Evolution in response to an abiotic stress shapes species coexistence

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Abstract

Adaptation to abiotic stresses is pervasive and generally relies on traits that are not independent from those affecting species interactions. Still, how such evolution affects species coexistence remains elusive. We addressed this using populations of two herbivorous spider mite species (*Tetranychus urticae* and *T. evansi*) evolving separately on tomato plants hyper-accumulating cadmium, a stressful environment for the mites, or on control plants with no-cadmium. Combinations of phenotypic analyses with structural stability theory predicted that adaptation of both species to cadmium allow them to coexist in that environment, whereas for cadmium-naïve mite populations the most likely outcome is competitive exclusion. The shift from exclusion to coexistence was due to an increase in structural niche differences caused by a simultaneous increase in intra and a decrease in interspecific competition. However, adaptation to cadmium did not affect species interactions and competitive outcomes in the cadmium-free environment, indicating that such evolutionary changes were environment-specific. Experimental outcomes of population dynamics with populations of the two species from each selection regime in each environment were fully compatible with model predictions. Therefore, evolution of single species in a new environment, even in absence of interspecific competitors, shapes species coexistence. Hence, population shifts to novel environments may have unforeseen evolutionary consequences for community composition and the maintenance of species diversity.

Significance Statement

The evolutionary consequences of strong selective pressures imposed by environmental changes on species interactions and their subsequent coexistence remain largely unknown. To fill this knowledge gap, we combined the power of experimental evolution and structural stability to analyze how evolution of two spider mite species on plants with or without cadmium affected their ability to coexist. We found that evolution in the high cadmium environment led to changes in growth rates, but also in the strength of intra- and interspecific competition, such that coexistence became possible, but only when the two species evolved in the new environment. Our study highlights that evolution under high pollutant concentrations, even in the absence of competitors, can shape long-term coexistence between species.

Introduction

Understanding how evolutionary dynamics shape and are shaped by long-term species persistence is key to predict community composition and biodiversity maintenance under global change. Character displacement in response to the presence of competitors is one of the classical examples of the role of natural selection in shaping trait evolution and species distribution[1–3]. In the last decades, the acknowledgment that ecology and evolution operate at similar timescales has led to the prediction that the presence of competitors selects for the evolution of competitive interactions (i.e., the negative per-capita effect of one species on another[4–6]), which in turn affects the ability of species to coexist[7–10]. In line with these predictions, empirical studies have shown that short term evolution in presence of competitors can modify competitive traits[11] and change coexistence patterns[6, 12–15].

Although the abovementioned studies have reinstated short-term evolution as an important force shaping ecological patterns, it may well be that we are still under-estimating the potential for evolution to impact species coexistence. An important yet overlooked possibility is that traits that affect species coexistence may be selected even in the absence of competitors. For example, species arriving in an environment before their interspecific competitors may adapt at a fast rate to that environment, which leads to an increased ability to monopolize it[16]. Likewise, prior adaptation to a given environment can lead to a lower relative growth rate of late arriving species[17]. In line with this, evidence that trait evolution affects species community composition has also been recently documented[18, 19]. However, to date no study has tested if and how trait evolution in response to an abiotic selection pressure modifies species coexistence through changes in both intra- and interspecific competition. Moreover, adaptation to one environment can lead to changes in competitive abilities in other environments[20] but the potential for this to translate into changes in coexistence patterns remains unclear. Hence, our knowledge of the role of evolution in response to abiotic stresses in shaping coexistence patterns is yet in its infancy.

The recent development of the complementary theoretical frameworks of modern coexistence theory and structural stability[21–23] provide clear mechanistic pathways to understand how competing species coexist in ecological time. Both theories agree that species can coexist whenever their niche differences, which arise when species specialize in different resources, overcome their differences in population growth in the presence of competitors[21, 24, 25], but structural stability, unlike modern coexistence theory, takes a probabilistic view of coexistence, which allows incorporating stochasticity and consider the

occurrence of both direct competition and other interactions such as mutualism[26]. This framework defines that species coexistence is possible when the opportunities for species to coexist, measured by the feasibility domain, can accommodate difference in intrinsic growth rates between species. Therefore, wider feasibility domains allow for the persistence of species combinations with more uneven intrinsic growth rates[22, 25]. To date, this tool has not been applied to address the role of evolution in shaping coexistence patterns.

Here, we assessed how evolution in response to an abiotic stress affects species coexistence by combining two powerful tools: experimental evolution and structural stability. We used a system composed of two closely-related spider-mite species, *Tetranychus urticae* and *T. evansi*, known to engage in competitive interactions when feeding on tomato plants (*Solanum lycopersicum*)[27, 28]. These plants can hyper-accumulate cadmium in their upper parts, which strongly reduces spider-mite fecundity and offspring survival, producing a strong selective pressure[29–31]. Each spider mite species evolved for 40 generations on plants with or without cadmium in absence of interspecific competitors (Figure S1). After this period, changes in intrinsic growth rates and in the strength of biotic interactions (intra- and interspecific competition) were assessed in both environments using a fully orthogonal experimental design: the evolved populations were exposed to a density gradient of intraspecific competitors (same species) that evolved in the same environment or of interspecific competitors (different species) that evolved in each environment (plants with or without cadmium; Figure S1). Subsequently, we combined structural stability theory with model parameterization to predict how evolution in response to an abiotic stress (cadmium) of each species, or both affected competitive outcomes. This allowed singling out the role of evolution of each species, as well as that of the environment where the interactions occurred. Finally, we empirically validated the short-term predictions of our models by conducting a population growth experiment with the same full orthogonal design.

Results

Evolution in cadmium changes the strength of interspecific competition

To verify that plants with cadmium represent a stress for these populations and thus pose a significant selection pressure, we first compared the intrinsic growth rate of naïve populations (evolving on plants without cadmium) on plants with cadmium. Populations of both mite species from the ‘no-cadmium’ selection regime had significantly lower growth rates in the

cadmium than in the no-cadmium environment (*Tetranychus urticae*: $\chi^2_{1,7}$= 71.985, P-value<0.001, *T. evansi*: $\chi^2_{1,7}$=117.15, P-value<0.001, Table S1), confirming our hypothesis.

