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 in cadmium-naïve mite populations the most likely outcome is exclusion. The shift from competitive exclusion to coexistence was due to an increase in niche differences accompanied by a decrease in fitness differences. A parallel population experiment validated these predictions. However, such changes were environment-specific, as spider-mite adaptation to cadmium did not affect species interactions and competitive outcomes in the cadmium-free environment. Our results suggest that evolution of single species in a new environment, even in absence of interspecific competitors, shapes species coexistence. Therefore, population shifts to novel environments may have unforeseen evolutionary consequences for community composition and the maintenance of species diversity.

Introduction

Understanding how evolutionary dynamics shape and are shaped by long-term species persistence is key to predict community composition and biodiversity maintenance. 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 interactions other than direct competition, such as mutualism[26]. This framework defines a feasibility domain, which is determined by the magnitude of niche differences. Coexistence is possible when the vectors defined by the intrinsic growth rates fall within this domain. Wider feasibility domains allow for the persistence of inferior competitors for species combinations with more uneven intrinsic growth rates[22,25]. To date, this tool has so far 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 and 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

Spider mites of both species evolved on plants with or without cadmium for *circa* 40 generations. 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), confirming our hypothesis.

After evolution, 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 on plants with cadmium was higher than that of populations evolving on plants with no cadmium, 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. 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), but not for *T. urticae* ($\chi^2_{1,6}$ = 0.131, P-value=0.717, Fig S3B). This resulted in a decreased predicted number of offspring produced by *T. evansi* in competition (relative to when growing alone) but only in the treatments in which *T. evansi* evolved in the cadmium environment (Fig. 1A, λ vs $\lambda+\alpha_{ii}$ for the yellow and blue vs the green and red dot). Moreover, evolving in the cadmium selection regime made *T. evansi* more sensitive to competition with *T. urticae* from the no cadmium selection regime (Fig. S4A, Table S1A, S1B, 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 S1A). 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, evolution in cadmium 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), 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) or interspecific competition (Fig. S7, Table S2). 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 (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). This occurs because evolution of both species in cadmium stabilized their interactions (i.e., increased their niche differences) and equalized their fitness differences (Fig. 2). Indeed, evolution in cadmium led to increased intraspecific competition only in *T. evansi*, the better competitor, (Fig S3) and to an increase in the intrinsic growth rate in both species, but with a smaller magnitude in *T. urticae* (Fig S2). This coexistence prediction is robust to relatively large variation in the species intrinsic growth rates, as reported by the distance between the species observed intrinsic growth rates and the edge of the feasibility domain (Fig. 3, S8, S9). 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, S9). In the case when *T. evansi* 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 from the *T. evansi* no cadmium selection regime but did not affect it (Fig. S4), which led to a prediction of exclusion in the cadmium environment (Fig. 4).

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), 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 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 S3). 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 (that equalized fitness differences between species) coupled with an increase in intraspecific competition in the superior, but not in the inferior competitor. 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.

198 We found that both species adapted to the cadmium environment after 40 generations of
199 experimental evolution in that environment, as evidenced by an increase in the intrinsic growth
200 rate of populations from the cadmium selection regime, compared to those from the no-
201 cadmium regime. At the 33rd generation of evolution, these population of *T. evansi* had not
202 shown signs of adaptation[31], thus the adaptation reported here occurred after that generation.
203 In line with this result, genetic variation for life-history traits on cadmium was absent in the
204 base population[31]. In addition, this previous study revealed lower performance of *T. evansi*
205 populations from the cadmium selection regime on the cadmium-free environment, compared
206 to populations evolving in the latter [31]. We do not recover this result, suggesting that extending
207 time for evolution to operate led to reduced costs of adaptation. This adaptation pattern is
208 compatible with the existence of cryptic genetic variation that is released upon evolution in the
209 cadmium environment[33]. Still, such adaptation may be too slow to enable the establishment of
210 mites in cadmium-contaminated sites. Indeed, assuming equal generation time in the lab and
211 in the field, one tomato growing season roughly corresponds to 15 generations in spider mites,
212 which is not sufficient to lead to genetic changes allowing adaptation to cadmium.
213 Evolution of *T. evansi* in cadmium also led to stronger sensitivity to intraspecific
214 competition in that environment, as compared to mites evolving on plants without cadmium.
215 Recently, evidence that intraspecific competitive ability may evolve during adaptation to a
216 novel abiotic environment has been accumulating[5,20,34]. There are many reasons to expect such
217 evolution. For example, individuals may become more efficient at extracting resources when
218 these are limiting[5], or higher population growth may lead to higher densities being reached
219 earlier, thus increasing intraspecific competition. Measuring intraspecific competition should
220 thus be mainstreamed in experimental evolution studies, which typically measure only
221 individual life-history traits such as fecundity and survival[35].
222 An overlooked logical follow-up of the fact that adaptation to abiotic selection pressures
223 may affect intraspecific interactions is that it may affect interspecific interactions as well.
224 Indeed, the latter are also based on (the relative values of) traits that may be modified by
225 adaptation in absence of competitors. Here, we shed light on this poorly studied issue by
226 documenting that evolving in a stressful environment without heterospecific competitors
227 affects competitive interactions between species in that environment. Specifically, when spider
228 mites evolved in cadmium, they affected their competitors as much or less than mites that
229 evolved on plants with no cadmium. This counter-intuitive result may be due to a growth-
230 competition trade-off, given that both species also increased their growth rate on plants with
231 cadmium upon evolution in that environment. Such trade-off has been an assumption of

232 theoretical models[36], and experimentally demonstrated in *Brassica nigra*[37,38]. Also, Limberger
233 and Fussmann[20] report that algae adapt more rapidly to abiotic selection pressures when they
234 evolve alone vs when in presence of competitors, which is compatible with the existence of
235 such trade-off. In sum, we show that interspecific competitive ability evolves in absence of
236 competitors, a result similar to when competing species coevolve[6,11,12,18]. The fact that we
237 recover such patterns even in the absence of a competitor in the environment implies both that
238 causality needs to be scrutinized in eco-evolutionary studies and that evolution has
239 consequences for community structure that are much more far reaching than initially thought.
240 Many empirical studies have shown that adaptation to an abiotic selection pressure can
241 change species interactions[18,20,39] but none have addressed how it affects species coexistence.
242 In fact, the only studies applying coexistence theory to evolutionary data concern adaptation to
243 the presence of a competitor[11,12,15]. In our study, we found that, in the cadmium environment,
244 species are only predicted to coexist when both competitors evolved separately in the presence
245 of cadmium. Such change from competitive exclusion to coexistence was not due to the large
246 change of a specific parameter but rather a small but full reorganization of how species grow
247 and interact with each other. Namely, coexistence was due to a combination of an increase in
248 the intrinsic growth rate of both species as they adapt to the cadmium environment, with
249 stronger intraspecific competition of the superior competitor, coupled with a reduced impact
250 of interspecific competition. Curiously, studies addressing how evolution in the presence of a
251 competitor affected species coexistence have not found such a radical effect of changing from
252 exclusion to coexistence. Instead, they found that coexistence is maintained when species
253 coevolve, albeit by mechanisms different than those operating in the absence of such
254 evolution[11,12,15]. We thus find that adaptation to an abiotic selection pressure may affect
255 community composition to a higher degree than direct adaptation to the presence of
256 competitors.
257 We also provide here a test for the accuracy of the theoretical predictions, by comparing
258 relative abundance of both species obtained by model predictions based on parameters
259 estimated from competition experiments with a short-term population dynamics experiment.
260 We found a high overlap between predicted and observed relative abundances of the two
261 species. Some mismatch was found when only one of the populations evolved in the cadmium
262 environment. This is probably due to stochasticity, as the experiment started with a small
263 number of individuals (six females of each species). Still, the strong correlation between
264 observed and predicted values suggests that our model parameterization is robust to changes in
265 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 with no cadmium. As found in studies with no evolution, we found that interactions are specific to a given environment[40–43]. 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 stabilizing niche differences[40–44]. However, our study tempers this statement by the finding that this is only true when both species have evolved in that stressful environment. |Moreover, the use of the structural stability framework allows incorporating sensitivity to changes in environmental conditions in these coexistence predictions[45–47]. In the no cadmium environment, 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. Our work provides significant advances in both evolutionary and ecological fields. On the one hand, it shows that traits such as the strength

300 of intra- and interspecific competition should be incorporated in the characterization of species
301 adaptation to novel environments. This is particularly true in experimental evolution studies,
302 which generally quantify evolution by measuring classical life-history traits such as fecundity
303 or offspring juvenile survival of single individuals[35,48], which may not reflect the conditions in
304 which populations evolve[49]. On the other hand, ecological studies benefit from incorporating
305 past evolutionary history, as such approach has the capacity to refine our understanding of how
306 species interact and coexist[36,50–52]. All in all, our work provides novel arguments for the need
307 to combine ecological and evolutionary perspectives and methodologies to understand
308 community composition.
309

