtaugh and Wrensch 1978). Here, in absence of competition for food, we found asymmetric reproductive interference between Tc and Tu, a result that is congruent with previous findings on pre- and post-mating reproductive isolation between the two populations used in this study (Cruz et al. 2021, 2024). This is also

compatible with earlier studies using Chinese populations of these species, both in the laboratory (Lu et al. 2017) and in the field (Lu et al. 2018), in which Tc consistently displaces Tu when at an even initial frequency. However, these studies also revealed that this outcome is reversed in the presence of pesticides, due to stronger pesticide resistance in Tu. In fact, any abiotic or biotic factor that affects the relative fitness of these two species, such as temperature (Riahi et al. 2013, Gotoh et al. 2015, Lu et al. 2017, Zou et al. 2018), host plants (Tomczyk et al. 1995, Witul and Kielkiewicz 1999, Huo et al. 2021), or natural enemies (Takabayashi et al. 2000), should also play a role in determining their persistence in natural settings. Yet, no study so far aimed at disentangling the relative role of such factors in shaping competitive outcomes, let alone the role of different intrinsic factors.

Previous theoretical work predicted that food competition and reproductive interference acting in opposite directions (*i.e.*, under a trade-off) may favour stable coexistence (Kishi and Nakazawa 2013). Although we found that competition for food balances out the advantage conferred by reproductive interference to Tc in the current study, coexistence was not predicted in any scenario. Instead, simulated population dynamics revealed that positive frequency dependence driven by reproductive interference occur even in the presence of food competition. This apparent discrepancy with previous predictions probably lies in the fact that we found negative niche differences in our system, a scenario yet unexplored despite several theoretical and empirical studies in the coexistence literature indicating that priority effects should be a common outcome of ecological interactions ((Ke and Letten 2018, Song et al. 2021, Spaak et al. 2021, Fragata et al. 2022) . Recent theoretical work suggests that changes in food competition intensity mediated by reproductive interference (*i.e.*, due to behavioural and/or evolutionary changes (Kishi and Tsubaki 2014, Noriyuki and Osawa 2016, Ruokolainen and Hanski 2016) could drive coexistence, by switching initially negative niche differences to positive (Yamamichi et al. 2023). However,

our experiments were specifically designed to test the opposite effect, that is, a change in the strength of reproductive interference in response to food competition, a possibility that has not yet been investigated, neither theoretically nor empirically.

An experimental test of the combined effects of reproductive interference and competition for food revealed that these are not equivalent to the combination of their independent effects. Whereas the intrinsic growth rate of both species and their sensitivity to competition were overall consistently lower due to unknown environmental effects (Experiment 3), the intensity of reproductive interference became similar for both species in the presence of food competition, while it was stronger for Tu than for Tc in its absence (Experiment 2). This could be explained by a positive correlation between traits involved in each of these interactions, or by the same traits being involved in (and affected by) both interactions (*e.g.*, body size, development time, locomotor activity, etc; Okuzaki et al. 2010, Maan and Seehausen 2011). For instance, food competition may affect trait values more severely in the offspring of the lower competitor, which in turn become more affected by reproductive interference (*e.g.*, because they produce mating signals of lower quality, become less competitive for mates, produce fewer/smaller/less mobile gametes, etc.). Alternatively, a change in the strength of reproductive interference in the presence of food competition could arise because the latter affects density, assuming that the strength of reproductive interference (β) varies with total population density (in the same way as the strength of food competition could change in the presence of reproductive interference if the α values are density-dependent, as proposed by Yamamichi et al.; 2023). However, for this hypothesis to explain our results, the β values would have to have a species-specific sensitivity to density.

