Genetic variance in spider mites reveals trade-offs: lines with higher growth rate suffer stronger competition but exert stronger reproductive interference

Authors

Miguel A. Cruz[1], Flore Zélé[2], Raquel Gaspar[1], Ricardo Santos[1], Leonor R. Rodrigues[1,3], Vitor C. Sousa[1,4], Sara Magalhães[1,4,5]

Affiliations

[1]Centre for Ecology, Evolution and Environmental Changes (CE3C) & CHANGE - Global Change and Sustainability Institute, Faculdade de Ciências da Universidade de Lisboa, Lisboa, Portugal

[2]ISEM, Université de Montpellier, CNRS, IRD, Montpellier, France

[3]Departamento de Biologia Vegetal, Faculdade de Ciências da Universidade de Lisboa, Lisboa, Portugal

[4]Departamento de Biologia Animal, Faculdade de Ciências da Universidade de Lisboa, Lisboa, Portugal[5]Wissenschaftskolleg zu Berlin, Institute for Advanced Study, Berlin, Germany.

Correspondence

Sara Magalhães (snmagalhaes@ciencias.ulisboa.pt)

Acknowledgements

We are grateful to Juan Lopez and Arianna Thomas-Cabianca for assistance with the maintenance of cultures and inbred lines and in data collection, and to Inês Fragata, João Picão Osório and Sofia Henriques for advice regarding the statistical analyses. This work was funded by an ERC Consolidator Grant (COMPCON, GA 725419) attributed to SM and by Fundação para a Ciência e Tecnologia (FCT) through CE3C - Centre for Ecology, Evolution and Environmental Changes unit funding (UIDB/00329/2025). MAC, VCS and SM acknowledge financial support provided by the FCT project GeneticBarriers (2022.03475.PTDC; PI: Alexandre Blanckaert). MAC was funded through an FCT PhD fellowship (SFRH/BD/136454/2018) and VCS and LRR by FCT contracts (CEECINST/00032/2018/CP1523/CT0008 and CEECIND/CP1715/CT0007). This is contribution ISEM-XXX of the Institute of Evolutionary Science of Montpellier (ISEM). Funding agencies did not participate in the design or analysis of experiments. For the purpose

of Open Access, a CC-BY 4.0 public copyright licence has been applied by the authors to the present document and will be applied to all subsequent versions up to the Author Accepted Manuscript arising from this submission.

Conflict of interest

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

Author contributions

FZ and SM conceived the study. FZ, MAC, SM and VCS designed the growth rate, competition for food and reproductive interference experiments. LRR designed the body size experiments. MAC, RG and RS collected the data. MAC analysed the data with significant input from FZ. FZ, SM and VCS supervised MAC. LRR supervised RG and RS. MAC and SM wrote the manuscript, with input from all authors. SM provided funding. All authors have approved the final version for publication.

Data availability

All datasets and R scripts used in this study are available at Zenodo (doi: 10.5281/zenodo.16753845).

Abstract

1. Within-species individual variation has been gaining increased attention in ecological studies of species interactions. Yet, these studies rarely consider genetic variation and potential genetic correlations among traits. Moreover, traits beyond those associated with trophic interactions are mostly overlooked. Filling these gaps is important to fully understand the relevance of trait variation in both the ecology and evolution of species interactions.

2. We addressed this using inbred lines of *Tetranychus cinnabarinus*, a spider mite species that often engages into antagonistic interactions with its congeneric *T. urticae*. Specifically, we measured (a) the intrinsic growth rate and the sex ratio in the absence of any interaction; (b) food competition against a density gradient of con- or heterospecific competitors in absence of reproductive interference; (c) reproductive interference in absence of food competition; and (d) body size of adult females and males.

3. We found significant genetic variance (*i.e.*, broad-sense heritability) among lines for all variables except for the effect of the focal species on its heterospecific competitor via food

competition. Moreover, we showed that lines with higher intrinsic growth were more sensitive to competition for food but exerted stronger reproductive interference on heterospecifics. In addition, the higher the proportion of sons produced by a line, the more resilient it was to reproductive interference but also the lower its growth rate. No genetic correlations were found between any variable and body size.
4. Our results indicate that considering the genetic architecture of traits involved in both trophic and sexual interactions is key to understanding the role of individual variation in species interactions.

Keywords
Broad-sense heritability; body size; food competition; genetic correlations; individual variation; reproductive interference.

Introduction

While community ecology has traditionally treated species as homogenous units based on mean trait values, approaches that acknowledge the importance of within-species variation for ecologically relevant traits have begun to gain traction (Jung et al. 2010; Bolnick et al. 2011; Violle et al. 2012; Des Roches et al. 2018; Westerband et al. 2021). Notably, numerous theoretical studies over the past two decades have investigated the effects of within-species individual variation on community assembly and attempted to integrate them into coexistence theory (Stump et al. 2022), often producing conflicting results. Depending on the specific trait being modelled, and whether individual trait variation is present for one or both species, such variation can either promote (*e.g.*, Lichstein et al. 2007; Crawford et al. 2019; Milles et al. 2020), hamper (*e.g.*, Courbaud et al. 2012; Hart et al. 2016; Stump et al. 2020), or have no impact (Banitz 2019) on species coexistence. Although attempts have been made to reconcile such disparate results under a broad framework (see Stump et al. 2022), accurately assessing the impact of individual variation on species coexistence may yet require accounting for more diverse types of traits, such as those involved in reproductive interactions.

Negative sexual interactions between species, known as reproductive interference (Gröning and Hochkirch 2008), have garnered recognition as important determinants of species coexistence (*e.g.*, Kishi 2015; Kyogoku 2015; Grether et al. 2017, 2024; Gómez-Llano et al. 2021). In particular, many empirical studies have shown that reproductive interference can hamper coexistence between ecologically similar species (*e.g.*, Hochkirch et al. 2007; Kishi

2015; Ting and Cutter 2018), and recent theoretical work has aimed at integrating these interactions into current coexistence theory (*e.g.*, Yoshimura and Clark 1994; Kishi and Nakazawa 2013; Schreiber et al. 2019; Yamamichi et al. 2023). These models predict that reproductive interference promotes coexistence under certain conditions, such as when competition for food and reproductive interference are asymmetric and affect each species in opposite ways (Kishi and Nakazawa 2013; Schreiber et al. 2019). Unlike traits involved in trophic interactions, individual variation for reproductive interference has received comparatively less attention. However, the well-established existence of within-population variation for traits such as mate preferences (*e.g.*, Widemo et al. 1999) and reproductive strategies (Gross 1996) suggests that such variation may also play a key role in species interactions.