After evolution for *circa* 40 generations, we estimated the offspring production of populations that had evolved on plants with or without cadmium when exposed to each of these environments, in presence or absence of intra- and interspecific competition. To this aim, we exposed mites from each species to a gradient of intra and interspecific competitors, then fitted the Ricker model [32] to estimate the intrinsic growth rate, intra and interspecific competition ability of each population in each environment. Using these estimated parameters, we predicted the number of females of each species in each environment. In the cadmium environment, the intrinsic growth rate of populations evolving in the cadmium selection regime was higher than that of populations evolving on plants without cadmium – the no-cadmium selection regime-, for both species (*T. urticae*: $\chi^2_{1,6}$=6.187, P-value=0.013, *T. evansi*: $Z_{1,7}$= 4.483, P-value=0.034, Fig 1, Fig. S2, Table S1). Associated with this increase, we also found an increase in the strength of intraspecific competition for *T. evansi* ($\chi^2_{1,7}$ = 8.570, P-value=0.003, Fig S3A, Table S1), but not for *T. urticae* ($\chi^2_{1,6}$ = 0.131, P-value=0.717, Fig S3B, Table S1). This resulted in a decreased predicted number of offspring produced by *T. evansi* in competition (relative to when growing alone) but only when *T. evansi* evolved in the cadmium selection regime (Fig. 1A, λ vs $\lambda+\alpha_{ii}$ for the yellow and blue vs the green and red dot). Moreover, evolving in cadmium made *T. evansi* more sensitive to competition with *T. urticae* from the no-cadmium selection regime (Fig. S4A, Table S2A, S2B, contrasts between the *T. evansi* cadmium *vs* no-cadmium selection regime, when exposed to *T. urticae* from the no-cadmium selection regime: T $ratio_{1,13}$= -0.053, P-value=0.003), reducing the predicted number of offspring produced after one generation of competition (Fig. 1A, $\lambda+\alpha_{ii}+\alpha_{ij}$, comparing yellow and red dots). Conversely, evolution in cadmium did not affect the impact of interspecific competition for *T. urticae* (Fig. S4B, Table S2A). Interestingly, we observe weak positive interactions within and between species in several replicates (i.e., negative alpha values, corresponding to facilitation) across selection regimes (Fig S3, S4), which reinforces the added value of using the structural stability framework.

In the no-cadmium environment, having evolved in the cadmium selection regime did not lead to significant changes in the intrinsic growth rate (Fig. S5, T. *evansi*: $\chi^2_{1,7}$=2.628, P-value=0.105; T. *urticae*: $\chi^2_{1,6}$= 0.121, P-value=0.728, Table S1), intraspecific competition (Fig. S6, *T. evansi:* $\chi^2_{1,7}$= 1.445, P-value=0.229; *T. urticae*: $\chi^2_{1,6}$= 0.003, P-value=0.998,

Table S1) or interspecific competition (Fig. S7, Table S1, S3). Still, the predicted growth rate of *T. evansi* populations from the cadmium selection regime was lower than that of populations evolved in the no-cadmium selection regime (Fig 1A, right panel, λ, comparing green and red to blue and yellow dots), a reduction not observed in *T. urticae* (Fig. 1B, right panel, λ). Weak intra- and interspecific facilitation was found for several replicates across selection regimes (Figs. S6, S7), similarly to what happened in the cadmium environment.

Evolution of both competitors increases the probability of long-term coexistence in the cadmium environment

We predicted competitive outcomes, either coexistence or competitive exclusion, for each combination of selection regimes, by coupling structural stability with the population model that describes the temporal dynamics of interacting species (the Ricker model). This framework led to the prediction that, in the cadmium environment, coexistence was more likely when both species had evolved in that environment (Fig. 2). Indeed, evolution of both species in cadmium led to an increase in the size of the feasibility domain via an increased intraspecific competition, only for *T. evansi*, the better competitor, (Fig S3). It also led to increased intrinsic growth rate of both species, but with a smaller magnitude in *T. urticae* (Fig S2). Our prediction of coexistence under evolution in cadmium environments is robust because it holds even after taking uncertainty (95% CI) into account when estimating the feasibility domain (Fig 2, light blue region), and the differences of species intrinsic growth rates (Fig. 3, S8).

When only one species evolved in the cadmium selection regime, coexistence was not possible in the cadmium environment (Figs 2, 3). Contrary to our initial expectation that the species that evolved in cadmium would be best suited to the new environment and thus would exclude competitors from the no-cadmium selection regimes, we observe that the species that evolved in cadmium was predicted to be excluded (Figs. 2, 3, S8). When *T. evansi* was the species that evolved in cadmium, this can be explained by increased intraspecific competition (Fig S3) and increased sensitivity to competition from *T. urticae* of the no-cadmium selection regime (Fig S4). In the reverse case, *T. urticae* that evolved in cadmium was negatively affected by interspecific competition with *T. evansi* from the no-cadmium selection regime but did not affect it (Fig. S4), which led to a prediction of exclusion in the cadmium environment (Fig. 4). Again, predictions, in this case of competition exclusion, are robust to variation in parameter estimation when accounting for lower and upper 95% confidence intervals.

In the no-cadmium environment, the two species were generally predicted to coexist except when *T. urticae* from the cadmium selection regime competed with *T. evansi* from the no-cadmium selection regime, in which case *T. urticae* was predicted to be excluded by *T. evansi* (Fig. 2). However, the minimum distance to the edges of the feasibility domain was short in all cases (Fig. 3, S9), especially when considering the feasibility domains with lower confidence intervals, indicating that small perturbations may shift the prediction from coexistence to exclusion and vice versa. Importantly, there was high heterogeneity among replicates but little effect of the evolutionary history of both species (Fig. 3). Our results thus indicate that, unlike what we observed in the cadmium environment, evolution in cadmium did not affect the range of opportunities for species to coexist in the no-cadmium environment (Fig. 2, S8).

Simulated competitive dynamics partially recover empirical patterns

To validate our approach, we compared theoretical predictions of ecological short-term dynamics with a population dynamic experiment in which the two species, in all possible combinations of selection regimes, were maintained for two generations. (Fig. S1). We observed a close agreement between model predictions and experimental observations (Fig. 4, slope pooled replicates: 0.707, P-value<0.001), despite some heterogeneity between replicate populations, especially in the no-cadmium environment (slope with replicates: 0.631, P-value<0.001, Table S4). Interestingly, predictions for *T. urticae* evolved in the cadmium selection regime were the most accurate in the no-cadmium environment, but the least in the cadmium environment (Fig. 4).