Irrespective of the mechanism underlying the interplay between competitive and sexual interactions, our simulations revealed that changes in the strength of reproductive interference in response to food competition (as compared to its absence) lead to a strong

increase in the threshold initial frequency under which the lower competitor for food (Tc) cannot persist. Then, using independent data for model parameterisation and validation, we demonstrated the importance of accounting for this effect to accurately predict population dynamics, thereby further highlighting the importance of understanding how trophic and sexual interactions affect each other. This last piece of work also shows that a simple model capturing the demographic effects of reproductive interference with only two parameters (β and θ; *cf.* Methods), hence not explicitly modelling each of its underlying mechanisms (or 'fitness components'; Kyogoku 2015), can generate very accurate predictions for the dynamics of two species in experimental cage populations. This ability to predict the system dynamics was further improved when accounting for the production of hybrid females that are less sensitive to food competition. While the ecological impact of hybrids is widely studied in the context of adaptation (Seehausen 2004, Gow et al. 2007), the demographic impact of 'unfit' hybrids on parental species has been largely overlooked. In fact, an increased frequency of hybrids in parental populations (as predicted and observed here) could generate unpredicted changes in population dynamics, especially if they strongly compete for food. However, consistent with observations made in our laboratory, our analyses instead revealed that hybrid females in our system may require much less resources than purebred females, possibly because most of them do not produce eggs (Cruz et al. 2021).

Our study is a first but rigorous attempt to delve into the complexity of evaluating experimentally ecological interactions taking into account competition for food and for mates. We have done the most we could perform given our logistical limitations, but larger studies could address additional aspects not covered in our study to fully comprehend their complex interactions. For instance, we could design experiments to determine whether the relationship between each species' sensitivity to intraspecific and interspecific competition also changes in the presence of reproductive interference, or whether and how reproductive

interference affects the intensity of food competition (*i.e.*, the opposite of what we tested here) as proposed by Yamamichi et al. (2023). Furthermore, the model could be developed to account for within-species negative reproductive interactions, such as male harassment, which may also play an important role in determining the strength of reproductive interference between populations (Kyogoku and Sota 2017). Such a theoretical development could ultimately allow for an expansion of the concept of niche differences not only to trophic, but also to sexual, interactions, leading to an even more powerful integration into a general coexistence framework (Gómez-Llano et al. 2021).

In conclusion, our study reveals that food competition can affect the strength of reproductive interference, with significant consequences for the dynamics of competing species. Given that reproductive interference is expected to often occur between species that compete for food (Servedio and Hermisson 2020), such outcome is likely a general feature of many ecological systems involving taxa that incidentally engage in sexual interactions (Weber and Strauss 2016), and it may have major consequences for the study of ecological coexistence (Germain et al. 2018, Gómez-Llano et al. 2021). Indeed, our study shows that addressing the effect of each interaction type alone might be insufficient to accurately predict population dynamics. This is because the co-occurrence of both types of interactions can change the shape of the trade-off between reproductive interference and competition for food, unbalancing the priority of one species over another in systems with negative niche differences (while potentially hampering coexistence in systems with positive niche differences). Understanding this interplay between feeding and sex is also crucial for speciation research, as the likelihood and duration of coexistence between closely-related species will determine the opportunities for reinforcement. Our findings may thus have far-reaching consequences for a recently growing field at the interface between speciation and coexistence theory (Germain et al. 2018, Kyogoku and Kokko 2020, Kyogoku and

Wheatcroft 2020, Grether et al. 2020, Boussens-Dumon and Llaurens 2021). Finally, despite species interactions being intensively studied for more than a century as a major determinant of species distribution and competitive outcomes, our results collectively show that we are still in the infancy of understanding how different mechanisms interact to determine the population dynamics of interacting species. Combining theoretical and empirical approaches is key to unveil how different types of interactions jointly impact species coexistence or exclusion.

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Author Contributions

MC, VS, OG, SM and FZ designed the experiments. MC collected data. MC and FZ performed modelling work and analysed data with advises and scripts from IF and OG. MC and FZ wrote the first draft of the manuscript, and all authors contributed substantially to revisions.

Conflict of interest

The authors declare that they have no conflict of interest with the content of this article.

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Figure Captions

Figure 1. Typical body colour of (A) *T. urticae*, (B) *T. cinnabarinus*, and (C) F1 hybrid females.