Acknowledging the importance of individual trait variation raises the question of whether such variation is more strongly determined by genetic (*e.g.*, Annicchiarico et al. 1999; Shuster et al. 2006) or environmental (*e.g.*, Johnson et al. 2008; Tian et al. 2022) factors. The origin of such variation is important. First, only when traits show genetic variation can evolution occur. Second, the very existence of genetic variance has relevance for predicted coexistence. Namely, some theoretical models predict that coexistence among competitors is possible when genetic variance for traits is low (Senthilnathan and Gavrilets 2021). However, genetic variance can also contribute to species coexistence via its effect on the evolutionary trajectories of populations (Vasseur et al. 2011; Barabás and D'Andrea 2016). Still, the impact of heritable trait variation on species coexistence may strongly depend on the strength and direction of potential correlations between traits (Schreiber et al. 2018). Empirical studies have provided evidence of such correlations. For example, Lankau and Strauss (2007) found a negative correlation between intra- and interspecific competition in *Brassica nigra* and negative correlations have also been found between intrinsic growth and sensitivity to competition (Hart et al. 2019; Pettersen et al. 2020). Genetic correlations may instead synergistically promote the evolution of strong competitors, for instance if individuals that are less negatively affected by competition with a heterospecific also exert a stronger competitive pressure on said heterospecific (Sakarchi and Germain 2023). However, the potential genetic correlations between traits underlying competition for food and reproductive interference remain unaddressed.

Here, we assessed genetic variance and potential correlations among traits associated with competition for food and reproductive interference using a system comprised of two closely related spider mite species, *Tetranychus cinnabarinus* and *T. urticae* (also known as

the red and green form of *T. urticae*; Auger et al. 2013). Both species have overlapping worldwide distributions and share the same host plants (Migeon and Dorkeld 2023). Thus, they may often engage in both trophic and sexual interactions in the field. This has been confirmed in a laboratory setting, where *T. urticae* excludes *T. cinnabarinus* through a combination of higher population growth when sharing food resources and heterospecific matings which result in the production of sterile hybrids (Cruz et al. 2021, 2025a). Here, we measured food competition and reproductive interference of inbred lines of *T. cinnabarinus* exposed to a single *T. urticae* line, as well as the growth rate, sex ratio and adult body size of *T. cinnabarinus* females and males in absence of heterospecifics. Then, we estimated the broad-sense heritability of these traits (Falconer and Mackay 1996). Finally, we tested for correlations among the traits found to be significantly heritable.

Methods

Establishment of spider mite inbred lines

As source populations to establish inbred lines, we used one genetically diverse population of *T. cinnabarinus* (abbreviated 'Tc' hereafter; population '*Wu.SS*' in Costa et al. 2023) originally created by merging six field-collected populations as described in Rodrigues et al. (2022), and one population of *T. urticae* ('Tu') for which the ecological consequences of competition for food and reproductive interference have previously been assessed (Cruz et al. 2025a). We derived inbred lines from each population following the sib-mating procedure described in Godinho and colleagues (2020), with some adjustments.

For the Tc population, 200 mated adult females were initially isolated on individual *ca*. 9 cm^2 bean leaf discs pressed onto water-soaked cotton in a Petri dish and allowed to lay eggs for 48h, after which they were discarded. The offspring on each leaf disc were then allowed to develop until adulthood, at which point mating occurred between siblings. From each leaf disc, 14 days after the installation of the initial female, 3 adult sib-mated females were isolated on individual leaf discs to establish the next generation, such that each line consisted of 3 replicate discs to reduce the probability of a line being lost. The procedure was then repeated as above. At each subsequent generation, a single replicate disc was selected per line to establish the next generation. After 15 generations of sib-mating, corresponding to a 93.6% probability of each line being fully inbred (Godinho et al. 2020), we obtained a total of 46 inbred lines of Tc. Of these, 29 were randomly selected for this study.

For the Tu population, we followed the same procedure but started with 20 adult females, to obtain a total of 9 inbred lines after 15 generations. One of these lines was then randomly selected for this study.

Lines were subsequently maintained on larger bean leaf patches at small population sizes (*ca*. 20 to 70 individuals) under the same controlled laboratory conditions as for the experiments (24±2ºC, 70% humidity, 16/8h L/D). This study did not require ethical approval for involving animals.

Measuring traits

We performed two distinct experiments, aimed at quantifying how the growth rate and the sex ratio of each Tc line, and how the former affected and was affected by the growth of the Tu line via (a) food competition in absence of reproductive interference and (b) reproductive interference in absence of food competition following procedures similar to those detailed in Cruz et al. (2025a). Prior to each experiment, lines were expanded by installing *ca*. 20 adult females of each line in individual small population cages (16 x 16 x 11 cm) containing 4 bean leaves. From these cages, we derived females to form age cohorts in each experiment. For the FC experiment (see below), we isolated females on large leaf fragments placed on water-soaked cotton in separate Petri dishes such that adult females would be ready for the experiment 12 days later (*i.e.*, average generation time in standard laboratory conditions). For the RI experiment, this procedure was adapted to obtain virgin males and females, needed in this experiment. To this aim, 2 * 50 virgin females undergoing their last moulting stage (henceforth 'quiescent' females), which will only produce sons as spider mites are arrhenotokous, and 3 * 50 mated females (which produced both daughters and sons), were isolated on large leaf fragments placed on water-soaked cotton in separate Petri dishes 11 days prior to the experiment. Two days prior to the experiment, 250 quiescent Tu females were isolated from the former age cohorts to remain virgin while emerging as adult, whereas on the day of the experiment, Tu males used in the experiment were directly taken from the virgin female age cohorts. As only a small number of individuals (10 females and 10 males) were needed from each Tc line, these were collected directly from their respective small breeding cages.

We also measured female and male body size. Details on the procedure to do so, including the creation of age cohorts, are provided in Supplementary Material II.

Food competition in absence of reproductive interference ('FC experiment')

To quantify the effect of intra- and interspecific food competition on population growth, focal Tc females of each line that had already mated with conspecific males were either tested alone (0 competitors; treatment 1), allowing determining the intrinsic growth rate (daily daughter production) of each line, or exposed to a range of densities of conspecific or heterospecific competitors (1 Tc focal female with 2, 7 or 19 Tc or Tu competitors; treatments 2 to 7), and focal Tu females from the single Tu line were either tested alone (treatment 8) or exposed to a range of densities of heterospecific competitors from each of the Tc lines (1 Tu focal female with 2, 7 or 19 heterospecific competitors; treatments 9 to 11). Fourteen days later, their female offspring was counted. As all 29 Tc lines could not be tested simultaneously, one experimental replicate of each density of conspecific or heterospecific competitors and 'focal female alone' were performed for a total of 20 Tc lines and the Tu line in a single temporal block. A total of 15 experimental blocks were carried out. This resulted in a total of 2915 replicates (10 replicates * 29 Tc lines * 10 treatments + 15 replicates * 1 Tu line * 1 treatment).

Reproductive interference in absence of food competition ('RI experiment')

To quantify the strength of reproductive interference, 5 virgin females of each Tc line and 5 virgin females of the Tu line interacted for 48 hours with both conspecific and heterospecific males (5 of each) before being isolated on individual food patches to lay eggs and for their offspring to develop. After development, when females were still undergoing their last moulting stage and thus were virgin but some males had already reached adulthood, the isolated offspring were merged together on a fresh interaction patch, and 2 days later 10 now-mated females were randomly isolated on fresh food patches to lay eggs and for their offspring to develop. Following this, the number of adult offspring (sons and daughters) of each isolated female was counted, to account for the costs resulting from the production of sterile F1 hybrids. This procedure allowed measuring the impact of reproductive interference while minimizing competition for food. The same procedure was also performed for each Tc line and the Tu line without installing heterospecifics, such that only 5 females and males of each species were initially installed (single species treatments). These allowed determining the intrinsic growth rate (daily daughter production per capita) and offspring sex ratio (proportion of sons in the brood) of each line. The experiment was carried out across 10 independent experimental blocks, with one experimental replicate of each treatment (both species together, Tc alone, and Tu alone) and Tc line per block. Hence, 10 experimental replicates were obtained per treatment per line, for a total of 880 replicates (10 experimental replicates * [29 tested Tc lines * 2 focal

species in the reproductive interference treatment + 29 Tc + 1 Tu lines in the single species treatment]).