Discussion

We used a combination of experimental evolution and structural stability theory to predict the impact of evolution in response to an abiotic selection pressure (cadmium) on coexistence patterns. We also tested how such evolution affected coexistence in an environment without cadmium. To obtain such theoretical predictions, we rigorously quantified the intrinsic growth rate of two spider-mite species and the strength of their intra- and interspecific competitive interactions across different environmental and selection scenarios. While evolutionary history had little effect on coexistence patterns in the environment with no-cadmium, the independent evolution of both species in response to cadmium led to a change from competitive exclusion to coexistence in that stressful environment. This was due to

differential changes in intrinsic growth rates, which equalized fitness between species, coupled with an increase in intraspecific competition in the superior, but not in the inferior competitor, that in turn, increase the potential range of fitness differences compatible with the persistence of both species. In sum, we here show for the first time that evolution in response to an abiotic selection pressure can change the interactions between and within populations and affect the probability of coexistence, even without a direct selection pressure posed by the presence of competitors.

We found that both species adapted to the cadmium environment after 40 generations of experimental evolution in that environment, as evidenced by an increase in the intrinsic growth rate of populations from the cadmium selection regime, compared to those from the no-cadmium regime. At the 33rd generation of evolution, these population of *T. evansi* had not shown signs of adaptation[31], hence the adaptation reported here occurred at a later time point. Moreover this previous study revealed lower performance of *T. evansi* populations from the cadmium selection regime on the environment without cadmium, compared to populations evolving in the no-cadmium selection regime [31]. We do not recover this result, suggesting that extending time for evolution to operate led to reduced costs of adaptation. This adaptation pattern is compatible with the existence of cryptic genetic variation that is released upon evolution in the cadmium environment[33]. This is also in line with the absence of genetic variation for traits associated to performance in the cadmium environment, found in the populations used to initiate the experimental evolution. Still, such adaptation may be too slow to enable the establishment of mites in cadmium-contaminated sites. Indeed, assuming equal generation time in the lab and in the field, one tomato growing season roughly corresponds to 15 generations in spider mites, which is not sufficient to lead to genetic changes allowing adaptation to cadmium.

Evolution of *T. evansi* in cadmium also led to stronger sensitivity to intraspecific competition in that environment, as compared to mites evolving on plants without cadmium. Recently, evidence that intraspecific competitive ability may evolve during adaptation to a novel abiotic environment has been accumulating[5, 20, 34]. There are many reasons to expect such evolution. For example, individuals may become more efficient at extracting resources when these are limiting[5], or higher population growth may lead to higher densities being reached earlier, thus increasing intraspecific competition. Measuring intraspecific competition should thus be mainstreamed in experimental evolution studies, which typically measure only individual life-history traits such as fecundity and survival[35].

An overlooked logical follow-up of the fact that adaptation to abiotic selection pressures may affect intraspecific interactions is that it may affect interspecific interactions as well. Indeed, the latter are also based on (the relative values of) traits that may be modified by adaptation in absence of competitors. Here, we shed light on this poorly studied issue by documenting that evolving in a stressful environment without heterospecific competitors affects competitive interactions between species in that environment. Specifically, when spider mites evolved in cadmium, they affected their competitors as much or less than mites that evolved on plants with no-cadmium. This counter-intuitive result may be due to a growth-competition trade-off, given that both species also increased their growth rate on plants with cadmium upon evolution in that environment. Such trade-off has been an assumption of theoretical models[36], and experimentally demonstrated in *Brassica nigra*[37, 38]. Also, Limberger and Fussmann[20] report that algae adapt more rapidly to abiotic selection pressures when they evolve alone vs when in presence of competitors, which is compatible with the existence of such trade-off. In sum, we show that interspecific competitive ability evolves in absence of competitors, a result similar to when competing species coevolve[6, 11, 12, 18]. The fact that we recover such patterns even in the absence of a competitor in the environment implies both that causality needs to be scrutinized in eco-evolutionary studies and that evolution has consequences for community structure that are much more far reaching than initially thought.

Many empirical studies have shown that adaptation to an abiotic selection pressure can change species interactions[18, 20, 39] but none have addressed how it affects species coexistence. In fact, the only studies applying coexistence theory to evolutionary data concern adaptation to the presence of a competitor[11, 12, 15]. In our study, we found that, in the cadmium environment, species are only predicted to coexist when both competitors evolved separately in the presence of cadmium. Such change from competitive exclusion to coexistence was not due to the large change of a specific parameter but rather a small but full reorganization of how species grow and interact with each other. Namely, coexistence was due to a combination of an increase in the intrinsic growth rate of both species as they adapt to the cadmium environment, with stronger intraspecific competition of the superior competitor, coupled with a reduced impact of interspecific competition. Although the ultimate mechanisms of these changes are unclear, we speculate that evolution improved the ability of spider-mites to feed on tomato plants that accumulated cadmium, likely by increasing resource uptake, which in turn decreased resources available to others (thus increasing intraspecific competition). This positive covariance between an increase in fitness

and an increase in self-limitation is congruent with theoretical predictions[40], which have been previously reported in other systems (e.g. [41]). Additionally, the observed reduction in interspecific competition (leading often to reciprocal facilitation) for the two species could be due to the increased benefits from plant defence suppression, which was found in both populations used [27]. Curiously, studies addressing how evolution in the presence of a competitor affected species coexistence have not found such a radical effect of changing from exclusion to coexistence. Instead, they found that coexistence is maintained when species coevolve, albeit by mechanisms different than those operating in the absence of such evolution[11, 12, 15]. We thus find that adaptation to an abiotic selection pressure may affect community composition to a higher degree than direct adaptation to the presence of competitors.

We also provide a test for the accuracy of the theoretical predictions, by comparing relative abundance of both species obtained by model predictions based on parameters estimated from competition experiments with a short-term population dynamics experiment. We found a high overlap between predicted and observed relative abundances of the two species. Some mismatch was found when only one of the populations evolved in the cadmium environment. This is probably due to stochasticity, as the experiment started with a small number of individuals (six females of each species). Still, the strong correlation between observed and predicted values suggests that our model parameterization is robust to changes in the number of individuals, resource and space availability, indicating that the combination of theoretical modelling and experimental estimation of competitive interactions has a high predictive power.