Figure 2. Experimental procedures used to quantify the effects of food competition and/or reproductive interference on offspring production. A - Food competition in absence of reproductive interference was measured by placing females mated with conspecific males on food patches where their juveniles would develop. The two different types of competition, intraspecific (left) and interspecific (right), are shown, with Tc focal females as an example. B - Reproductive interference in absence of food competition was measured across two consecutive generations (G0 to G2) to account for the costs resulting from the production of sterile F1 hybrids. Common mating patches were used for virgin females and males of the two species, but individual food patches were used for ovipositing females and developing juveniles. C - The combined effect of food competition and reproductive interference was measured across three consecutives generations (G1 to G3) by using common (food and mating) patches for the entire life cycle of the individuals. As females mated with males from their own population were used at the onset of the experiment, the first generation (G0 to G1) allowed re-estimating food competition in absence of reproductive interference (to correct for testing time). The next generations of offspring (G2 to G3) allowed re-estimating reproductive interference in the presence of food competition. FC: food competition; RI: reproductive interference; Red: *T. cinnabarinus* (Tc) females; Blue: *T. urticae* (Tu) females; Purple: hybrid females.

Figure 3. Experimental procedure used to follow the dynamics of both species and their hybrids in cage populations. Common plants were used for the entire life cycle of individuals

of both species. This procedure enabled food competition between females and developing juveniles, as well as reproductive interference between virgin adult individuals of both species. The figure depicts the initial installation of mated females (G0), the transfer of 400 randomly sampled mated females to start each subsequent discrete generation (G1 onward), as well as random sampling and phenotyping of 50 virgin females every two generations. FC: food competition; RI: reproductive interference; Red: *T. cinnabarinus* (Tc) females; Blue: *T. urticae* (Tu) females; Purple: hybrid females.

Figure 4. Three experiments teasing apart the single and combined effects of food competition and reproductive interference on the per capita growth rate of each species. In A, lines represent the average number of females produced daily under increasing densities of either conspecific or heterospecific competitors (dark and light colours, respectively) on a common food patch, but in absence of reproductive interference. Shaded areas represent the 95% confidence intervals. In B and C, violin plots show the distribution of the mean number of females produced daily in the absence or presence of heterospecific individuals (dark and light colours, respectively) on a common mating patch, but in absence of food competition B; or when individuals shared the same food and mating patches over 3 generations C. Within each violin plot, boxplots represent the median and 1st and 3rd quartiles, and whisker length corresponds to 1.5 times the interquartile range. In all panels, individual points show the mean number of females produced daily by a single female (per capita) for each of 5 different replicate populations, and *T. cinnabarinus* (Tc) and *T. urticae* (Tu) are displayed in red and blue, respectively.

Figure 5. The strength and symmetry of reproductive interference change in the presence of food competition. A - Intrinsic growth rate (λ) estimated as the mean number of

daughters produced daily by single isolated females, averaged for Experiments 1 and 2, or measured in Experiment 3. B - Per capita effect of food competition (α) estimated from Experiments 1 and 3. C - Per capita effect of reproductive interference (β) estimated from Experiments 2 and 3. In all panels, dots show parameter values (± 95% confidence intervals) estimated across 5 replicate populations, dark and light colours represent within- and between-species effects, respectively, and parameter values for *T. cinnabarinus* (Tc) and *T. urticae* (Tu) are displayed in red and blue, respectively.

Figure 6. Changes in the strength of reproductive interference under food competition affect the predicted competitive outcomes. A - Predicted outcomes depending on the niche and fitness differences between species when populations only compete for food. The two solid black lines define the space in which species can coexist (grey area) or enter alternative stable states (priority effects; white area). Otherwise, the species with higher fitness will exclude the other. B - Predicted outcomes depending on the relative strength of reproductive interference and fecundity differences when populations only interact during reproduction. Due to priority effects, coexistence is possible if reproductive interference equalises the fecundity differences between populations at equal initial frequency of both species (solid line: $N_{Tu}/N_{Tc} = 1$), or if the initial frequency of the inferior competitor is sufficiently high to compensate for the combined effects of its fecundity disadvantage and/or higher sensitivity to reproductive interference (dotted lines from left to right: N_{Tu}/N_{Tc} = 2/1, 4/3, 3/4, and 1/2). C and D - Predicted outcomes for different initial absolute densities and relative frequencies of the two species using interaction strength measured when both types of interaction occurred separately or when they acted jointly, respectively. In both panels, heat maps show that the identity of the remaining species after 20 generations depends on the initial conditions due to priority effects. In all panels, but only for equal initial frequency of the two species in panel