310 # Material and methods

311 **Overview of the experimental system and maintenance conditions**

312 We used a system composed of *Tetranychus urticae*, a generalist spider mite species that feeds
313 on several economically important crops[53], *T. evansi*, a solanaceous specialist that has recently
314 invaded Europe[54], and tomato plants (*Solanum lycopersicum*, *var* MoneyMaker). Population
315 maintenance and experiments were performed in a climatic room under controlled conditions
316 (25:20 ºC, 65% of humidity, light:dark = 16:8). Outbred populations of each species were
317 maintained using 5-week-old plants, having at least five fully expanded leaves. Plants used in
318 all experiments (including in the experimental evolution) were watered twice a week with 100
319 mL of tap water for a duration of two weeks, then transplanted to new pots and watered each
320 week once with 100mL tap water and another two times with either 100mL of distilled water
321 or 100mL of 2mM cadmium chloride solution during another three weeks. This cadmium
322 concentration is highly detrimental to both spider-mite species, resulting in at least a 50%
323 reduction in fecundity as compared to mites on metal-free plants[29,30].
324

325 **Experimental evolution**

326 Outbred populations, formed via controlled, one-on-one crosses of 400 individuals (200
327 females and 200 males of each population) of three and four field populations of *T. urticae* and
328 *T. evansi* spider mites, respectively[55] were used to initiate experimental evolution populations.
329 The latter were created by transferring 220 adult females of each species from the outbred
330 populations to an experimental box (size: 26 x 18 x 13.5 cm) covered with a lid with a gauze-
331 covered opening to allow air circulation. The box contained four tomato leaves with their stems
332 in a small pot with water (two leaves per pot, two pots per box), replenished every week. To

333 minimize differences among plants and leaves, each box contained the second, third, fourth
334 and fifth leaves to develop (starting from the cotyledons), each taken from a different plant.
335 We established five populations for each of four selection regimes: *T. urticae* or *T. evansi*,
336 exposed to leaves from plants watered either with a 2mM cadmium solution or with water only.
337 Every two weeks (corresponding to a mite generation), 220 adult females were transferred by
338 aspiration to a new experimental box containing four new tomato leaves (i.e., populations were
339 maintained in discrete generations). The remaining mites were transferred to a new box with
340 two tomato leaves to create a T-1 box. These mites were used as a back-up when less than 220
341 females were found in the experimental box. If still this did not reach 220 mites, females from
342 the base population were added until 220 adult females were reached (cf. [55] for details).
343 Prior to the experiments, individuals from all regimes were placed in a common garden of
344 cadmium-free tomato leaves for two generations. For that, 200 females from each population
345 were transferred to new boxes (100 per box) with two tomato leaves, except for replicate 2 of
346 *T. urticae* evolving on leaves with cadmium, which did not produce enough individuals and
347 was thus not used in subsequent experiments. After three weeks, six cohorts of 50 adult females
348 were transferred to petri dishes with cotton soaked in water and two tomato leaflets. Females
349 were left to oviposit for four days, and then killed Two weeks later, the adult female offspring
350 was collected for the experiment. Experiments were performed at generation 40 (empirical
351 estimation of competitive ability and intrinsic growth rate) and 42 (estimation of the growth
352 rate of populations in cages with interspecific competitors).
353
354 **Empirical estimation of competitive abilities and intrinsic growth rate**
355 To test if evolution on plants with or without cadmium affected the probability of coexistence
356 between *T. urticae* and *T. evansi* in environments with or without cadmium, we estimated the
357 intrinsic growth rates and the strength of intra- and interspecific interactions of mites from each
358 selection regime in each environment (Fig. S1). The data generated was then used to estimate
359 parameters to be used in a population model that describes the dynamics of interacting species
360 (cf. below).
361 To quantify changes in growth rate with different competition scenarios and estimate the
362 parameters to predict coexistence outcomes, we followed the methodology described in [56]. For
363 that, we cut leaf discs (18mm diameter) from tomato plants grown with or without cadmium
364 and placed one female alone or with 1, 3 or 9 females (i.e., competitors). To estimate
365 intraspecific competition, the competitor females were from the same species, selection regime
366 (with or without cadmium) and replicate population as the focal female. To estimate

interspecific competition, the focal females and the competitor females were necessarily from different species, and all possible combinations of selection regime (with or without cadmium - full factorial design) with matching population replicates (replicate 1 of *T. urticae* was exposed to replicate 1 of *T. evansi*, etc) were performed. 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 (cadmium or no cadmium) 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 the growth rate of populations exposed to 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).

401 **Theoretical estimation of competition and growth parameters**

402 Data collected in the intrinsic growth rate and competitive ability experiment was used to
403 parameterize a model, from which we quantified niche and fitness differences to predict the
404 outcome of competition between the two spider mite species. We assume that the population
405 dynamics in our experiment can be described by Ricker's competition model[32], which has been
406 used in a previous study with spider mites[57]. Unlike the often-used Beverton-Holt model[58], this
407 model allows incorporating positive interactions (i.e., facilitation), which based on initial data
408 scrutiny, were likely to occur in our system. The Ricker model is described by the following
409 equation (equation 1):

410

411 $$(1)\ \frac{N_{i,t+1}}{N_{i,t}} = \lambda_i e^{-\alpha_{ii}N_{i,t}-\alpha_{ij}N_{j,t}}$$

412

413 where $N_{i,t+1}$ is the number of individuals of species i in the next generation, λ_i the intrinsic
414 growth rate of species i in absence of competitors, α_{ii} is the coefficient representing the
415 strength of intraspecific competition, that describes the per-capita effect of species i on itself,
416 α_{ij} is the coefficient representing the strength of interspecific competition, which describes the
417 per-capita effect of species j on species i, and $N_{i,t}$, $N_{j,t}$ are the number of competitors of species
418 i and j, respectively, in the current generation. To fit the Ricker model, we used the function
419 cxr_pm_fit from the "cxr" package[59] in R to estimate λ_i, α_{ii} and α_{ij} for all selection regimes.
420 The parameters were estimated for each replicate population separately. To ensure enough
421 statistical power (as survival in the cadmium environment was low), we also pooled the data
422 of all replicates associated with each selection regime. As most females (across replicate
423 populations) did not produce offspring in the cadmium environment, and the cxr package does
424 not allow the inclusion of zeros, we transformed our data by summing one to each of our
425 datapoints (since the logarithm of one is zero, we can this way include the filtering effect of
426 cadmium in our data). Initial parameters were set at λ_i=1, α_{ii}=α_{ij}=0.1 and confidence intervals
427 were estimated using 1000 bootstrap samples. Visual inspection of the model fitting was done
428 for each combination of parameters, selection regimes and environments by plotting predicted
429 vs. observed values (figures available in the git repository). To estimate the relative impact of
430 intra and interspecific competition in the offspring production of the two spider mite species,
431 we simulated the number of offspring produced after one generation using the estimated
432 parameters and the Ricker model. We used as a starting condition 10 individuals of each species

433 and simulated one generation assuming population growth following only the intrinsic growth
434 rate, the joint effects of intrinsic growth rate and of intraspecific competition and joint effects
435 of the intrinsic growth rate and intra and interspecific competition. Confidence intervals for the
436 predictions were calculated using the lower and upper estimates of the intrinsic growth rate and
437 intra and interspecific competition indexes obtained from the cxr package.
438
439 **Structural stability approach to predict coexistence**
440 Estimates obtained from the Ricker model (Fig. S4, S5, S7, S8) showed widespread weak
441 positive interspecific interactions (i.e., facilitation) across selection regimes and replicate
442 populations. Since Modern Coexistence Theory does not incorporate facilitation between
443 species, we used the structural stability framework[22,45,60] to predict the outcomes of species
444 interactions in our system. This approach generates a cone shaped feasibility domain
445 representing the range of species interactions (including competition and facilitation) that allow
446 for coexistence. If the vector with the species intrinsic growth rate falls within the feasibility
447 domain, coexistence is possible, otherwise there is exclusion. Using the parameters estimated
448 with the Ricker model (cxr package), we calculated the structural niche differences (Ω), defined
449 as the normalized solid angle of the cone that represents the feasibility domain of a system[22],
450 and structural fitness differences (θ), as the angle between the vector of intrinsic growth rates
451 of the species (r) and the centroid of the feasibility cone (r_c). For a system to be feasible (that
452 is, for coexistence to occur) the fitness differences (measured as a degree) must be 45 times
453 lower than the niche differences. This corresponds to individuals of each species affecting
454 conspecifics more than heterospecifics and fitness differences being smaller than niche
455 differences. Note that these are also the conditions for coexistence to occur in Modern
456 Coexistence Theory[22,25].
457 To measure each species' vulnerability to exclusion, we tested how resistant coexistence is
458 to perturbations. Following a recently-developed approach[45,61], we estimated the species
459 exclusion distance, which corresponds to the minimal distance between the vector of intrinsic
460 growth rates ($r = [\lambda_I, \lambda_j]$) and the edges of the feasibility domain (vectors corresponding to either
461 $[0, \frac{\alpha_{22}}{\alpha_{12}}]$ or $[0, \frac{\alpha_{21}}{\alpha_{11}}]$) as a proxy for how strong a perturbation must be to change the coexistence
462 outcome in each system. To calculate the distance between the vector of intrinsic growth rates
463 and the edges of the feasibility domain, we applied the following formula[45]:
464 (2) $dist_i = \cos^{-1} \begin{bmatrix} \lambda_i \\ \lambda_j \end{bmatrix} . \begin{bmatrix} \alpha_{21} \\ \alpha_{11} \end{bmatrix}$

465

466 **Model validation**

467 We validated our approach by comparing model predictions with the empirical results from the
468 population growth experiment. For that, we used the Ricker model to predict the relative
469 abundance of each species after two generations in each treatment. Then, we performed a
470 general linear regression with a binomial error distribution to compare the mean observed
471 proportions of each competitor in all replicates of all treatments with the model proportion
472 estimates.