Theoretical approach for parameter estimation

Interaction coefficients were estimated from data obtained in each of the above experiments, for the different treatments, as described in detail in Supplementary Section S4 in Cruz et al. (2025a). Briefly, for the purpose of parameter estimation, we adapted a discrete-time population model (Schreiber et al. 2019), which predicts the number of individuals of species i (N_i) that interact through food competition and reproductive interference with individuals of species j (N_j), at each generation t:

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

The leftmost component of the expression ($N_{i,t}\,\delta\,\lambda_i$) represents population growth in absence of limiting interactions, where δ is the oviposition period in days and λ_i is the per capita daily intrinsic growth rate of species i. The middle component represents the effect of food competition, where α_{ii} and α_{ij} are, respectively, the per capita effects of food competition with $N_{i,t}$ competitors of species i and $N_{j,t}$ competitors of species j (*i.e.*, intraspecific and interspecific competition, respectively) at generation t. The rightmost component represents the per capita effect of reproductive interference, where β_{ij} is the per capita effect of interspecific sexual interactions with $N_{j,t}$ individuals of species j at generation t.

The procedure followed here differs from that in Cruz et al. (2025a) in that we used the optimization method 'L-BFGS-B' for estimation, which enables bounding parameter values. Here, for all α and β, we did not set any maximum value, but a minimum value of 0.0001 to prevent the estimation from converging to negative values, indicative of facilitation (Godoy and Levine 2014), as these are not supported by the framework proposed by Schreiber and colleagues.

Statistical analyses

Analyses were carried out using the R statistical software (v4.2.2). To assess genetic variance for the effect of trophic and sexual interactions on Tc population growth, we estimated the broad-sense heritability (H^2) of each parameter (λ_{Tc}, α_{TcTc}, α_{TcTu}, α_{TuTc}, β_{TcTu}, and β_{TuTc}) as well as three other variables measured in the single species treatments of the RI experiment: the number of adult sons produced; the total offspring production, computed as the sum of adult sons and daughters; and the offspring sex ratio, computed as the proportion of sons (*i.e.*,

number of sons divided by total offspring production; see Supplementary Table SI1 and Figure SI1, SI2). To obtain H^2 estimates, we fitted generalized linear mixed models with a Markov chain Monte Carlo approach using the *MCMCglmm* package (Hadfield 2010) for each combination of focal and competitor individuals; see Table SI2 for detailed information). As response variables, we fit either the daily per capita daughter production (for λ, α and β), number of sons, proportion of sons (*i.e.*, offspring sex ratio), or total adult offspring (sum of sons and daughters), all of which were log-transformed, with a Gaussian error structure. Tc line identity and experimental block were fit as random explanatory variables. For the FC experiment, where lines were tested under different densities of competitors, the total density of individuals on a patch was also fit as a fixed explanatory variable. H^2 were then computed using the variances estimated by the models following the formula:

$$H^2 = \frac{V_L}{V_L + (V_B + V_R)} \qquad \text{(Eq. 2)}$$

where V_L corresponds to the variance among inbred lines, V_B to the variance among experimental blocks, and V_R to the residual variance of the model. Statistical significance of heritability estimates was determined by comparing the deviance information criterion (DIC) of a model with line identity as random factor and a model without line identity; differences in DIC (ΔDIC) greater than 2 were considered to be significant. All models ran for 300000 iterations, with a burn-in of 10000 iterations and a thinning of 100, and were always provided with a flat, non-informative prior (V=1 and nu=0.002). All models were checked for convergence and auto-correlation, and no issues were found.

Next, we tested for correlations among all parameters which were found to be significantly heritable (*i.e.*, all except α_{TuTc}; see Figure 1). For each pair of parameters, the observed Pearson correlation coefficient (PCC) between estimated parameter values across Tc lines was computed with the R function *cor*. Statistical significance of each correlation was determined by performing 1000 bootstrap permutations on the list of values of one parameter out of the pair, then computing the corresponding PCCs. P-values associated with each correlation were calculated as the percentage of bootstrapped PCCs lower than or equal to the observed PCC, and then were subjected to an FDR correction to account for multiple testing (Benjamini and Hochberg 1995). For correlations involving the parameter β_{TuTc}, line 42 was considered an outlier and excluded due to an unusually high mean value and confidence interval.

The adult female and male body size of 18 of the inbred lines were also measured and assessed for genetic correlations with traits involved in trophic and sexual interactions (see Supplementary Material II).

Results

Most traits showed significant broad-sense heritability

We found significant broad-sense heritability for the intrinsic growth rate of the Tc lines in both the FC and RI experiments (λ_{FC}, H^2 = 0.13, ΔDIC = 20.79; and λ_{RI}, H^2 = 0.20, ΔDIC = 44.47, respectively; Figure 1; Table SI2), and for the offspring sex ratio ('SR', H^2 = 0.14, ΔDIC = 31.56), production of sons ('Sons', H^2 = 0.05, ΔDIC = 4.56), and total production of adult offspring ('Total offspring', H^2 = 0.16, ΔDIC = 22.74) in the RI experiment. Moreover, significant broad-sense heritability was found for sensitivity to food competition, whether intraspecific (α_{TcTc}, H^2 = 0.07, ΔDIC = 49.57) or interspecific (α_{TcTu}, H^2 = 0.06, ΔDIC = 36.63), and to reproductive interference (β_{TcTu}, H^2 = 0.06, ΔDIC = 7.22). There was also significant genetic and/or maternal variance among Tc lines for their negative effect on Tu growth through reproductive interference (β_{TuTc}, H^2 = 0.05, ΔDIC = 3.91), but not through competition for food (α_{TuTc}, H^2 = 0.01, ΔDIC = -0.38). Finally, male and female body sizes showed significant broad-sense heritability (see Supplementary Material II).

Lines with higher intrinsic growth are more sensitive to competition for food

The Tc lines most affected by intraspecific competition (*i.e.*, with individuals of the same line) were also those that were most affected by interspecific competition with the Tu line (α_{TcTc} and α_{TcTu}, PCC = 0.86, P < 0.0001; Figure 2A; Table SI3). Moreover, both competition coefficients were positively correlated with the intrinsic growth rate of Tc lines (α_{TcTc} and λ_{FC}, PCC = 0.89, P < 0.0001 and α_{TcTu} and λ_{FC}, PCC = 0.78, P < 0.0001; Figures 2B, C; Table SI3), indicating a trade-off between growth and the ability to cope with competition for food (*i.e.*, the more a line is able to grow in absence of competition, the more it is negatively affected by competitors).