B, and for predictions based on all data in panels C and D, the red area defines the space in which *T. cinnabarinus* (Tc) excludes *T. urticae* (Tu), and the blue area defines the space in which Tu excludes Tc. In A and B, grey circles and black diamonds are means (± 95% confidence intervals) for each replicate population and for all data, respectively. In C and D, dashed grey lines and solid black lines delimit the space in which each species excludes the other, using parameters estimated with each replicate population and with all data, respectively (but note that negative parameter values and confidence intervals are not displayed).

Figure 7. The model predictions accurately fit the dynamics observed in cage populations. A - Observed proportions of *Tetranychus urticae* (Tu), *T. cinnabarinus* (Tc), and hybrid females in experimental cage populations over 8 generations. Dots show the observed proportions in each replicate population, and coloured lines and shaded areas show the averages and associated 95% confidence intervals across all replicate populations. B - Predicted proportions of females assuming that food competition affects the strength of reproductive interference (parameters estimated from Experiment 3 across all replicate populations) and that hybrid females are 15 times less affected by food competition than purebred females. Coloured lines and shaded areas show predicted proportions and associated upper and lower limits that could be obtained given all possible combinations between the 95% confidence intervals of each parameter of the model. C - Linear regression between observed and predicted proportions. Dots show each replicate population, the solid line shows the linear regression between observed and predicted proportions, and the dashed line shows the 1:1 relationship between values in the two axes. Blue: Tu females; Red: Tc females; Purple: hybrid females.