473

474 **Statistical analyses**

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

480 To test how evolution on plants with cadmium affected population growth and competition
481 in cadmium we used general linear models with a gamma distribution for the intrinsic growth
482 rate, and with a normal distribution for the coefficients for intra and interspecific competition.
483 All models were applied separately for both species, and normality and dispersion of the
484 residuals was inspected for all models tested. For all models, we included the parameters
485 estimated with the cxr package for each replicate as a dependent variable and Selection Regime
486 as fixed factor (with two levels corresponding to selection regimes evolving in cadmium or no
487 cadmium environment). For the strength of interspecific competition, the model included the
488 selection regime of the focal and of the competitor species, as well as their interaction as fixed
489 factors. Whenever the interaction term was significant, we applied contrasts to compare
490 estimates for all combinations of focal and competitor selection regimes. Similar models were
491 applied to test the impact of evolution in cadmium on the performance in the no cadmium
492 environment.

493 All analyses were done using R 4.2.1 version[62], statistical analysis were done using the
494 package "glmmTMB"[63], contrasts were performed using the "emmeans" package[64], analyses
495 of residuals were done for each model using the DHARMa package[65], and graphical
496 representation was done using "ggplot2"[66]. All data scripts and data used for the analyses is
497 available in the git repository https://github.com/irfragata/evolution_cadmium_coexistence.

498

Authorship Statement

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

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Figures

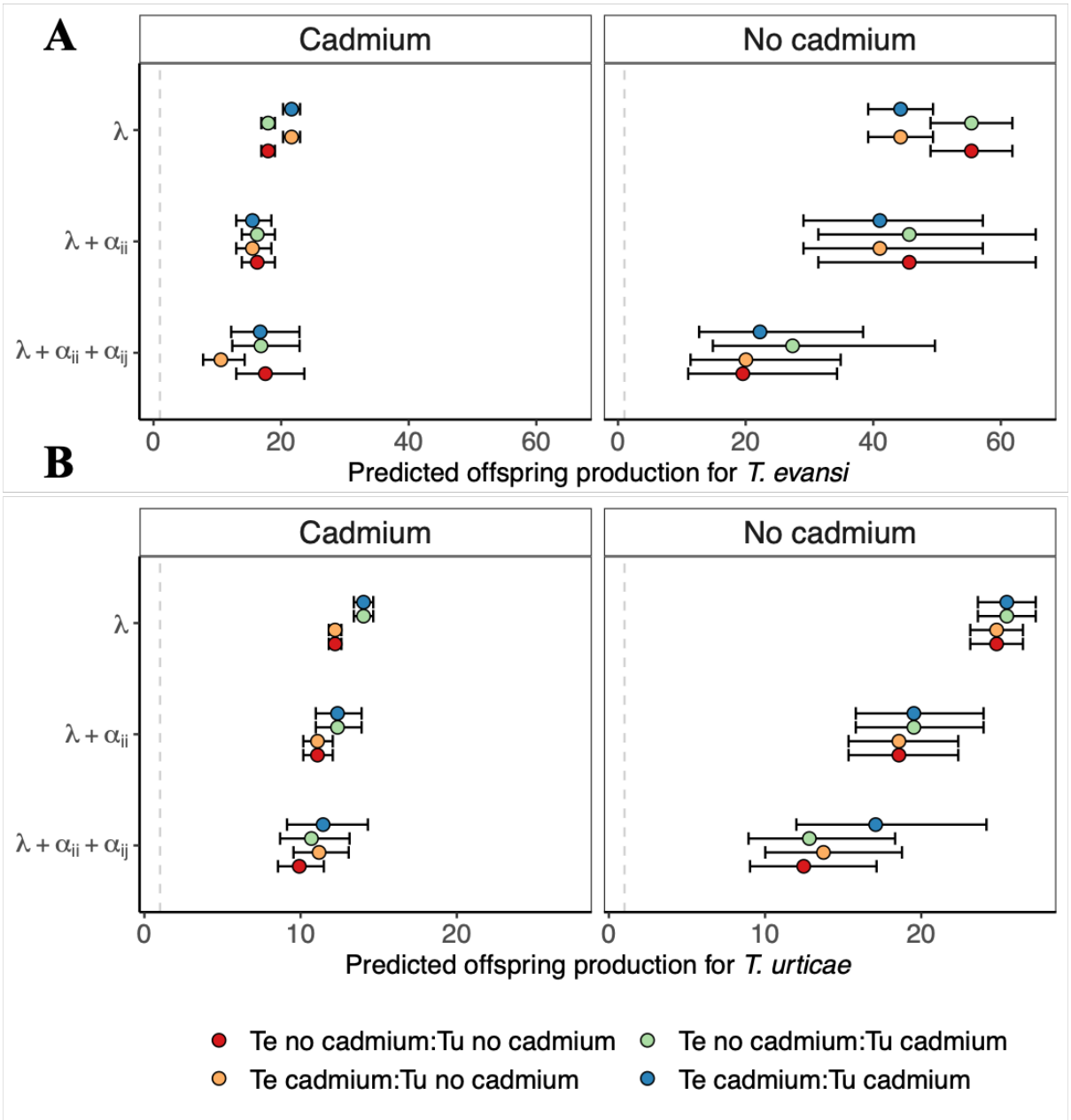


Figure 1 – Predicted number of female offspring after one generation for A) *T. evansi* (Te) and B) *T. urticae* (Tu), to estimate the relative effect of competition on the growth rate of the different populations. Estimate were done for the intrinsic growth rate (λ), the intrinsic growth rate plus intraspecific competition ($\lambda + \alpha_{ii}$) and the latter plus interspecific competition ($\lambda + \alpha_{ii} + \alpha_{ij}$) 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 ($i.e., \lambda$ and $\lambda + \alpha_{ii}$).

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).

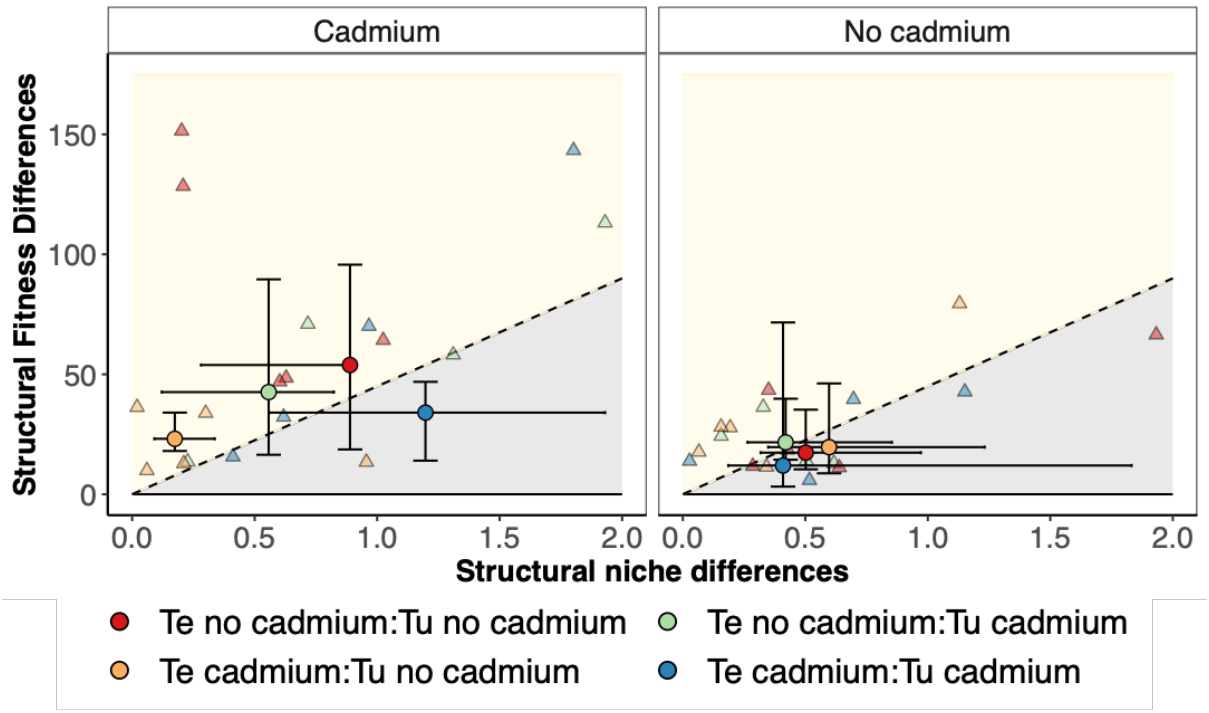


Figure 2 – Predicted structural niche and fitness differences in cadmium (left panel) and no cadmium (right panel) environments for the different treatments (i.e. combinations of cadmium or no cadmium selection regimes). The dark grey region indicates the coexistence region (i.e., the feasibility domain) and light yellow delimitates the region for exclusion (i.e., the unfeasibility domain). Treatments correspond to combinations of *T. evansi* (Te) and *T. urticae* (Tu) selection regimes (cf. colour code). In the unfeasibility domain (light yellow area), Te always excludes Tu except in the treatment Te cadmium: Tu no cadmium (orange circle). Circles correspond to the parameters estimated from all replicates pooled and triangles to parameters estimated from each 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.