Another strength of our study is that, unlike previous studies, we applied a full factorial design, in which we explore how different combinations of evolutionary histories of both competitors affect the probability of coexistence. We find that coexistence in an environment with cadmium is only possible when both species evolved in that environment. Hence, evolution does not follow a linear path, being contingent on the evolutionary history of the two species. This suggests that asynchronies in the arrival to cadmium-contaminated sites may lead to species exclusion, due to evolution of the species first colonizing that environment. Coexistence is only possible when the two species are both adapted, which can be achieved either if they arrive simultaneously to a site with cadmium, then adapt at a similar pace, or if they are already adapted upon arrival. Hence, as previously shown for short-term differences in arrival time[27], we here show that historical contingencies affect species coexistence also via their effect on evolution.

We also explored how evolution in response to cadmium affects coexistence outcomes in an environment without cadmium. As reported in other studies with no evolution, we found that interactions are specific to a given environment[42–45]. In general, our results align well with previous work showing that stable coexistence is fostered in stressful environments via a reduction in growth rate that equalize fitness differences, and by a shift from interspecific to intraspecific competition, which increases the range of coexistence opportunies (i.e. increases the size of the feasibility domain)[42–46], and stabilize the population dynamics of interacting species. However, our study tempers this statement by the finding that this is only true when both species have evolved in that stressful environment and that such stabilization only occurs in the environment where they evolved. Moreover, the use of the structural stability framework allows incorporating sensitivity to changes in environmental conditions in these coexistence predictions[47–49]. In the environment without cadmium, we found a small distance to the edge (i.e., low robustness) across all selection regimes, suggesting that changes in competitive outcomes are likely to occur due to stochastic events. Instead, in the cadmium environment, the distance to the edge was higher in all cases, suggesting that communities in that environment are more long-lasting. Thus, our results suggest that communities in cadmium-free environments will be modulated by small environmental changes, whereas those in environments with cadmium will be more shaped by evolution.

Our study highlights the added value of combining experimental evolution and controlled experiments with ecological theory, by providing novel insights of how species adaptation to an abiotic stressor affects their ability to coexist. This work provides significant advances in both evolutionary and ecological fields. On the one hand, it shows that traits such as the strength of intra- and interspecific competition should be incorporated in the characterization of species adaptation to novel environments. This is particularly true in experimental evolution studies, which generally quantify evolution by measuring classical life-history traits such as fecundity or offspring juvenile survival of single individuals[35, 50], which may not reflect the conditions in which populations evolve[51]. On the other hand, ecological studies benefit from incorporating past evolutionary history, as such approach has the capacity to refine our understanding of how species interact and coexist[36, 52–54]. All in all, our work provides novel arguments for the need to combine ecological and evolutionary perspectives and methodologies to understand community composition.

Material and methods

Overview of the experimental system and maintenance conditions

We used a system composed of *Tetranychus urticae*, a generalist spider mite species that feeds on several economically important crops[55], *T. evansi*, a solanaceous specialist that has recently invaded Europe[56], and tomato plants (*Solanum lycopersicum, var* MoneyMaker). Population maintenance and experiments were performed in a climatic room under controlled conditions (25:20 ºC, 65% of humidity, light:dark = 16:8). Outbred populations of each species were maintained using 5-week-old plants, having at least five fully expanded leaves. Plants used in all experiments (including in the experimental evolution) were watered twice a week with 100 mL of tap water for a duration of two weeks, then transplanted to new pots and watered each week once with 100mL tap water and another two times with either 100mL of distilled water or 100mL of 2mM cadmium chloride solution during another three weeks. This cadmium concentration is highly detrimental to both spider-mite species, resulting in at least a 50% reduction in fecundity as compared to mites on metal-free plants[29, 30].

Experimental evolution

Outbred populations, formed via controlled, one-on-one crosses of 400 individuals (200 females and 200 males of each population) of three and four field populations of *T. urticae* and *T. evansi* spider mites, respectively[57] were used to initiate experimental evolution populations. The latter were created by transferring 220 adult females of each species from the outbred populations to an experimental box (size: 26 x 18 x 13.5 cm) covered with a lid with a gauze-covered opening to allow air circulation. The box contained four tomato leaves with their stems in a small pot with water (two leaves per pot, two pots per box), replenished every week. To minimize differences among plants and leaves, each box contained the second, third, fourth and fifth leaves to develop (starting from the cotyledons), taken from a different plant. We established five populations for each of four selection regimes: *T. urticae* or *T. evansi*, exposed to leaves from plants watered either with a 2mM cadmium solution (the cadmium selection regime) or with water only (the no-cadmium selection regime). Every two weeks (corresponding to a mite generation), 220 adult females were transferred by aspiration to a new experimental box containing four new tomato leaves (i.e., populations were maintained in discrete generations). The remaining mites were transferred to a new box with two tomato leaves to create a T-1 box. These mites were used as a back-up when less than 220 females were found in the experimental box. If still this did not reach 220 mites, females

from the base population were added until 220 adult females were reached (cf. [57] for details).

Prior to the experiments, individuals from all regimes were placed in a common garden of cadmium-free tomato leaves for two generations. For that, 200 females from each population were transferred to new boxes (100 per box) with two tomato leaves, except for replicate 2 of *T. urticae* evolving on leaves with cadmium, which did not produce enough individuals and was thus not used in subsequent experiments. After three weeks, six cohorts of 50 adult females were transferred to petri dishes with cotton soaked in water and two tomato leaflets. Females were left to oviposit for four days, and then killed. Two weeks later, the adult female offspring was collected for the experiment. Experiments were performed at generation 40 (empirical estimation of competitive ability and intrinsic growth rate) and 42 (estimation of the growth rate of populations in cages with interspecific competitors).

Empirical estimation of competitive abilities and intrinsic growth rate

To test if evolution on plants with or without cadmium affected the probability of coexistence between *T. urticae* and *T. evansi* in environments with or without cadmium, we estimated the intrinsic growth rates and the strength of intra- and interspecific interactions of mites from each selection regime in each environment (Fig. S1). The data generated was then used to estimate parameters of a population model that describes the dynamics of interacting species (cf. below).