Figures

Figure 1

Figure 2

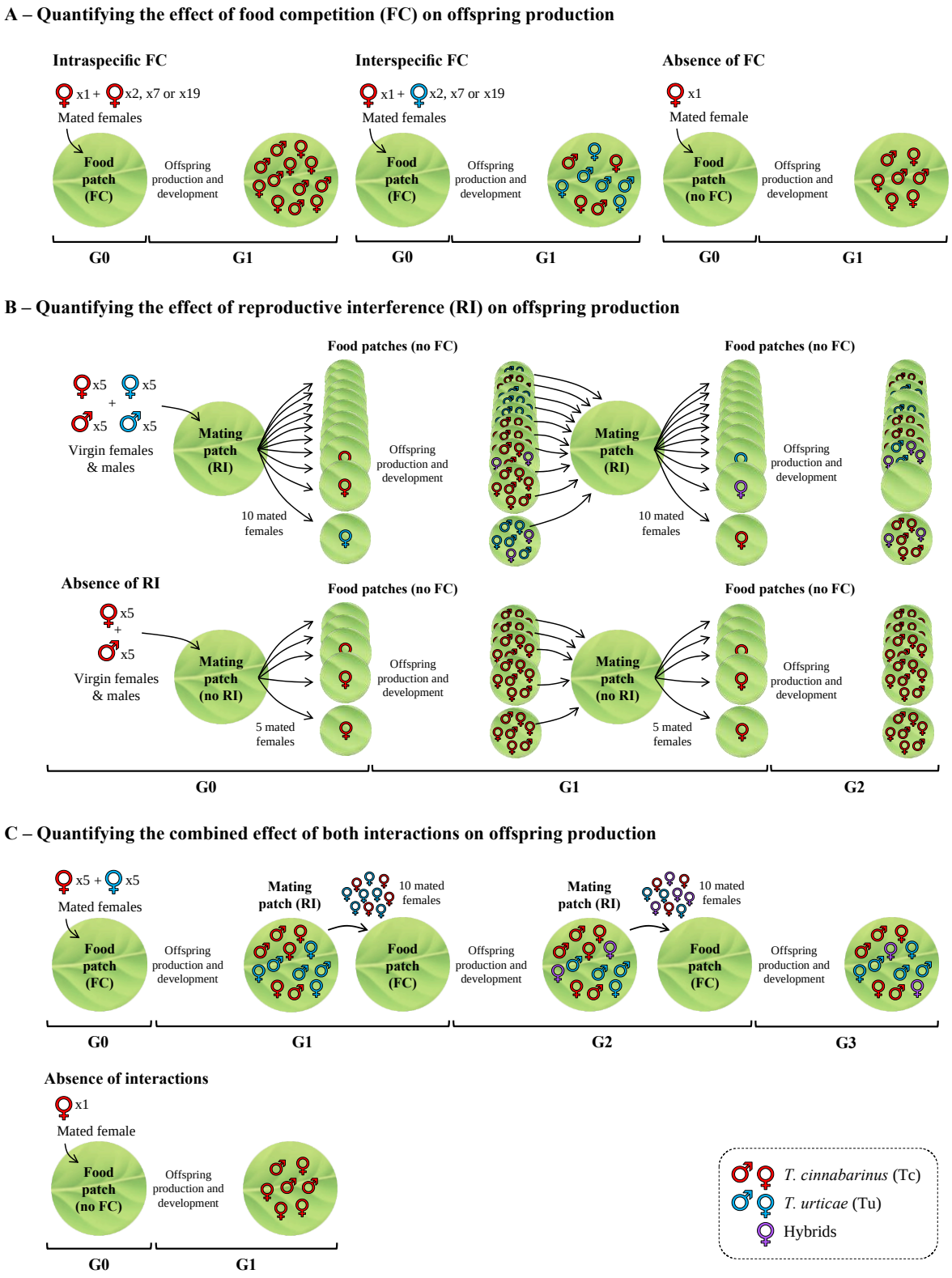

A – Quantifying the effect of food competition (FC) on offspring production
Intraspecific FC
x1 + x2, x7 or x19
Mated females
Food patch (FC)
Offspring production and development
G0
G1
Interspecific FC
x1 + x2, x7 or x19
Mated females
Food patch (FC)
Offspring production and development
G0
G1
Absence of FC
x1
Mated female
Food patch (no FC)
Offspring production and development
G0
G1
B – Quantifying the effect of reproductive interference (RI) on offspring production
x5 x5 + x5 x5
Virgin females & males
Mating patch (RI)
10 mated females
Food patches (no FC)
Offspring production and development
Absence of RI
x5 + x5
Virgin females & males
Mating patch (no RI)
5 mated females
Food patches (no FC)
Offspring production and development
G0
G1
G2
C – Quantifying the combined effect of both interactions on offspring production
x5 + x5
Mated females
Food patch (FC)
Offspring production and development
Mating patch (RI)
10 mated females
G0
G1
G2
G3
Absence of interactions
x1
Mated female
Food patch (no FC)
Offspring production and development
G0
G1
T. cinnabarinus (Tc)
T. urticae (Tu)
Hybrids