Lines with higher intrinsic growth induce stronger reproductive interference on Tu

The sensitivity to reproductive interference of Tc to Tu was not correlated to that of Tu to Tc (β_{TcTu} and β_{TuTc}, PCC = -0.22, P = 0.21; Figure 3A; Table SI3). A lack of correlation was also found between the sensitivity of Tc lines to reproductive interference and their intrinsic growth rate (β_{TcTu} and λ_{RI}, PCC = 0.31, P = 0.08; Figure 3B; Table SI3). However, a significant positive

correlation was found between the intrinsic growth rate of the Tc lines and the harm they caused to Tu via reproductive interference (β_{TuTc} and λ_{RI}, $PCC = 0.35$, $P = 0.05$; Figure 3B; Table SI3).

The Tc lines most sensitive to intraspecific competition induce the strongest reproductive interference on Tu

The sensitivity of Tc to reproductive interference by Tu did not significantly correlate with its sensitivity to intraspecific or interspecific competition for food (β_{TcTu} and α_{TcTc}, $PCC = -0.09$, $P = 0.38$ and β_{TcTu} and α_{TcTu}, $PCC = -0.04$, $P = 0.41$, respectively; Figure 4A, B; Table SI3), nor did the latter correlate with the effect of Tc on Tu through reproductive interference (α_{TcTu} and β_{TuTc}, $PCC = 0.17$, $P = 0.26$; Figure 4D; Table SI3). However, the Tc lines most affected by intraspecific competition were also the ones with the greatest negative effect on the Tu line through reproductive interference (α_{TcTc} and β_{TuTc}, $PCC = 0.42$, $P = 0.04$; Figure 4C; Table SI3).

Correlations between food competition and reproductive interference may be explained by the offspring sex ratio

To investigate a possible explanation for the relationship between reproductive interference and food competition, we also tested for correlations between these parameters and the offspring sex ratio. Although producing more or fewer daughters does not affect the strength of reproductive interference experienced by Tc (see β_{TcTu} and λ_{RI}; Figure 3B), we found a significant negative correlation between offspring sex ratio and the sensitivity of Tc to reproductive interference by Tu (*sex ratio* and β_{TcTu}, $PCC = -0.38$, $P = 0.03$; Figure 5A; Table SI3). Thus, independently of the absolute number of females produced, Tc lines that produce a higher proportion of sons were less affected by reproductive interference. This is not due to an increase in the absolute number of sons, but specifically the relative proportion of sons to daughters, as while both intrinsic growth and offspring sex ratio correlate with total offspring production (*total offspring* and λ_{RI}, $PCC = 0.96$, $P < 0.0001$; and *sex ratio* and *total offspring*, $PCC = -0.42$, $P = 0.03$, respectively; Figure S4A, B; Table SI3), total offspring was unrelated to the overall number of sons (*total offspring* and *sons*, $PCC = 0.15$, $P = 0.29$; Figure S4C; Table SI3).

Moreover, although we found a positive correlation between the intrinsic growth of the Tc lines and the harm they inflicted on Tu through reproductive interference (see β_{TuTc} and λ_{RI}; Figure 3C), offspring sex ratio (as proportion of males) was negatively correlated with the former (*sex ratio* and λ_{RI}, $PCC = -0.64$, $P < 0.0001$; Figure 5C; Table SI3), and not correlated

with the latter (*sex ratio* and β_{TuTc}, *PCC* = -0.07, *P* = 0.39; Figure 5B; Table SI3). Thus, offspring sex ratio did not directly explain the harm caused by Tc to Tu via reproductive interference, but only indirectly through its relationship to the number of daughters produced.

Male and female body sizes did not correlate with any other variable (see Supplementary Material II).

Discussion

In this study, we assessed the genetic variance for and potential genetic correlations between traits associated with food competition and reproductive interference in the spider mite *T. cinnabarinus* interacting with a closely-related species, *T. urticae*. We found significant genetic (and/or maternal) variance for all estimated parameters except for the negative effect that Tu has on Tc via food competition. Moreover, we found significant positive correlations between the sensitivity of Tc lines to intraspecific and to interspecific competition, and between both these sensitivities and the intrinsic growth rate of Tc. We also found a positive correlation between all aforementioned parameters and the negative effect that Tc has on Tu via reproductive interference. Finally, the sensitivity of Tc lines to reproductive interference was also found to correlate negatively with the proportion of males.

Genetic variance for biotic interactions was significant, but low

The values of broad-sense heritability estimated for each parameter were overall low. This is consistent with the relatively low contributions of genetic (and/or maternal) factors to determining competitive interactions identified in other systems (*e.g.*, Johnson et al. 2008; Tian et al. 2022, but see Annicchiarico et al. 1999; Shuster et al. 2006). These low values could be due to a high environmental variance for these traits, as found for other behavioural traits (Dochtermann et al. 2019). Alternatively, they are closer to evolutionary stability due to their close relationship to fitness, and thus genetic variance for them has already been depleted by prior selection (Hoffmann et al. 2016). The remaining genetic variance that we detected may have been maintained through the existence of trade-offs between traits, as those found here. Indeed, quantitative genetics predicts that trade-offs among traits may contribute to the maintenance of their genetic variance (Falconer and Mackay 1996).

Intrinsic growth rates correlate with sensitivity to both biotic interactions

The lines that had higher intrinsic growth rates also experienced stronger negative effects of competition for food. This is in agreement with past studies showing trade-offs between intrinsic growth rates and the ability to cope with competition in other systems (*e.g.*, Luckinbill 1979; Hart et al. 2019; Pettersen et al. 2020). We hypothesized that body size may underlie this correlation, as lines that produce more offspring may produce smaller individuals, more sensitive to competition (Blanckenhorn et al. 2011). Still, such evolutionary constraints are not necessarily ubiquitous, as hinted at by a study in grass species for which interspecific competitive ability evolved independently of intrinsic growth rates (Sakarchi and Germain 2023).

We found a positive correlation between the intrinsic growth rate and the strength of reproductive interference that Tc inflicts on Tu. This may be due to reproductive interference being density dependent, as its strength increases with more encounters with heterospecifics (Kuno 1992; Gröning and Hochkirch 2008; Kyogoku and Sota 2017). However, the lack of correlation between intrinsic growth and sensitivity of Tc to reproductive interference by Tu runs counter to these same expectations. This mismatch may stem from other traits involved in these reproductive interactions, such as the sex ratio.

Offspring sex ratio as a mediator of correlations among food competition and reproductive interference coefficients

Offspring sex ratio was negatively correlated with intrinsic growth rate, such that lines which produced more offspring also produced fewer sons. Furthermore, we found a negative relationship between the harm suffered by Tc from reproductive interference inflicted by Tu and offspring sex ratio. This suggests that it is the proportion of males that matters most for reproductive interference in this system, and not the number of females which was captured with our estimation of intrinsic growth rates. Indeed, in species such as spider mites, where males take a more active role in reproduction than females by being choosier (Rodrigues et al. 2020; Cruz et al. 2025b), the relative proportion of conspecific to heterospecific males is expected to be an important factor in determining vulnerability to heterospecific matings (as in frogs; Hettyey and Pearman 2003).

Positive correlations between biotic interactions could hamper coexistence

Coexistence between species competing for shared resources is expected to be facilitated by a trade-off between intra- and interspecific competition (Vasseur et al. 2011). Such trade-offs have been shown for both plant and animal species (*e.g.*, Lankau 2008; Liendo et al. 2018; but

see Miller and Schemske 1990), but this was not the case in our system. However, we tested competition between these species in an environment containing only one type of resource and very limited space. Perhaps under conditions allowing for access to more varied resource types and for spatial segregation, stabilizing mechanisms could arise and foster coexistence.