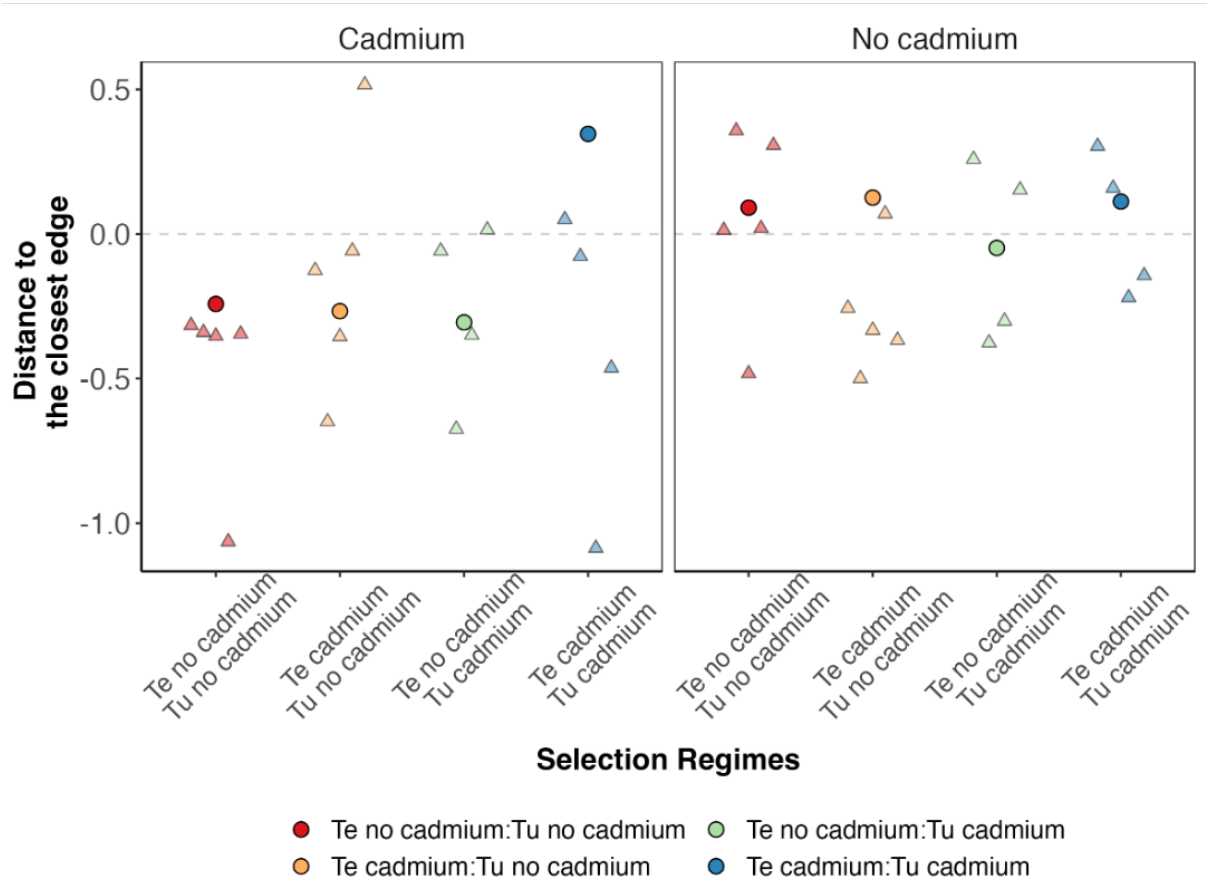


Figure 3 – Distance between the vector of intrinsic growth rates and the closest edge of the feasibility domain in the cadmium (left panel) and no cadmium (right panel) environments for the different treatments (i.e. combinations of no cadmium or cadmium selection regimes for *T. evansi* (Te) and for *T. urticae* (Tu); cf. colour code). Positive distances indicate that the vector of growth rates is inside of the feasibility domain (i.e., coexistence is possible), and negative distances indicate that the vector is outside of the feasibility domain (i.e., exclusion is predicted). Circles correspond to the distance calculated with data from all replicates pooled and triangles to distance calculated per replicate.

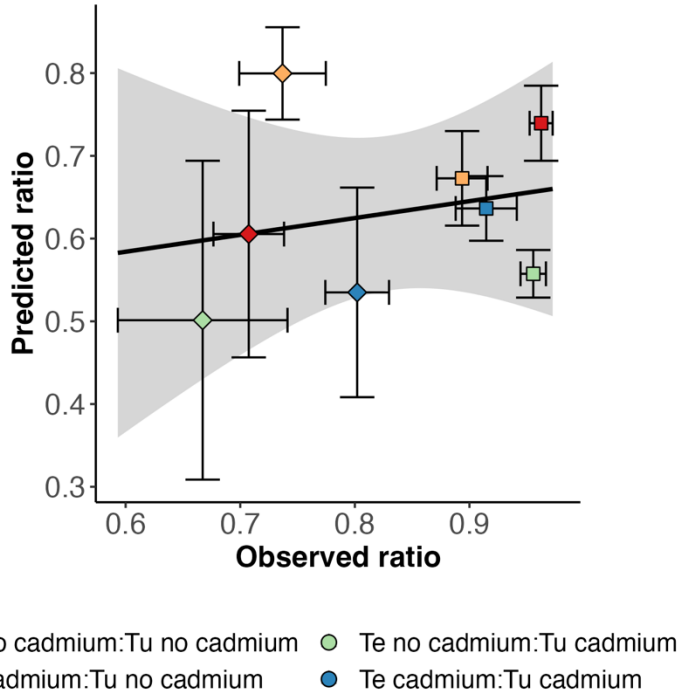


Figure 4 – Correlation between predicted and observed relative abundance of *T. evansi* after 2 generations in an independent population growth experiment in which the two species are released at equal densities. Squares indicate data from the cadmium environment and diamonds indicate data from the no-cadmium environment. Predicted error bars were calculated based on standard error obtained from 1000 bootstrap samples and observed error bars were obtained from replicate populations. Treatments correspond to combinations of *T. evansi* (Te) and *T. urticae* (Tu) selection regimes (cf. colour code).

Supplementary Tables & Figures

Supplementary Tables

Table S1 – Analyses of differences in interspecific competition in the cadmium environment. (A) Summary of the ANOVA (type III) to estimate the effect of evolving on plants with cadmium on the strength of interspecific competition for *T. urticae* and *T. evansi*. (B) Contrasts between the strength of interspecific competition for the *T. evansi* cadmium and non-cadmium selection regimes. Contrasts were obtained using the emmeans function, from the linear model including the selection regimes of focal and competitor individuals as well as their interaction.

A)

	T. urticae		***T. evansi***	
Parameter	**Chisq**	**Pr(>Chisq)**	**Chisq**	**Pr(>Chisq)**
Tu Regime	0.1325	0.7158	0.9035	0.3419
Te Regime	1.3768	0.2407	13.0935	0.0003***
Tu Regime: Te Regime	0.1188	0.7304	5.6283	0.018*

* 0.05 >= P-value > 0.01; ** 0.01 >= P-value > 0.001; *** P-value < 0.001

B)

Contrasts *T. evansi*	**Estimate**	**T ratio**	**P-value**
Te no cadmium: Tu no cadmium - Te cadmium: Tu no cadmium	-0.0531	-3.6180	0.0031***
Te no cadmium: Tu no cadmium - Te no cadmium: Tu cadmium	-0.0148	-0.9510	0.3592
Te no cadmium: Tu no cadmium - Te cadmium: Tu cadmium	-0.0157	-1.0070	0.3323
Te cadmium: Tu no cadmium - Te no cadmium: Tu cadmium	0.0383	2.4610	0.0286 *
Te cadmium: Tu no cadmium - Te cadmium: Tu cadmium	0.0374	2.4050	0.0318 *
Te no cadmium: Tu cadmium - Te cadmium: Tu cadmium	-0.0009	-0.0540	0.9581

* 0.05 >= P-value > 0.01; ** 0.01 >= P-value > 0.001; *** P-value < 0.001

Table S2 – Analyses of differences in interspecific competition in the no-cadmium environment. Summary of the ANOVA (type III) to estimate the effect of evolving in cadmium on the strength of interspecific competition for *T. urticae* and *T. evansi*. The linear model included the selection regimes of focal and competitor individuals as well as their interaction.