Figure 3

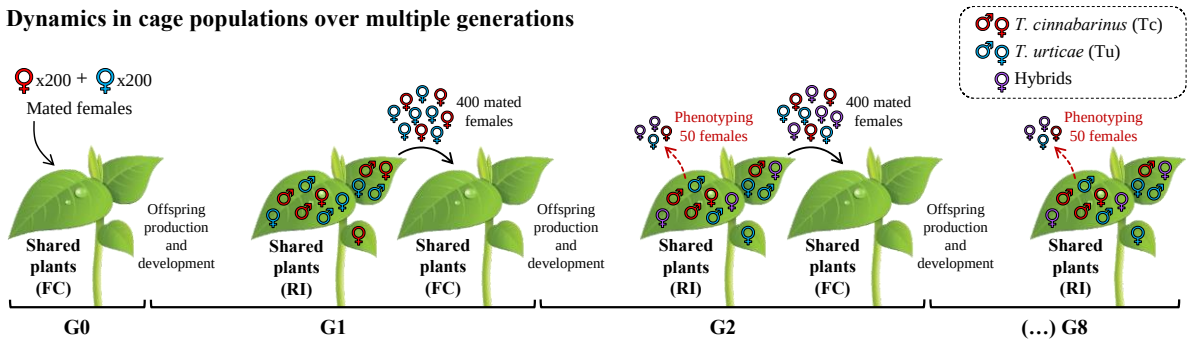

Dynamics in cage populations over multiple generations
T. cinnabarinus (Tc)
T. urticae (Tu)
Hybrids
x200 + x200
Mated females
Shared plants (FC)
Offspring production and development
400 mated females
Shared plants (RI)
Shared plants (FC)
Offspring production and development
Phenotyping 50 females
400 mated females
Shared plants (RI)
Shared plants (FC)
Offspring production and development
Phenotyping 50 females
Shared plants (RI)
G0
G1
G2
(…) G8

Figure 4

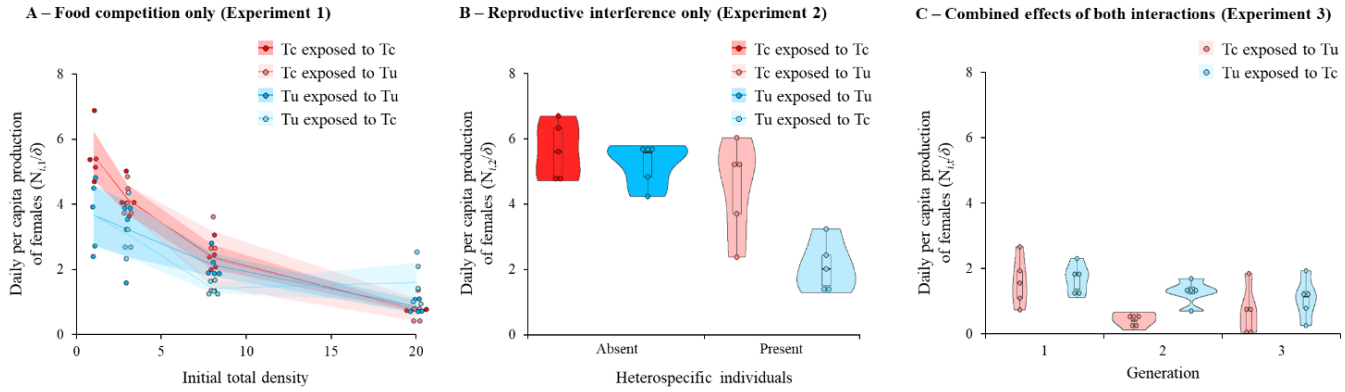

A – Food competition only (Experiment 1)
Tc exposed to Tc
Tc exposed to Tu
Tu exposed to Tu
Tu exposed to Tc
Daily per capita production of females ($N_{i,t}/\delta$)
Initial total density
B – Reproductive interference only (Experiment 2)
Absent
Present
Heterospecific individuals
C – Combined effects of both interactions (Experiment 3)
Generation