Theory also predicts that coexistence between species engaging in both trophic and sexual interactions simultaneously should be possible if there is a trade-off between interaction strengths, such that the superior competitor for food is inferior in reproductive interference and vice-versa (Kishi and Nakazawa 2013). However, an empirical study shows that these interactions may instead jointly promote exclusion (Gómez-Llano et al. 2023). Here, we find no evidence of a trade-off between the sensitivity of the Tc lines to food competition and their sensitivity to reproductive interference, but instead a puzzling trade-off between the ability of Tc lines to cope with (both intra- and interspecific) food competition and the harm they inflict to the Tu line through reproductive interference. While it is likely that a common mechanism underlies both interactions, we expected these to act in the same direction (Cruz et al. 2025a). For instance, a reduction in adult body size following development in competition for limited resources (Li and Zhang 2018) could result in smaller Tc males, which would exert less reproductive interference on Tu instead (by being weaker competitors for mates; Potter et al. 1976; Enders 1993 see also the Discussion in Cruz et al. (2025a). Here, we failed to uncover any significant genetic correlations involving body size, whether in females or in males, that could validate this hypothesis (see Supplementary Material II). Thus, it seems more likely that this positive correlation is a by-product of how each of these parameters is individually correlated with intrinsic growth and, by extension, with the offspring sex ratio. The former explanation involving body size is also compatible with this, as body size may be correlated with food competition and reproductive interference through physiology rather than genetics: females developing under competition for limited resources may be smaller and, consequently, may lay smaller eggs on average (as observed in seed beetles; Yanagi et al. 2013). These eggs may then have a lower likelihood of being fertilized (hence to become female in haplodiploids), as has been shown in spider mites (Macke et al. 2011). This, in turn, may translate into a higher sensitivity to food competition (as fewer females are produced) but more harm caused to the other species (as more males are produced).

Finally, while coexistence between these species is unlikely given the estimated parameter values, it is still possible that the extent of variation among lines itself could foster coexistence. Indeed, theoretical studies have shown that coexistence can occur between species which would otherwise exclude each other, when there is more intrapopulation variation for

the inferior competitor relative to the superior competitor (Begon and Wall 1987; Lichstein et al. 2007; Hart et al. 2016), a hypothesis that would require investigating variation for these traits in Tu as well. Thus, our findings set the stage for future experimental studies investigating the impact of individual variation on coexistence between species engaging in both food competition and reproductive interference. In sum, we show that most parameters involved in competition for food and reproductive interference are genetically correlated, and that several of these correlations are underlain by the population growth rate and sex ratio. These results have important consequences for both species coexistence and their evolutionary potential.

References

Annicchiarico, P., E. Piano, and I. Rhodes. 1999. Heritability of, and genetic correlations among, forage and seed yield traits in Ladino white clover. Plant Breed. 118:341–346.

Auger, P., A. Migeon, E. A. Ueckermann, L. Tiedt, and M. Navajas. 2013. Evidence for synonymy between *Tetranychus urticae* and *Tetranychus cinnabarinus* (Acari, Prostigmata, Tetranychidae): review and new data. Acarologia 53:383–415.

Banitz, T. 2019. Spatially structured intraspecific trait variation can foster biodiversity in disturbed, heterogeneous environments. Oikos 128:1478–1491.

Barabás, G., and R. D'Andrea. 2016. The effect of intraspecific variation and heritability on community pattern and robustness. Ecol. Lett. 19:977–986.

Begon, M., and R. Wall. 1987. Individual Variation and Competitor Coexistence: A Model. Funct. Ecol. 1:237–241.

Benjamini, Y., and Y. Hochberg. 1995. Controlling the False Discovery Rate: A Practical and Powerful Approach to Multiple Testing. J. R. Stat. Soc. Ser. B Stat. Methodol. 57:289–300.

Blanckenhorn, W. U., P. E. A. Hoeck, C. Reim, and Y. Teuschl. 2011. A cost of being large: genetically large yellow dung flies lose out in intra-specific food competition. Evol. Ecol. 25:875–884.

Bolnick, D. I., P. Amarasekare, M. S. Araújo, R. Bürger, J. M. Levine, M. Novak, V. H. W. Rudolf, S. J. Schreiber, M. C. Urban, and D. A. Vasseur. 2011. Why intraspecific trait variation matters in community ecology. Trends Ecol. Evol. 26:183–192.

Costa, S. G., S. Magalhães, and L. R. Rodrigues. 2023. Multiple mating rescues offspring sex ratio but not productivity in a haplodiploid exposed to developmental heat stress. Funct. Ecol. 37:1291–1303.

Courbaud, B., G. Vieilledent, and G. Kunstler. 2012. Intra-specific variability and the competition–colonisation trade-off: coexistence, abundance and stability patterns. Theor. Ecol. 5:61–71.

Crawford, M., F. Jeltsch, F. May, V. Grimm, and U. E. Schlägel. 2019. Intraspecific trait variation increases species diversity in a trait-based grassland model. Oikos 128:441–455.

Cruz, M. A., O. Godoy, I. Fragata, V. C. Sousa, S. Magalhães, and F. Zélé. 2025a. Competition for food affects the strength of reproductive interference and its consequences for species coexistence. Funct. Ecol. 39:1982–1997.

Cruz, M. A., S. Magalhães, M. Bakırdöven, and F. Zélé. 2025b. *Wolbachia* strengthens the match between premating and early postmating isolation in spider mites. Evolution 79:203–219.

Cruz, M. A., S. Magalhães, É. Sucena, and F. Zélé. 2021. *Wolbachia* and host intrinsic reproductive barriers contribute additively to postmating isolation in spider mites. Evolution 75:2085–2101.

Des Roches, S., D. M. Post, N. E. Turley, J. K. Bailey, A. P. Hendry, M. T. Kinnison, J. A. Schweitzer, and E. P. Palkovacs. 2018. The ecological importance of intraspecific variation. Nat. Ecol. Evol. 2:57–64.

Dochtermann, N. A., T. Schwab, M. Anderson Berdal, J. Dalos, and R. Royauté. 2019. The Heritability of Behavior: A Meta-analysis. J. Hered. 110:403–410.

Enders, M. M. 1993. The effect of male size and operational sex ratio on male mating success in the common spider mite, *Tetranychus urticae* Koch (Acari: Tetranychidae). Anim. Behav. 46:835–846.

Falconer, D. S., and T. F. C. Mackay. 1996. Introduction to Quantitative Genetics. 4th ed. Longmans Green, Harlow, Essex, UK.

Godinho, D. P., M. A. Cruz, M. Charlery de la Masselière, J. Teodoro-Paulo, C. Eira, I. Fragata, L. R. Rodrigues, F. Zélé, and S. Magalhães. 2020. Creating outbred and inbred populations in haplodiploids to measure adaptive responses in the laboratory. Ecol. Evol. 10:7291–7305.

Godoy, O., and J. M. Levine. 2014. Phenology effects on invasion success: insights from coupling field experiments to coexistence theory. Ecology 95:726–736.

Gómez-Llano, M., W. A. Boys, T. Ping, S. P. Tye, and A. M. Siepielski. 2023. Interactions between fitness components across the life cycle constrain competitor coexistence. J. Anim. Ecol. 92:2297–2308.