To quantify changes in growth rate with different competition scenarios and estimate the parameters to predict coexistence outcomes, we followed the methodology described in [58]. For that, we cut leaf discs (18mm diameter) from tomato plants grown with or without cadmium and placed one female alone or with 1, 3 or 9 females (i.e., competitors). To estimate intraspecific competition, the competitor females were from the same species, selection regime (cadmium or no-cadmium) and replicate population as the focal female. To estimate interspecific competition, the focal females were exposed to females from different species, either from the same or a differnet selection regime (full factorial design) with matching population replicates (replicate 1 of *T. urticae* was exposed to replicate 1 of *T. evansi*, etc). The intrinsic growth rate was estimated from leaf discs with single females.

Leaf discs were placed on water-saturated cotton inside a square petri dish (size: 12.6 x 12.6 x 1.6 cm, with a gauze covered top to allow for ventilation), with 16 or 12 discs (for intra and interspecific assays, respectively). Each petri dish corresponded to one environment (leaf discs from plants exposed to cadmium or not) and one treatment (a combination of the

selection regimes of the focal female and of the competitors – in the case of intraspecific competition these are the same), with each density of competitors being allocated to one row within the petri dish (i.e., each petri dish had four replicates of all densities). Females were left to oviposit for three days and then killed. Two weeks later, the number of adult females per patch was counted. Ten replicates per intra- or intraspecific treatment were done for each of the five experimental populations. The experiment was done in six blocks, the first three with replicate populations one to three and the last with replicate populations four and five.

Empirical estimation of population growth rate in presence of interspecific competitors

To test if evolution on plants with or without cadmium affected the population growth rate under competition, we placed six females of the two species from different selection regimes on plants with or without cadmium (full factorial design, *T. urticae* cadmium / no-cadmium * *T. evansi* cadmium / no-cadmium * plants with / without cadmium, i.e., eight experimental conditions) and measured the number of adult females produced after two generations. For that, we placed two leaves from plants with or without cadmium in plastic boxes (size: 16.5 x 16.5 x 12 cm) covered with a lid with a gauze-covered opening to allow air circulation. The petiole of the leaves was in a small pot with water. After one mite generation (two weeks), another two leaves were added to the box. By the end of the second generation, we counted the number of adult females from each species in each box. Ten experimental replicates were tested per experimental condition. The experiment was done in five blocks, each including one replicate population of each selection regime. This data was then compared with model predictions based on parameters estimated from the intrinsic growth rate and competitive abilities (cf. Model validation section below).

Theoretical estimation of competition and growth parameters

Data collected in the intrinsic growth rate and competitive ability experiment was used to parameterize a model, from which we quantified niche and fitness differences to predict the outcome of competition between the two spider mite species. We assume that the population dynamics in our experiment can be described by Ricker's competition model[32], which has been used in a previous study with spider mites[59]. Unlike the often-used Beverton-Holt model[60], this model allows incorporating positive interactions (i.e., facilitation), which based on initial data scrutiny, were likely to occur in our system. The Ricker model is described by the following equation (equation 1):

$$(1)\ \frac{N_{i,t+1}}{N_{i,t}} = \lambda_i e^{-\alpha_{ii}N_{i,t}-\alpha_{ij}N_{j,t}}$$

where $N_{i,t+1}$ is the number of individuals of species *i* in the next generation, λ_i the intrinsic growth rate of species *i* in absence of competitors, α_{ii} corresponds to the strength of intraspecific competition, which describes the per-capita effect of species *i* on itself, α_{ij} is the strength of interspecific competition, which describes the per-capita effect of species *j* on species *i*, and $N_{i,t}$, $N_{j,t}$ are the number of competitors of species *i* and *j*, respectively, in the current generation. To fit the Ricker model, we used the function cxr_pm_fit from the "cxr" package[61] in R to estimate λ_i, α_{ii} and α_{ij} for all selection regimes. The parameters were estimated for each replicate population separately. To ensure enough statistical power (as survival in the cadmium environment was low), we also pooled the data of all replicates associated with each selection regime. As most females (across replicate populations) did not produce offspring in the cadmium environment, and the cxr package does not allow the inclusion of zeros, we transformed our data by summing one to each of our datapoints (since the logarithm of one is zero, we can this way include the filtering effect of cadmium in our data). Initial parameters were set at λ_i=1, α_{ii}=α_{ij}=0.1 and confidence intervals were estimated using 1000 bootstrap samples. Visual inspection of the model fitting was done for each combination of parameters, selection regimes and environments by plotting predicted vs. observed values (figures available in the git repository). To test if the cxr package produced the best fit to our data, we compared the euclidean distance between predicted and observed estimates for 5 different fitting algorithms (Fig. S9), all using the Ricker model (details are available in the git repository). To estimate the relative impact of intra- and interspecific competition in the offspring production of the two spider mite species, we simulated the number of offspring produced after one generation using the estimated parameters and the Ricker model. We used as a starting condition 10 individuals of each species and simulated one generation assuming population growth following only the intrinsic growth rate, joint effects of intrinsic growth rate and of intraspecific competition and joint effects of the intrinsic growth rate and both intra- and interspecific competition. Confidence intervals for the predictions were calculated using the lower and upper estimates of the intrinsic growth rate and intra and interspecific competition indexes obtained from the cxr package [61].

Structural stability approach to predict coexistence

Estimates obtained from the Ricker model (Fig. S4, S5, S7, S8) showed widespread weak positive interspecific interactions (i.e., facilitation) across selection regimes and replicate populations. Since Modern Coexistence Theory does not incorporate facilitation between species, we used the structural stability framework[22, 47, 62] to predict the outcomes of species interactions in our system. Based on the strength and sign of species interactions (including competition and facilitation), this approach calculates the feasibility domain. The size of this domain measures the range of species intrinsic growth rates compatible with species coexistence (bounded by the vectors [0, $\frac{\alpha_{22}}{\alpha_{12}}$] and [0, $\frac{\alpha_{21}}{\alpha_{11}}$] when considering only positive growth rates). The main prediction from structural stability is that if the vector containing differences between species in their intrinsic growth rate falls within the feasibility domain, coexistence is possible, otherwise there is exclusion. In principle, the feasibility domain can include both negative and positive intrinsic growth rates, however, we restricted our coexistence predictions only to positive values, as our experimental design would not allow for negative growth rates. Finally, to account for uncertainty in the estimation of model parameters, we also estimated the feasibility domain bound by the upper (lower) 95% of the vector [0, $\frac{\alpha_{22}}{\alpha_{12}}$] and the lower (upper) boundaries of the [0, $\frac{\alpha_{21}}{\alpha_{11}}$] to obtain the widest (narrowest) conditions under which species are able to coexist.