Figure 5

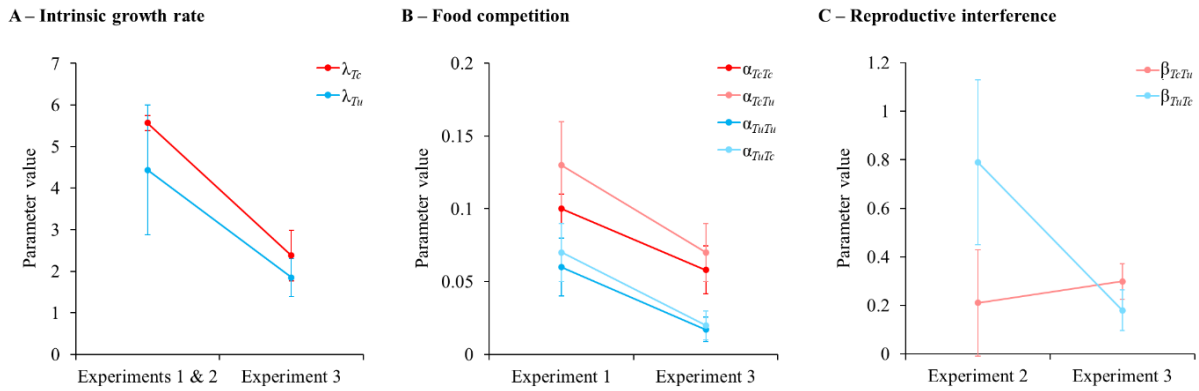

A – Intrinsic growth rate
Parameter value
0
1
2
3
4
5
6
7
Experiments 1 & 2
Experiment 3
λTc
λTu
B – Food competition
Parameter value
0
0.05
0.1
0.15
0.2
Experiment 1
Experiment 3
αTcTc
αTcTu
αTuTu
αTuTc
C – Reproductive interference
Parameter value
0
0.2
0.4
0.6
0.8
1
1.2
Experiment 2
Experiment 3
βTcTu
βTuTc

Figure 6

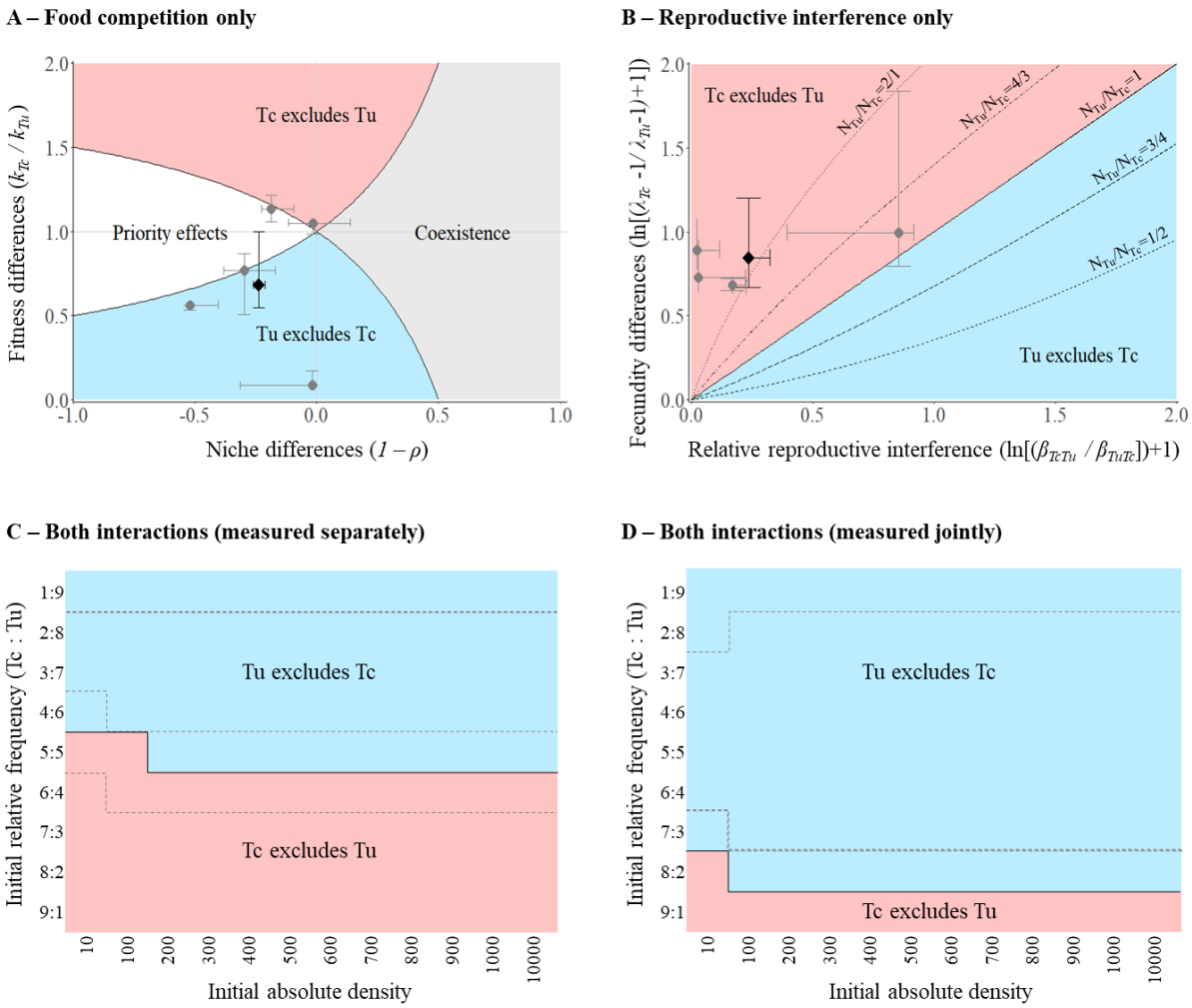
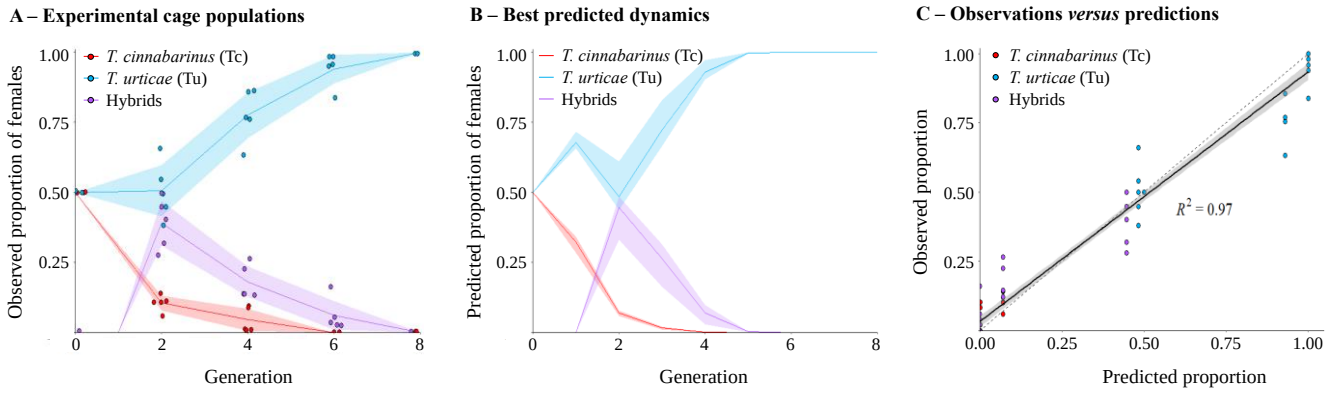