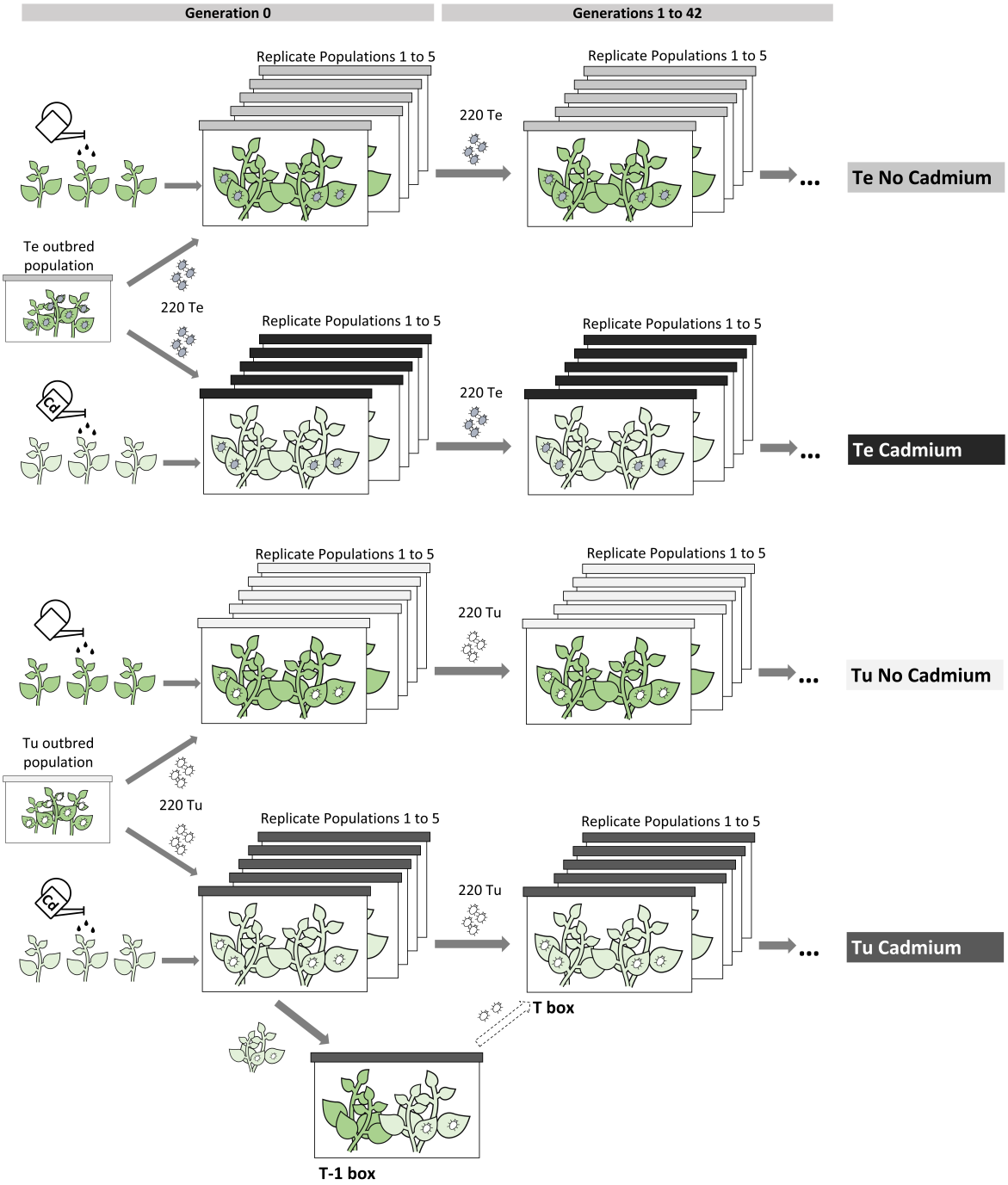
	T. urticae		***T. evansi***	
Parameter	**Chisq**	**Pr(>Chisq)**	**Chisq**	**Pr(>Chisq)**
Tu Regime	0.026	0.872	1.233	0.267
Te Regime	0.321	0.571	0.141	0.708
Tu Regime: Te Regime	0.452	0.502	0.029	0.865

Table S3 – Average proportion of *T. evansi* females obtained from the experiment to estimate the growth rate of populations with both intra and interspecific competitors. Each treatment corresponds to a combination of selection regimes (no Cadmium Te: no-cadmium Tu, cadmium Te: no-cadmium Tu, no-cadmium Te: cadmium Tu and cadmium Te: cadmium Tu) and was composed of 10 replicate populations. Each box was initialized with 6 females of the two species.

Replicate population	**Selection Regime Te**	**Selection Regime Tu**	**Environment**	**Proportion of Te females** (per replicate)
1	No Cadmium	No Cadmium	Cadmium	0.9578
2	No Cadmium	No Cadmium	Cadmium	0.9992
3	No Cadmium	No Cadmium	Cadmium	0.9381
4	No Cadmium	No Cadmium	Cadmium	0.9622
5	No Cadmium	No Cadmium	Cadmium	0.9555
1	No Cadmium	No Cadmium	No Cadmium	0.6236
2	No Cadmium	No Cadmium	No Cadmium	0.6481
3	No Cadmium	No Cadmium	No Cadmium	0.7699
4	No Cadmium	No Cadmium	No Cadmium	0.7732
5	No Cadmium	No Cadmium	No Cadmium	0.7222
1	Cadmium	No Cadmium	Cadmium	0.9704
2	Cadmium	No Cadmium	Cadmium	0.9148
3	Cadmium	No Cadmium	Cadmium	0.9794
4	Cadmium	No Cadmium	Cadmium	0.9567
5	Cadmium	No Cadmium	Cadmium	0.9571
1	Cadmium	No Cadmium	No Cadmium	0.6015
2	Cadmium	No Cadmium	No Cadmium	0.5636
3	Cadmium	No Cadmium	No Cadmium	0.9167
4	Cadmium	No Cadmium	No Cadmium	0.5065
5	Cadmium	No Cadmium	No Cadmium	0.7478
1	No Cadmium	Cadmium	Cadmium	0.9184
3	No Cadmium	Cadmium	Cadmium	0.8195
4	No Cadmium	Cadmium	Cadmium	0.9129
5	No Cadmium	Cadmium	Cadmium	0.9236
1	No Cadmium	Cadmium	No Cadmium	0.6484
3	No Cadmium	Cadmium	No Cadmium	0.6837
4	No Cadmium	Cadmium	No Cadmium	0.7885
5	No Cadmium	Cadmium	No Cadmium	0.8269
1	Cadmium	Cadmium	Cadmium	0.8336
3	Cadmium	Cadmium	Cadmium	0.9518
4	Cadmium	Cadmium	Cadmium	0.9070
5	Cadmium	Cadmium	Cadmium	0.9662
1	Cadmium	Cadmium	No Cadmium	0.7917
3	Cadmium	Cadmium	No Cadmium	0.7228
4	Cadmium	Cadmium	No Cadmium	0.8712
5	Cadmium	Cadmium	No Cadmium	0.8225

Supplementary Figures

A)