To measure each species' vulnerability to exclusion, we tested how resistant coexistence is to perturbations. Following a recently-developed approach[47, 63], we estimated the species exclusion distance, which corresponds to the minimal distance between the vector of intrinsic growth rates (r= [λ_I, λ_j]) and the edges of the feasibility domain (vectors corresponding to either [0, $\frac{\alpha_{22}}{\alpha_{12}}$] or[0, $\frac{\alpha_{21}}{\alpha_{11}}$]) as a proxy for how strong a perturbation must be to change the coexistence outcome in each system. To calculate the distance between the vector of intrinsic growth rates and the edges of the feasibility domain, we applied the following formula[47]:

$$(2)\ dist_i = \cos^{-1}\begin{bmatrix}\lambda_i\\ \lambda_j\end{bmatrix}\cdot\begin{bmatrix}\alpha_{21}\\ \alpha_{11}\end{bmatrix}$$

Model validation

We validated our approach by comparing model predictions with the empirical results from the population growth experiment. For that, we used the Ricker model to predict the relative abundance of each species after two generations in each treatment, starting with the initial conditions of the experiment. Then, we performed a general linear regression with a binomial error distribution to estimate the correlation between the mean observed proportions of each

competitor (using the cbind function to join the number of *T. evansi* and *T. urticae* females) in all replicates of all treatments with the model proportion estimates (Number of *T. evansi* females/Number of *T. urticae* females) obtained for each replicate.

Statistical analyses

To test the impact of the cadmium environment on the intrinsic growth rate of *T. urticae* and *T. evansi* populations that were naïve to this selection pressure, we applied, for each species, a linear model using as dependent variables the estimates of the intrinsic growth rate (estimated from the cxr, see above) of the five experimental replicates of the no-cadmium selection regimes and environment as fixed factor (with two levels, cadmium and no-cadmium)

To test how evolution on plants with cadmium affected population growth and competition in the cadmium environment, we used general linear models with a gamma distribution for the intrinsic growth rate, and with a normal distribution for the coefficients for intra- and interspecific competition. All models were applied separately for both species, and normality and dispersion of the residuals was inspected for all models tested. For all models, we included the parameters estimated with the cxr package for each replicate as a dependent variable and Selection Regime as fixed factor (with two levels corresponding to selection regimes evolving in cadmium or no-cadmium environment). For the strength of interspecific competition, the model included the selection regime of the focal and of the competitor species, as well as their interaction as fixed factors. Whenever the interaction term was significant, we applied contrasts to compare estimates for all combinations of focal and competitor selection regimes. Similar models were applied to test the impact of evolution in cadmium on the performance in the no-cadmium environment. Finally, we applied a bootstrap technique to estimate the likelihood of finding differences between environments/selection regimes randomly. For that, we randomized (with replacement) the parameter estimates within environments/selection regimes (depending on the model) and applied the same models as described above. Then, we estimated the probability of randomly obtaining a significant p-value (below or equal to 0.05) with our data set by computing the number of times a significant result was obtained. For each model we performed 1000 samples.

All analyses were done using R 4.2.1 version[64], statistical analyses were done using the package “glmmTMB”[65], contrasts were performed using the “emmeans” package[66], analyses of residuals were done for each model using the DHARMa package[67], and

graphical representation was done using “ggplot2”[68]. All data scripts and data used for the analyses are available in the git repository https://github.com/irfragata/evolution_cadmium_coexistence.

Authorship Statement

IF, OG and SM conceived the original idea. IF, DG, OG and SM designed the experiments. IF, DG, LRR and MC performed the experiments and collected the data. IF performed the modelling work and analysed data, with advice from FZ and OG. IF produced the figures, with help from LRR. IF, SM and OG wrote the first draft of the manuscript, and all authors contributed substantially to revisions. SM supervised DG, IF, LRR and MC, FZ supervised MC, OG supervised IF. SM provided the financial support for the experiments, SM and OG for the meetings to analyze the data and write the paper.

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Competing interests: Authors declare no competing interests.