A – Food competition only
Tc excludes Tu
Priority effects
Coexistence
Tu excludes Tc
Fitness differences (k_{Tc} / k_{Tu})
Niche differences ($1-\rho$)
B – Reproductive interference only
Tc excludes Tu
N_{Tu}/N_{Tc}=2/1
N_{Tu}/N_{Tc}=4/3
N_{Tu}/N_{Tc}=1
N_{Tu}/N_{Tc}=3/4
N_{Tu}/N_{Tc}=1/2
Tu excludes Tc
Fecundity differences (ln[(λ_{Tc} -1/ λ_{Tu}-1)+1])
Relative reproductive interference (ln[(β_{TcTu} / β_{TuTc}])+1)
C – Both interactions (measured separately)
Tu excludes Tc
Tc excludes Tu
Initial relative frequency (Tc : Tu)
Initial absolute density
D – Both interactions (measured jointly)
Tu excludes Tc
Tc excludes Tu
Initial relative frequency (Tc : Tu)
Initial absolute density

Figure 7


A – Experimental cage populations
Observed proportion of females
1.00
0.75
0.50
0.25
T. cinnabarinus (Tc)
T. urticae (Tu)
Hybrids
0
2
4
6
8
Generation
B – Best predicted dynamics
Predicted proportion of females
1.00
0.75
0.50
0.25
T. cinnabarinus (Tc)
T. urticae (Tu)
Hybrids
0
2
4
6
8
Generation
C – Observations versus predictions
Observed proportion
1.00
0.75
0.50
0.25
T. cinnabarinus (Tc)
T. urticae (Tu)
Hybrids
R2 = 0.97
0.00
0.25
0.50
0.75
1.00
Predicted proportion