Gómez-Llano, M., R. M. Germain, D. Kyogoku, M. A. McPeek, and A. M. Siepielski. 2021. When ecology fails: how reproductive interactions promote species coexistence. Trends Ecol. Evol. 36:610–622.

Grether, G. F., A. E. Finneran, and J. P. Drury. 2024. Niche differentiation, reproductive interference, and range expansion. Ecol. Lett. 27:e14350.

Grether, G. F., K. S. Peiman, J. A. Tobias, and B. W. Robinson. 2017. Causes and consequences of behavioral interference between species. Trends Ecol. Evol. 32:760–772.

Gröning, J., and A. Hochkirch. 2008. Reproductive interference between animal species. Q. Rev. Biol. 83:257–282.

Gross, M. R. 1996. Alternative reproductive strategies and tactics: diversity within sexes. Trends Ecol. Evol. 11:92–98.

Hadfield, J. D. 2010. MCMC methods for multi-response generalized linear mixed models: The MCMCglmm R package. J. Stat. Softw. 33:1–22.

Hart, S. P., S. J. Schreiber, and J. M. Levine. 2016. How variation between individuals affects species coexistence. Ecol. Lett. 19:825–838.

Hart, S. P., M. M. Turcotte, and J. M. Levine. 2019. Effects of rapid evolution on species coexistence. Proc. Natl. Acad. Sci. 116:2112–2117.

Hettyey, A., and P. B. Pearman. 2003. Social environment and reproductive interference affect reproductive success in the frog *Rana latastei*. Behav. Ecol. 14:294–300.

Hochkirch, A., J. Gröning, and A. Bücker. 2007. Sympatry with the devil: reproductive interference could hamper species coexistence. J. Anim. Ecol. 76:633–642.

Hoffmann, A. A., J. Merilä, and T. N. Kristensen. 2016. Heritability and evolvability of fitness and nonfitness traits: Lessons from livestock. Evolution 70:1770–1779.

Johnson, M. T. J., R. Dinnage, A. Y. Zhou, and M. D. Hunter. 2008. Environmental Variation Has Stronger Effects than Plant Genotype on Competition among Plant Species. J. Ecol. 96:947–955.

Jung, V., C. Violle, C. Mondy, L. Hoffmann, and S. Muller. 2010. Intraspecific variability and trait-based community assembly. J. Ecol. 98:1134–1140.

Kishi, S. 2015. Reproductive interference in laboratory experiments of interspecific competition. Popul. Ecol. 57:283–292.

Kishi, S., and T. Nakazawa. 2013. Analysis of species coexistence co-mediated by resource competition and reproductive interference. Popul. Ecol. 55:305–313.

Kuno, E. 1992. Competitive exclusion through reproductive interference. Popul. Ecol. 34:275–284.

Kyogoku, D. 2015. Reproductive interference: ecological and evolutionary consequences of interspecific promiscuity. Popul. Ecol. 57:253–260.

Kyogoku, D., and T. Sota. 2017. A generalized population dynamics model for reproductive interference with absolute density dependence. Sci. Rep. 7:1–8.

Lankau, R. 2008. A chemical trait creates a genetic trade-off between intra- and interspecific competitive ability. Ecology 89:1181–1187.

Lankau, R. A., and S. Y. Strauss. 2007. Mutual feedbacks maintain both genetic and species diversity in a plant community. Science 317:1561–1563.

Li, G. Y., and Z. Q. Zhang. 2018. Does size matter? Fecundity and longevity of spider mites (*Tetranychus urticae*) in relation to mating and food availability. Syst. Appl. Acarol. 23:1796–1808.

Lichstein, J. W., J. Dushoff, S. A. Levin, and S. W. Pacala. 2007. Intraspecific Variation and Species Coexistence. Am. Nat. 170:807–818.

Liendo, M. C., M. A. Parreño, J. L. Cladera, M. T. Vera, and D. F. Segura. 2018. Coexistence between two fruit fly species is supported by the different strength of intra- and interspecific competition. Ecol. Entomol. 43:294–303.

Luckinbill, L. S. 1979. Selection and the r/K Continuum in Experimental Populations of Protozoa. Am. Nat. 113:427–437.

Macke, E., S. Magalhães, H. D.-T. Khan, A. Luciano, A. Frantz, B. Facon, and I. Olivieri. 2011. Sex allocation in haplodiploids is mediated by egg size: evidence in the spider mite *Tetranychus urticae* Koch. Proc. R. Soc. B Biol. Sci. 278:1054–1063.

Migeon, A., and F. Dorkeld. 2023. Spider Mites Web: a comprehensive database for the Tetranychidae.

Miller, T. E., and D. W. Schemske. 1990. An Experimental Study of Competitive Performance in *Brassica rapa* (cruciferae). Am. J. Bot. 77:993–998.

Milles, A., M. Dammhahn, and V. Grimm. 2020. Intraspecific trait variation in personality-related movement behavior promotes coexistence. Oikos 129:1441–1454.

Pettersen, A. K., M. D. Hall, C. R. White, and D. J. Marshall. 2020. Metabolic rate, context-dependent selection, and the competition-colonization trade-off. Evol. Lett. 4:333–344.

Potter, D. A., D. L. Wrensch, and D. E. Johnston. 1976. Aggression and Mating Success in Male Spider Mites. Science 193:160–161.

Rodrigues, L. R., A. R. T. Figueiredo, T. Van Leeuwen, I. Olivieri, and S. Magalhães. 2020. Costs and benefits of multiple mating in a species with first-male sperm precedence. J. Anim. Ecol. 89:1045–1054.

Rodrigues, L. R., F. Zélé, I. Santos, and S. Magalhães. 2022. No evidence for the evolution of mating behavior in spider mites due to *Wolbachia*-induced cytoplasmic incompatibility. Evolution 76:623–635.

Sakarchi, J., and R. M. Germain. 2023. The evolution of competitive ability. Am. Nat. 201:1–15.

Schreiber, S. J., S. Patel, and C. terHorst. 2018. Evolution as a Coexistence Mechanism: Does Genetic Architecture Matter? Am. Nat. 191:407–420.

Schreiber, S. J., M. Yamamichi, and S. Y. Strauss. 2019. When rarity has costs: coexistence under positive frequency-dependence and environmental stochasticity. Ecology 100:1–13.

Senthilnathan, A., and S. Gavrilets. 2021. Ecological consequences of intraspecific variation in coevolutionary systems. Am. Nat. 197:1–17.

Shuster, S. M., E. V. Lonsdorf, G. M. Wimp, J. K. Bailey, and T. G. Whitham. 2006. Community heritability measures the evolutionary consequences of indirect genetic effects on community structure. Evolution 60:991–1003.

Stump, S. M., J. H. Marden, N. G. Beckman, S. A. Mangan, and L. S. Comita. 2020. Resistance Genes Affect How Pathogens Maintain Plant Abundance and Diversity. Am. Nat. 196:472–486.

Stump, S. M., C. Song, S. Saavedra, J. M. Levine, and D. A. Vasseur. 2022. Synthesizing the effects of individual-level variation on coexistence. Ecol. Monogr. 92:e01493.