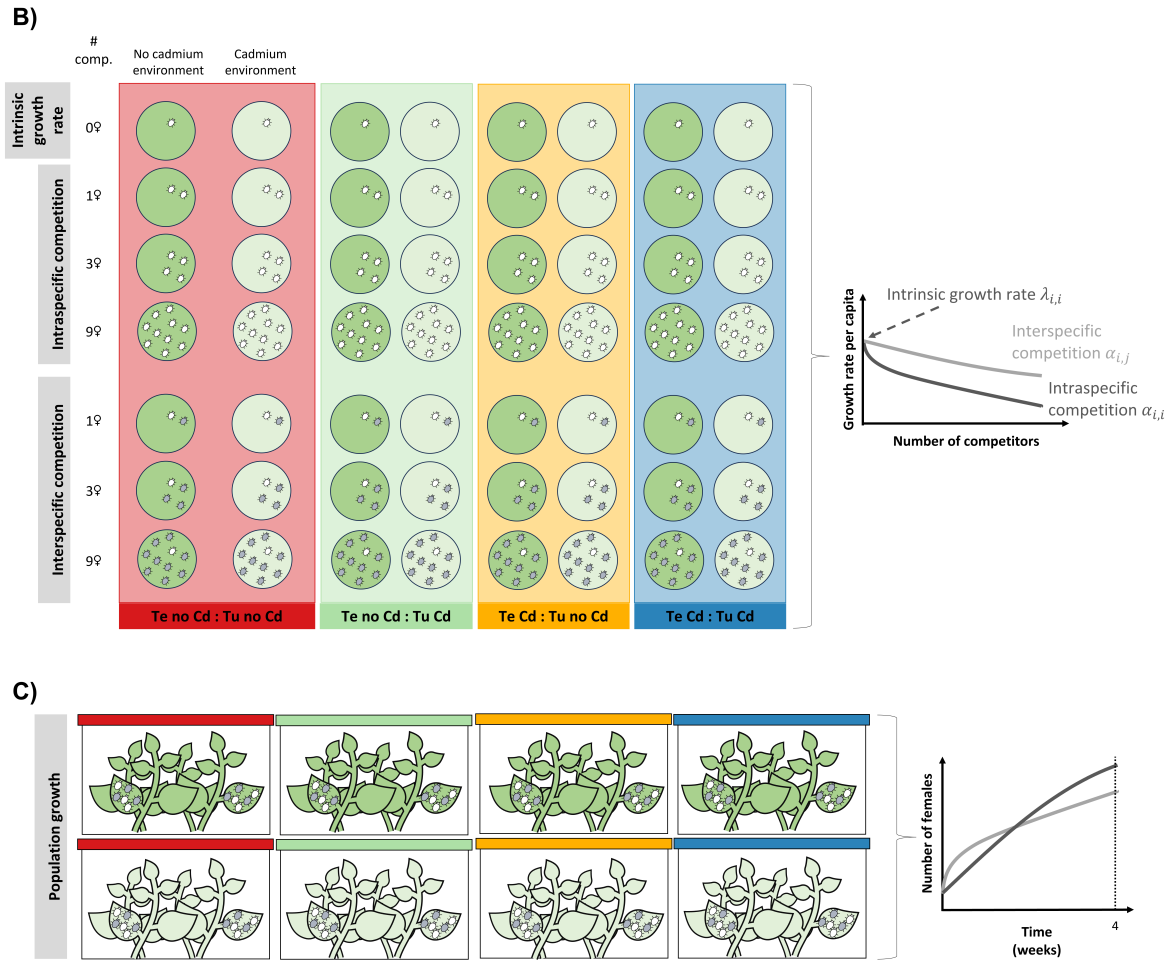


Figure S1 – Overview of the experimental procedure. A) Experimental evolution: 220 females from the *T. urticae* (Tu) or *T. evansi* (Te) outbred populations were transferred to create four experimental regimes: No cadmium (plants grown in soil without cadmium, dark green) and Cadmium (plants grown in soil with 2mM cadmium, light green), for each mite species evolved in absence of interspecific competition. Each selection regime was replicated five times. Every two weeks (roughly corresponding to one spider mite generation), 220 females were transferred from each population to a new box with the same plant treatment. This procedure was followed for 42 mite generations. **B) Experiment to estimate strength of competition**: Females from the four experimental regimes were placed on a leaf disk of a plant grown with (light green) or without (dark green) cadmium. Focal females were exposed to a gradient of intraspecific or interspecific female competitors stemming from the cadmium or no-cadmium selection regimes. In total, four possible combinations of cadmium and no-cadmium selection regimes were performed: Te no cadmium: Tu no cadmium (red), Te no cadmium: Tu cadmium (green), Te cadmium: Tu no cadmium (yellow), Te cadmium: Tu cadmium (blue). The number of adult female offspring was measured after two weeks to calculate the per capita offspring production (total number of offspring divided by the number of focal females initially added to the patch). These data were then used to parameterize a Ricker model to estimate the intrinsic growth rate and the intra and interspecific competition coefficients. The parameters were then used to estimate 1) the relative impact of intra and interspecific competition by predicting the number of offspring produced under different

scenarios (cf. Figure 1 in main text) and 2) the long-term coexistence outcomes of competition between the different selection regimes (cf. Figure 2 in main text). **C) Population experiment:** Six females from each experimental evolution selection regime were placed in a box with two leaves from plants grown with or without cadmium. Boxes were created for the four possible combinations of cadmium and no-cadmium selection regimes: Te no cadmium: Tu no cadmium (red), Te no cadmium: Tu cadmium (green), Te cadmium: Tu no cadmium (yellow), Te cadmium: Tu cadmium (blue). After two weeks, two more plants were added, and the number of adult female offspring of each mite species was counted four weeks later. Figure adapted from Godinho et al (2024).

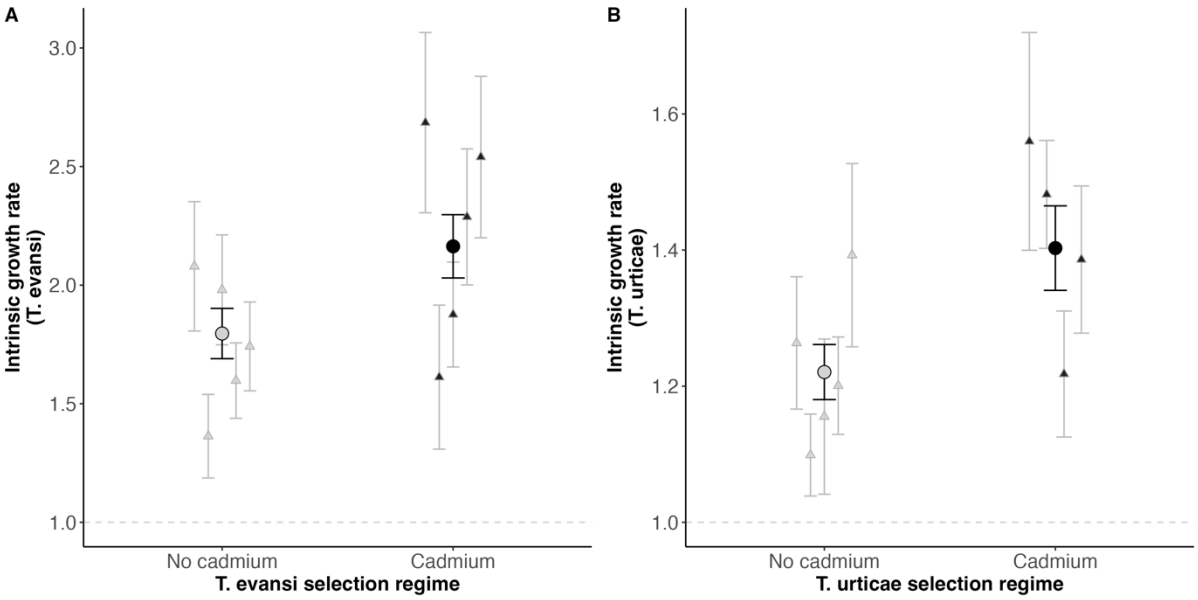


Figure S2 – Intrinsic growth rate of *T. evansi* (A) and *T. urticae* (B) from the cadmium and the no cadmium selection regimes, when tested in the cadmium environment. No cadmium and cadmium selection regimes are represented in light and dark colours, respectively. Error bars were calculated based on standard error obtained from 1000 bootstrap samples. Circles correspond to the parameters estimated from all replicates pooled and triangles to parameters estimated from each replicate. Note that the scales are different between the two panels.

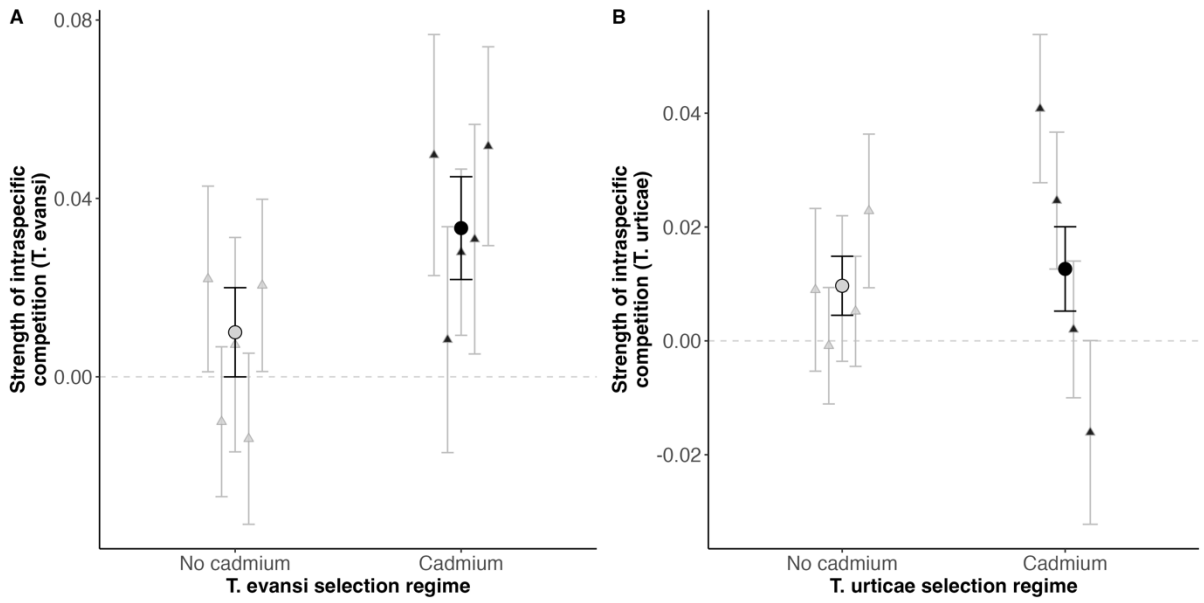


Figure S3 – Strength of intraspecific competition for *T. evansi* (A) and *T. urticae* (B) selection regimes in the cadmium environment. No cadmium and cadmium selection regimes are represented in light and dark colours, respectively. Error bars were calculated based on standard error obtained from 1000 bootstrap samples. Circles correspond to the parameters estimated from all replicates pooled and triangles to parameters estimated from each replicate. Note that the scales are different between the two panels.