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Figures

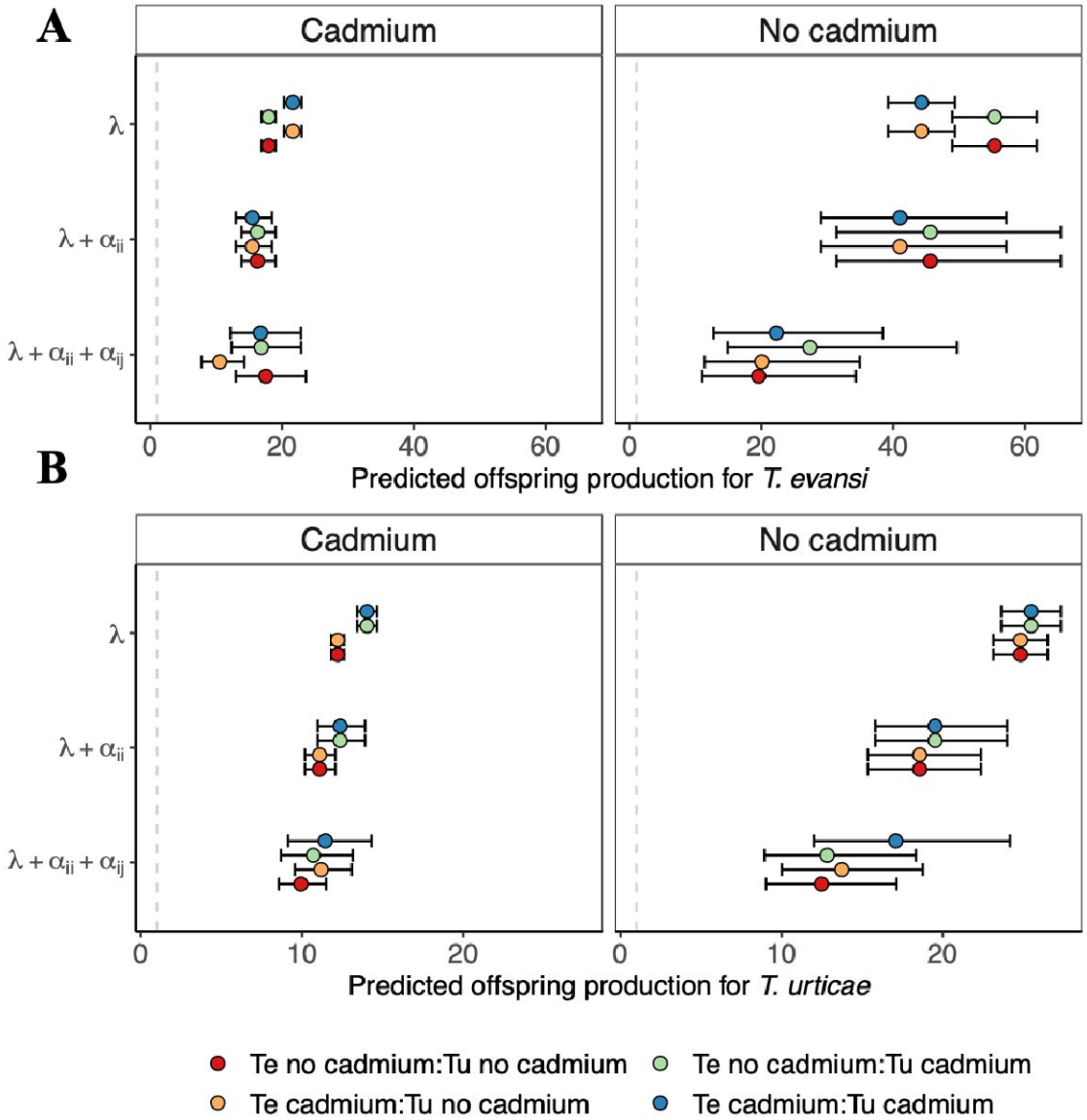

Figure 1 – Predicted number of female offspring after one generation for A) *T. evansi* (Te) and B) *T. urticae* (Tu), to estimate the relative effect of competition on the growth rate of the different populations. To represent the cumulative impact of each parameter on the offspring production, predictions were done using only the intrinsic growth rate), the intrinsic growth rate plus intraspecific competition (and the latter plus interspecific competition (in an environment with or without cadmium (left and right panels, respectively). Note that since two of the four treatments share the same focal selection regime (e.g. for *T. urticae* the orange and red dots correspond to the focal control regime "Tu no-cadmium"), two colours are duplicates for the metrics without interspecific competition

(and). Starting conditions for simulations: 10 individuals of each species. Treatments correspond to combinations of *T. evansi* (Te) and *T. urticae* (Tu) selection regimes (cf. colour code). Note the different scales on the X-axis between the two species (A vs B).

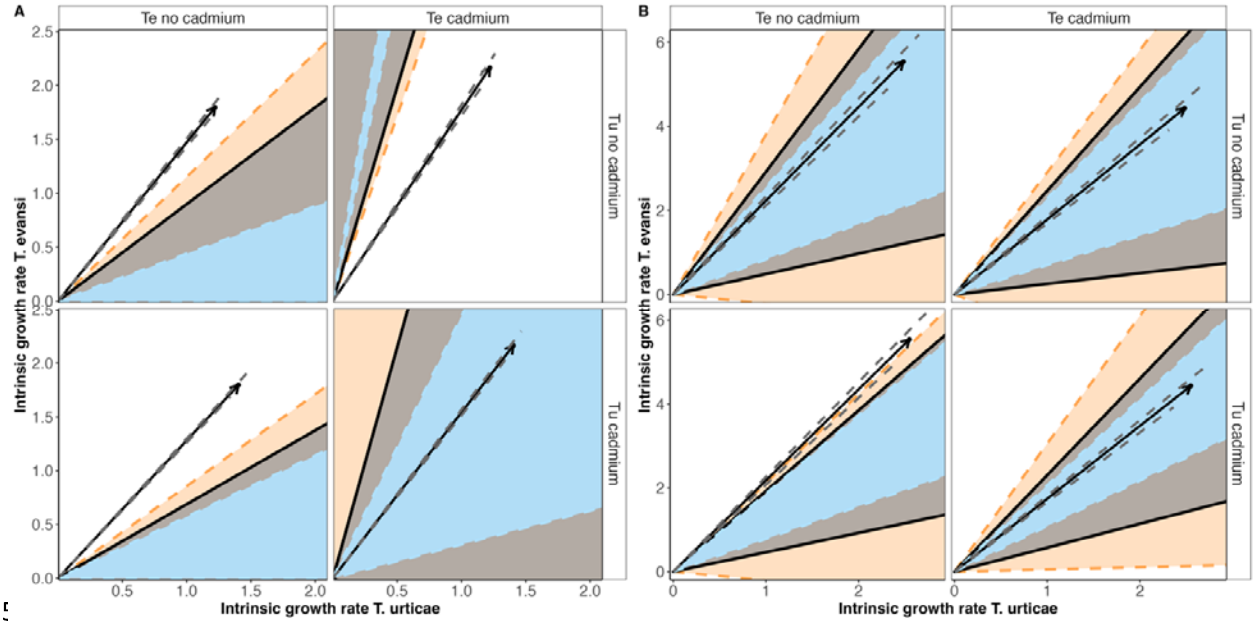

Figure 2 – Feasibility domain predicted for enviromments with (A) or without (B) cadmium for the different combinations of cadmium and no-cadmium selection regimes in which *T. urticae* (Tu) or *T. evansi* (Te) have evolved. The x and y axis correspond to the positive intrinsic growth rate for *T. urticae* and *T. evansi*, respectively. The arrow represents the vector of intrinsic growth for each of the two species and the edges of the cone delimiting the area under which the isoclines cross at positive abundances (i.e., the feasibility domain, in which coexistence is possible). Dashed lines surrounding the arrows represent the 95% confidence interval of the intrinsic growth rate. Arrows that fall outside of the cone indicate that Te excludes Tu, except in the upper-right panel of figure A (Te cadmium- Tu no-cadmium), in which Tu excludes Te. The dark grey region indicates the feasibility domain obtained from the ratio of intra and interspecific competitive abilities estimated with data from all replicates pooled (see methods). This region has been evaluated only under the conditions in which both species show positive intrinsic growth rates. The light blue and light orange regions delimitate the smallest and largest feasibility domains obtained from the lower and upper 95% confidence interval of the competitive ability parameters. White regions denote the unfeasibility domain (region for exclusion). Confidence intervals of the estimates

were obtained by performing 1000 bootstrap samples with replacement when estimating parameters from the data of all experimental replicates pooled.