Tian, X., H. Ohtsuki, and J. Urabe. 2022. Competitive consequences determined by phenotypic but not genetic distance: A study with asexual water flea genotypes. Funct. Ecol. 36:2152–2162.

Ting, J. J., and A. D. Cutter. 2018. Demographic consequences of reproductive interference in multi-species communities. BMC Ecol. 18:1–13.

Vasseur, D. A., P. Amarasekare, V. H. W. Rudolf, and J. M. Levine. 2011. Eco-evolutionary dynamics enable coexistence via neighbor-dependent selection. Am. Nat. 178:E96–E109.

Violle, C., B. J. Enquist, B. J. McGill, L. Jiang, C. H. Albert, C. Hulshof, V. Jung, and J. Messier. 2012. The return of the variance: Intraspecific variability in community ecology. Trends Ecol. Evol. 27:244–252.

Westerband, A. C., J. L. Funk, and K. E. Barton. 2021. Intraspecific trait variation in plants: a renewed focus on its role in ecological processes. Ann. Bot. 127:397–410.

Widemo, F., S. A. Sæther, F. Widemo, S. A. Sæther, F. Widemo, S. A. Sæther, F. Widemo, S. A. Sæther, F. Widemo, and S. A. Sæther. 1999. Beauty is in the eye of the beholder: causes and consequences of variation in mating preferences. Trends Ecol. Evol. 14:26–31.

Yamamichi, M., K. Tsuji, S. Sakai, and E. I. Svensson. 2023. Frequency-dependent community dynamics driven by sexual interactions. Popul. Ecol. 65:204–219.

Yanagi, S., Y. Saeki, and M. Tuda. 2013. Adaptive egg size plasticity for larval competition and its limits in the seed beetle Callosobruchus chinensis. Entomol. Exp. Appl. 148:182–187.

Yoshimura, J., and C. W. Clark. 1994. Population dynamics of sexual and resource competition. Theor. Popul. Biol. 45:121–131.

Tables and Figures

Figure 1. Estimates of heritability for each parameter and trait obtained in the FC and RI experiments. Broad-sense heritability was estimated from the offspring production data obtained for each combination of focal individuals and competitors (FC experiment) or heterospecific mates (RI experiment) (see Table S2). Dots represent mean broad-sense heritability for each parameter. Error bars represent the 95% highest posterior density interval (HPDI). Asterisks indicate values of heritability significantly different from 0 at the 5% level.

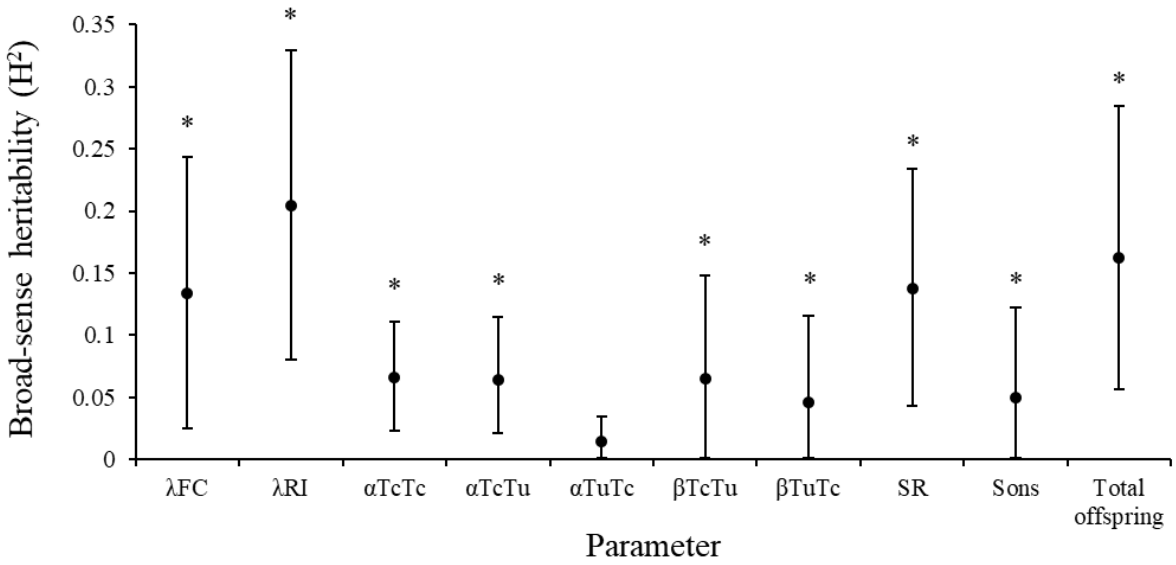

Figure 2. Correlations among parameters estimated from the offspring production data obtained in the FC experiment. Each dot represents, for one Tc line, mean values estimated from the FC experiment for (A) sensitivity to intra- and interspecific competition, α_{TcTc} and α_{TcTu}; (B) sensitivity to intraspecific competition, α_{TcTc}, and intrinsic growth rates, λ_{FC}; and (C) sensitivity to interspecific competition, α_{TcTu}, and intrinsic growth rates, λ_{FC}. Error bars represent the 95% confidence interval. As the sensitivity of Tu to interspecific competition, α_{TuTc}, was not heritable, this parameter was not tested for significant correlations. Red trendlines indicate a significant positive correlation between parameters at the 5% level.

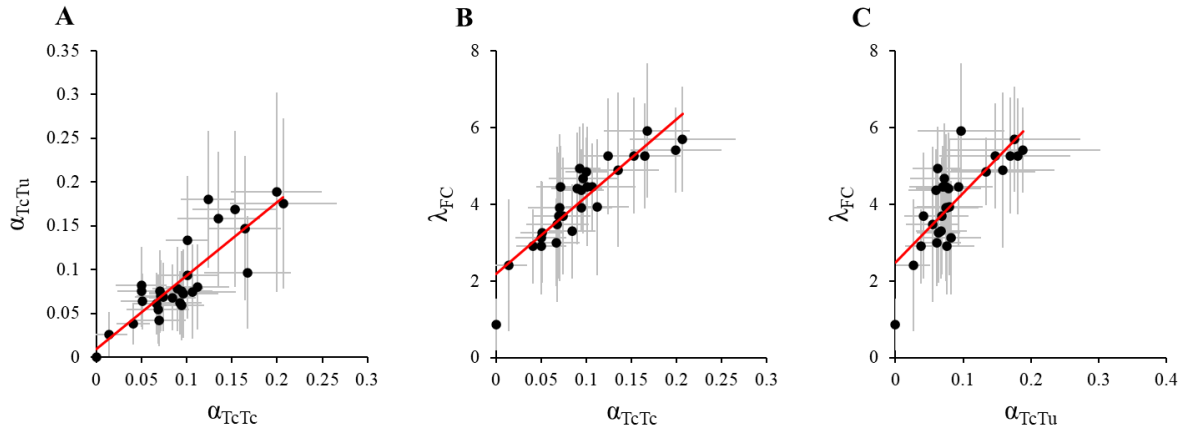

Figure 3. Correlations among parameters estimated from the offspring production data obtained in the RI experiment. Each dot represents, for one Tc line, mean values estimated from the RI experiment for (A) sensitivity to heterospecific mates by Tc, β_{TcTu}, and the effect of Tc on heterospecific mates, β_{TuTc}; (B) sensitivity to heterospecific mates by Tc, β_{TcTu}, and intrinsic growth rates, λ_{RI}; and (C) the effect of Tc on heterospecific mates, β_{TuTc}, and intrinsic growth rates, λ_{RI}. Error bars represent the 95% confidence interval. The red trendline indicates a significant positive correlation between parameters at the 5% level.