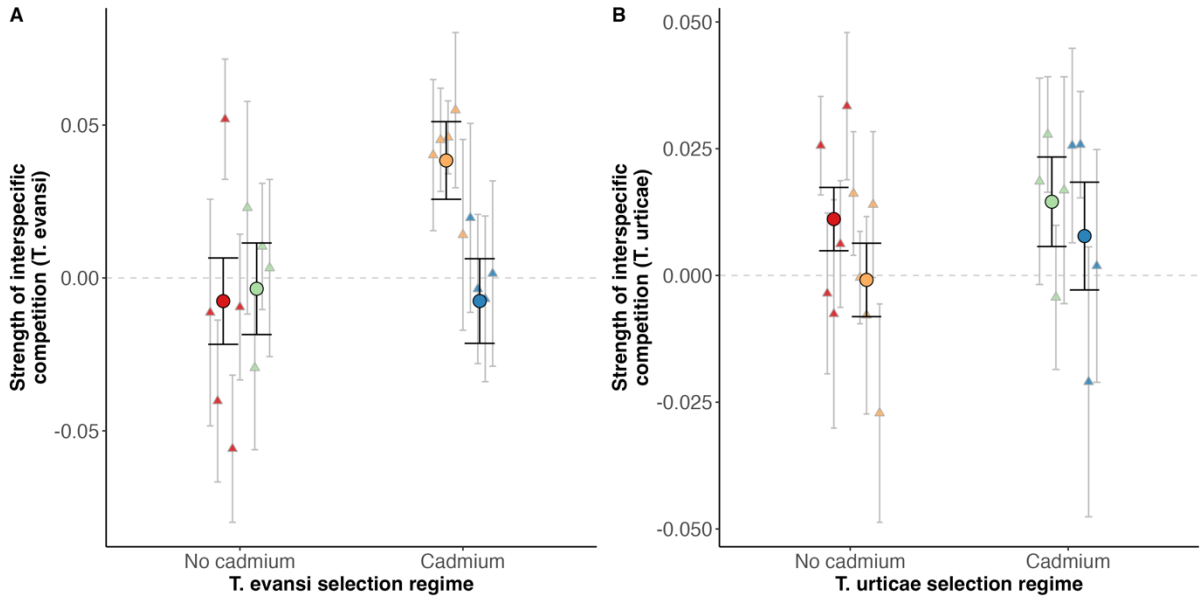


Figure S4 – Strength of interspecific competition for *T. evansi* (A) and *T. urticae* (B) selection regimes in the cadmium environment. Colors indicate the four possible combinations of cadmium and no-cadmium selection regimes were performed: Te no cadmium: Tu no cadmium (red), Te no cadmium: Tu cadmium (green), Te cadmium: Tu no cadmium (yellow), Te cadmium: Tu cadmium (blue). Error bars were calculated based on standard error obtained from 1000 bootstrap samples. Circles correspond to the parameters estimated from all replicates pooled and triangles to parameters estimated from each replicate. Note that the scales are different between the two panels.

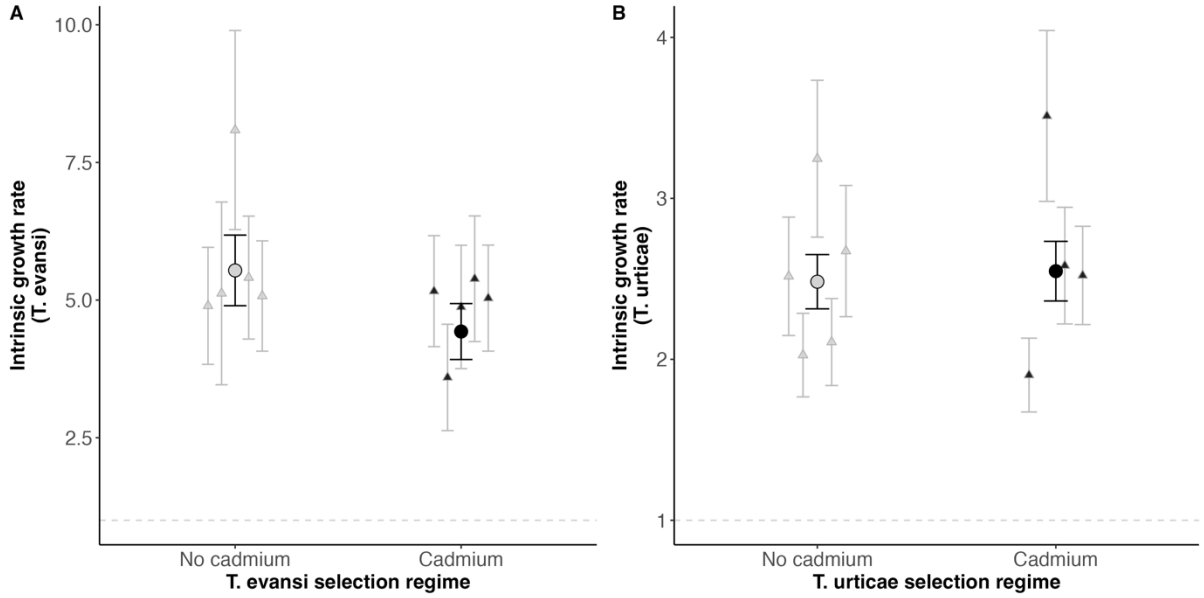


Figure S5 – Intrinsic growth rate for *T. evansi* (A) and *T. urticae* (B) selection regimes in the no cadmium environment. No cadmium and cadmium selection regimes are represented in light and dark colours, respectively. Error bars were calculated based on standard error obtained from 1000 bootstrap samples. Circles correspond to the parameters estimated from all replicates pooled and triangles to parameters estimated from each replicate. Note that the scales are different between the two panels.

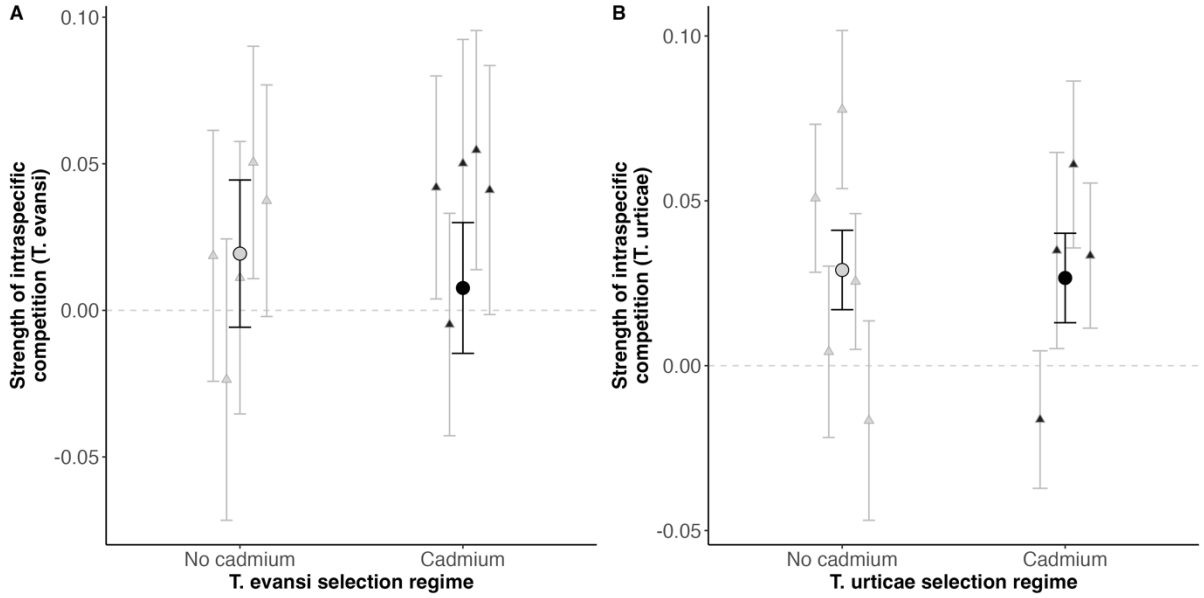


Figure S6 – Strength of intraspecific competition for *T. evansi* (A) and *T. urticae* (B) selection regimes in the no cadmium environment. No cadmium and cadmium selection regimes are represented in light and dark colours, respectively. Error bars were calculated based on standard error obtained from 1000 bootstrap samples. Circles correspond to the parameters estimated from all replicates pooled and triangles to parameters estimated from each replicate. Note that the scales are different between the two panels.