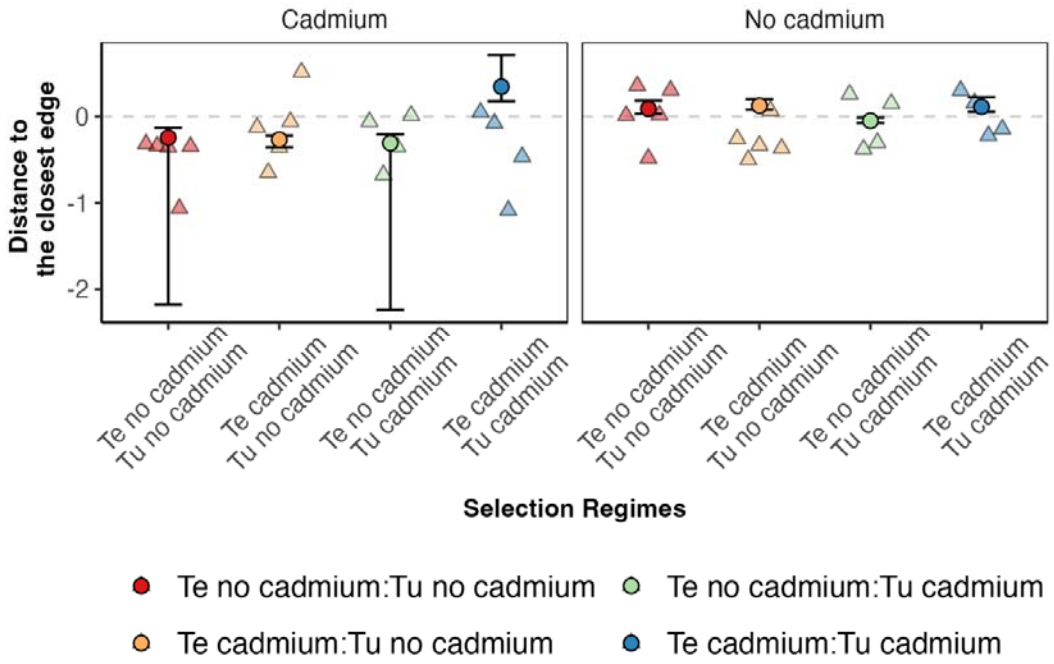

Figure 3 – Distance between the vector of intrinsic growth rates and the closest edge of the feasibility domain in environments with (left panel) or without (right panel) cadmium for the different combinations of no-cadmium or cadmium selection regimes for *T. evansi* (Te) and for *T. urticae* (Tu) (cf. colour code). Positive distances indicate that the vector of growth rates is inside the feasibility domain (i.e., coexistence is possible), and negative distances indicate that the vector is outside the feasibility domain (i.e., exclusion is predicted). Circles correspond to the distance calculated with data from all replicates pooled and triangles to distances calculated per replicate. Error bars were obtained by performing 1000 bootstrap samples with replacement when estimating parameters and were obtained from the data of all experimental replicates pooled.

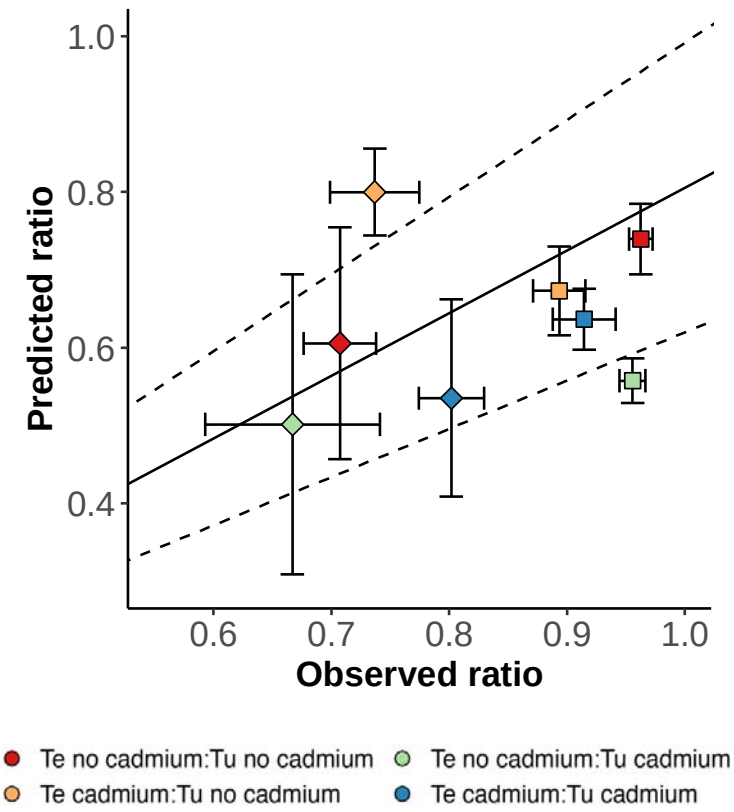

Figure 4 – Correlation between predicted and observed relative abundance of *T. evansi* (Number of *T. evansi* females/Number of females of both species) after 2 generations in an independent population growth experiment in which the two species are released at equal densities. Squares indicate data from the cadmium environment and diamonds data from the no-cadmium environment. Predicted error bars were calculated based on standard error obtained from 1000 bootstrap samples and observed error bars were obtained from replicate populations. The full line represents the slope obtained from a general linear model (see methods) and the dashed lines to the standard error obtained from the model. Treatments correspond to combinations of selection regimes to which *T. evansi* (Te) and *T. urticae* (Tu) were exposed (cf. colour code).