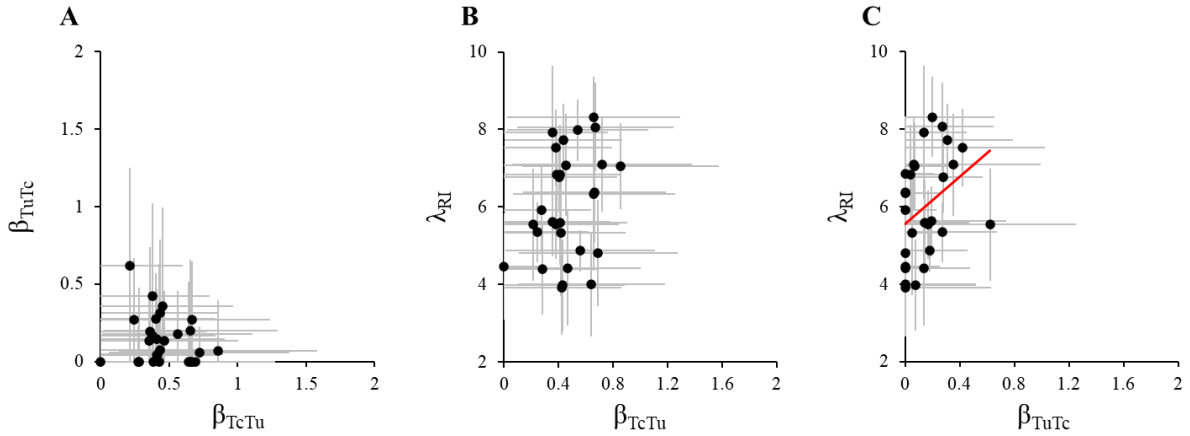

Figure 4. Correlations among parameters related to competition for food and to reproductive interference. Each dot represents, for one Tc line, mean values estimated for (A) sensitivity to conspecific competitors, α_{TcTc}, and to heterospecific mates, β_{TcTu}; (B) sensitivity to heterospecific competitors, α_{TcTu}, and to heterospecific mates, β_{TcTu}; (C) sensitivity to conspecific competitors, α_{TcTc}, and the effect of Tc on heterospecific mates, β_{TuTc}; and (D) sensitivity to heterospecific competitors, α_{TcTu}, and the effect of Tc on heterospecifics mates, β_{TcTu}. Error bars represent the 95% confidence interval. As the sensitivity of Tu to interspecific competition, α_{TuTc}, was found to not be heritable, this parameter was not tested for significant correlations. Red trendlines indicate a significant positive correlation between parameters at the 5% level.

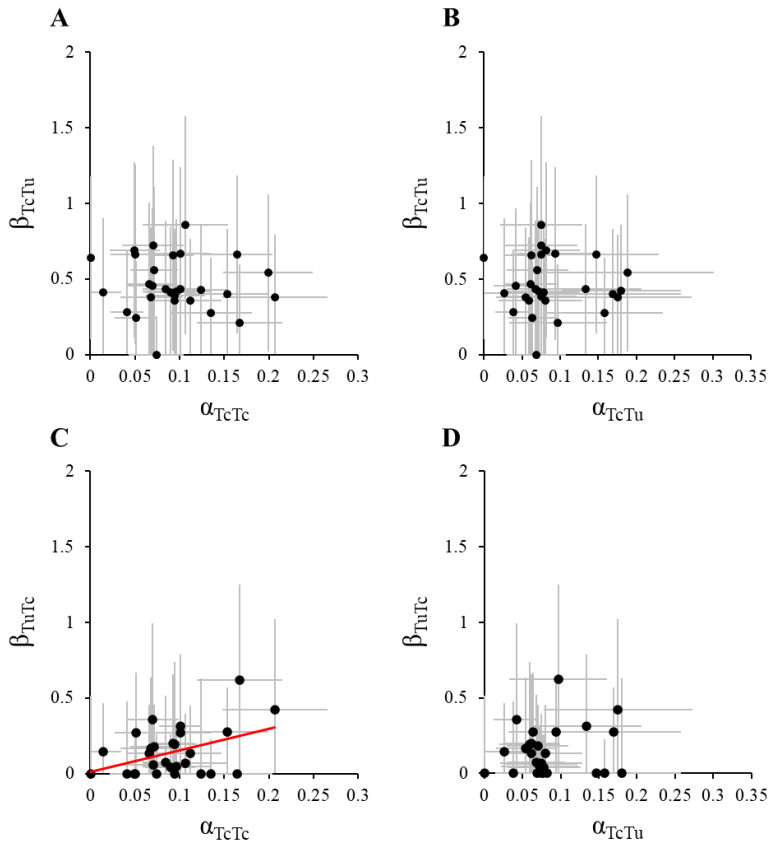
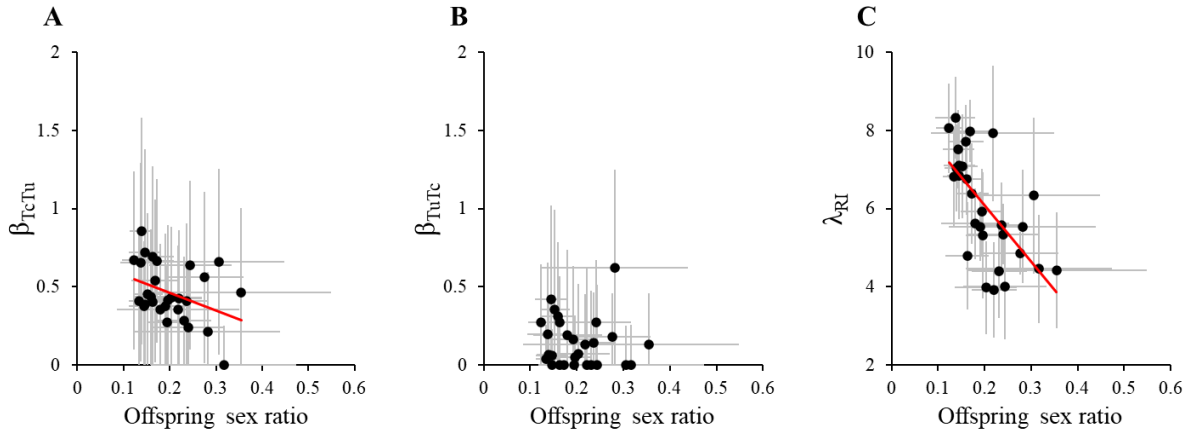
Figure 5. Correlations between offspring sex ratio, sensitivity to, and harm caused via reproductive interference in the RI experiment. Each dot represents, for one Tc line, the mean estimated values of the offspring sex ratio (computed as the proportion of sons) and (A) the sensitivity of Tc to reproductive interference, β_{TcTu}, (B) the effect of Tc on Tu via reproductive interference, β_{TuTc}, and (C) the intrinsic growth rate of Tc. Error bars represent the 95% confidence interval. The red trendline indicates a significant negative correlation between parameters at the 5% level.