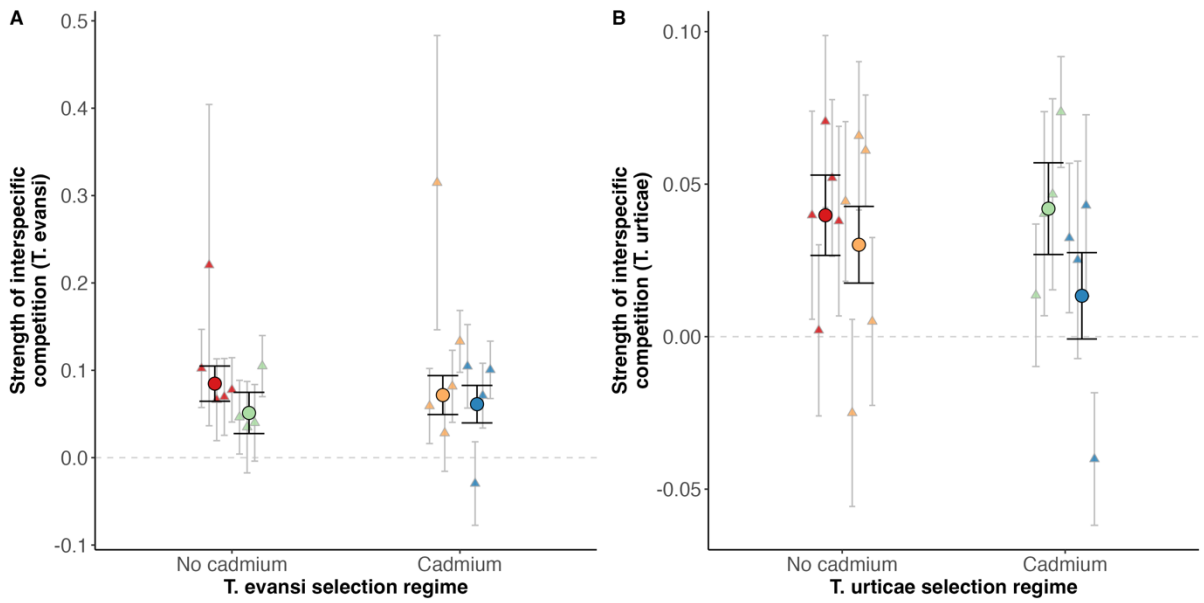


Figure S7 – Strength of interspecific competition for *T. evansi* (A) and *T. urticae* (B) selection regimes in the no cadmium environment. Colors indicate the four possible combinations of cadmium and no-cadmium selection regimes were performed: Te no cadmium: Tu no cadmium (red), Te no cadmium: Tu cadmium (green), Te cadmium: Tu no cadmium (yellow), Te cadmium: Tu cadmium (blue). Error bars were calculated based on standard error obtained from 1000 bootstrap samples. Circles correspond to the parameters estimated from all replicates pooled and triangles to parameters estimated from each replicate. Note that the scales are different between the two panels.

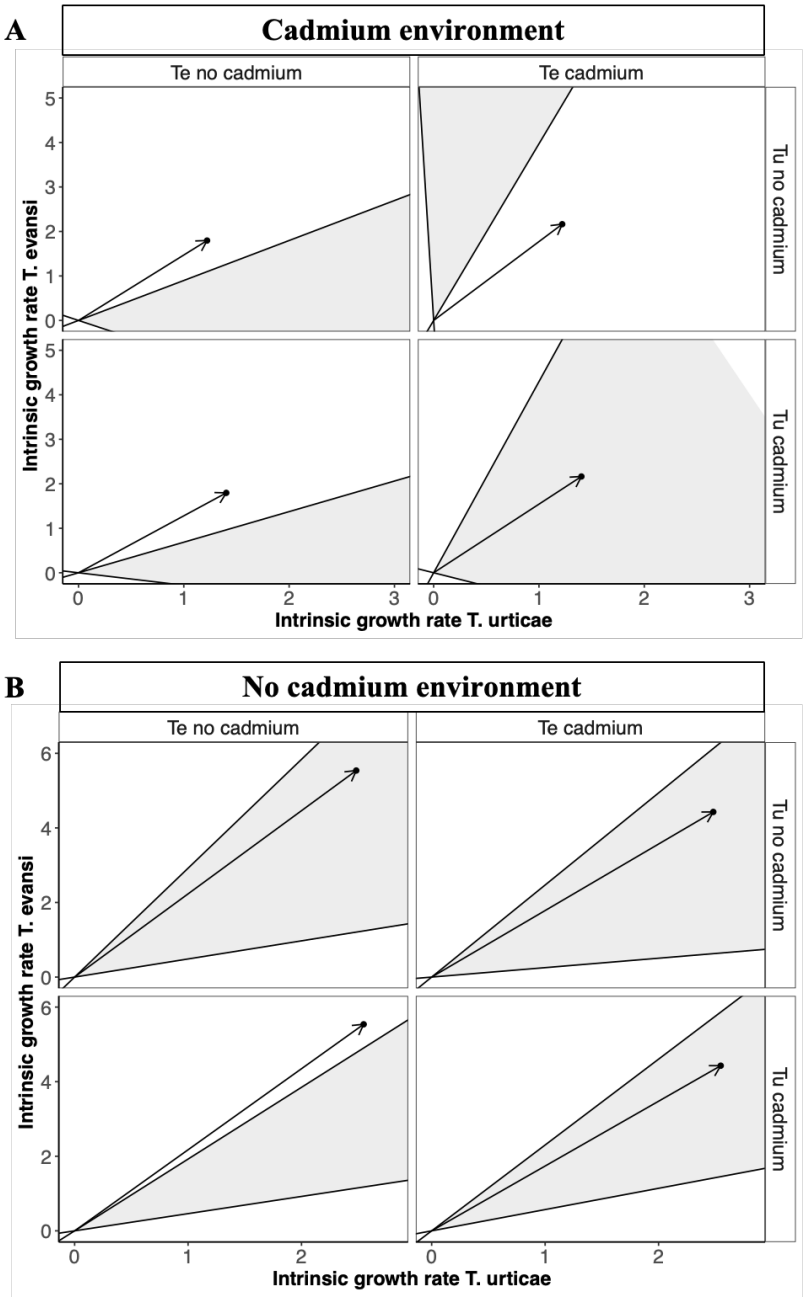


Figure S8 – Feasibility domain for the different combinations of selection regimes (cadmium and no-cadmium) in which *T. urticae* (Tu) or *T. evansi* (Te) have evolved in (A) the cadmium and (B) the no cadmium environment. The arrow represents the vector of intrinsic growth for each of the two species and the edges of the cone delimit the area under which the isoclines cross at positive abundances (i.e., the feasibility domain, in which coexistence is possible). 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.

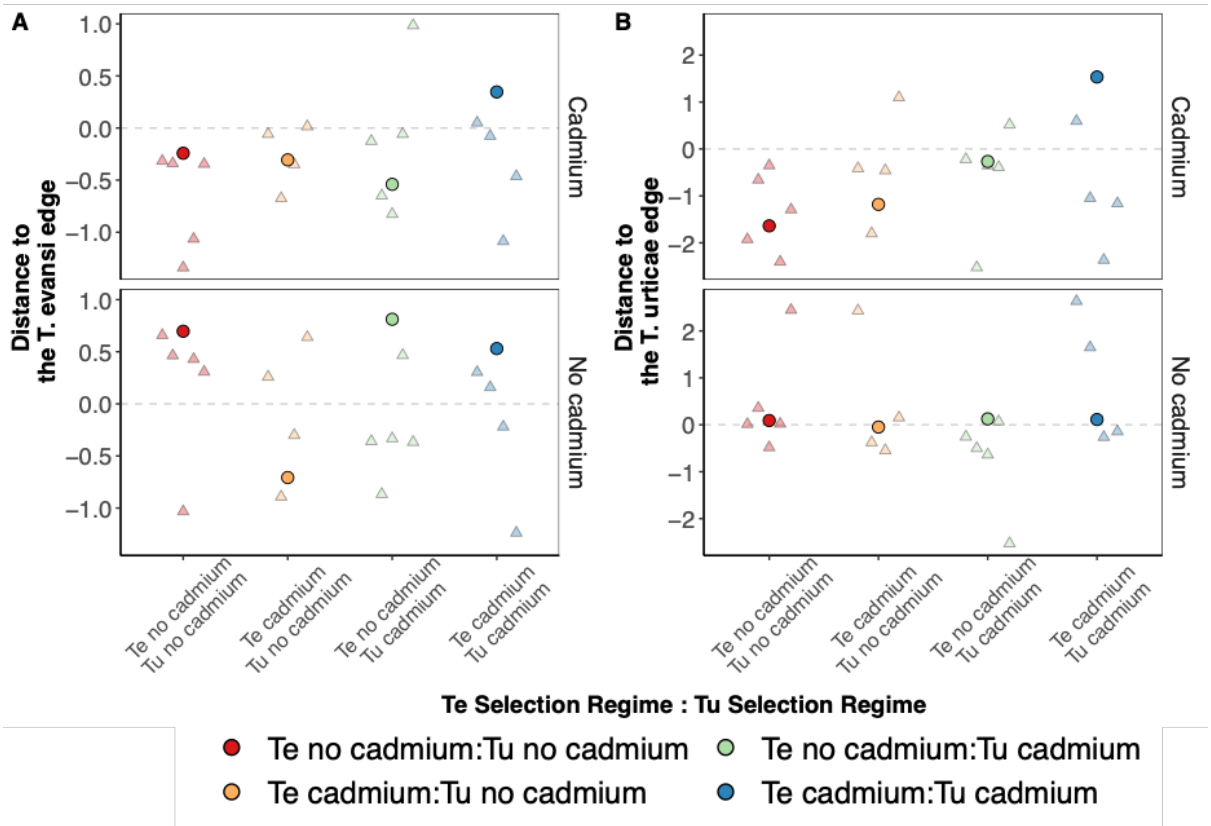


Figure S9 – Distance to the two edges of the feasibility domain for the different combinations of cadmium and no-cadmium selection regimes of *T. urticae* (Tu) and *T. evansi* (Te) in the cadmium and the no-cadmium environment. Distance between the realized growth rates and the edges of the feasibility domain for which Te (A) or Tu (B) will be excluded, in the cadmium (upper panels) and no-cadmium (lower panels) environments for the different treatments (i.e. combinations of no cadmium or cadmium selection regimes, cf. colour codes). Positive distances indicate that the vector of growth rates is inside of the feasibility domain (i.e., coexistence is possible), and negative distances indicate that the vector is outside of the feasibility domain (one species is excluded). Circles correspond to the distance calculated with data from all replicates pooled and triangles to distance calculated per replicate. Note the differences in scales between panels.