Order of arrival promotes coexistence via spatial niche preemption by the weak competitor

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Abstract

Historical contingency, such as the order of species arrival, can modify competitive outcomes via niche modification or preemption. However, how these mechanisms ultimately modify stabilising niche and average fitness differences remains largely unknown. By experimentally assembling two congeneric spider mite species feeding on tomato plants during two generations, we show that order of arrival interacts with species' competitive ability to determine competitive outcomes. Contrary to expectations, we did not observe that order of arrival cause priority effects. In fact, coexistence was predicted when the inferior competitor (*Tetranychus urticae*) arrived first. In that case, *T. urticae* colonized the preferred feeding stratum (leaves) of *T. evansi* leading to spatial niche preemption, which equalized fitness but also increased niche differences, driving community assembly to a close-to-neutrality scenario. Our study demonstrates how the spatial context of competitive interactions interact with species competitive ability to influence the effect of order of arrival on species coexistence.

Introduction

Priority effects are broadly defined as the process by which historical contingencies in community assembly (e.g. order and/or timing of arrival) change the outcome of interspecific interactions (Chase 2003; Fukami 2015). Priority effects can be inhibitory if earlier arrival by one species inhibits the growth of the species arriving subsequently (Chase 2003; Fukami 2015; Ke & Letten 2018). Such effects are expected to result in alternative stable states and hamper coexistence (Fukami 2015; Ke & Letten 2018). In turn, facilitative priority effects occur when population growth is higher when individuals arrive after the settlement of a first species. Facilitative effects are expected to foster coexistence (Fukami 2015), but they have been less often identified in natural communities (Queijeiro-Bolaños *et al.* 2017; Clay *et al.* 2019; Halliday *et al.* 2020).

Two major mechanisms are predicted to cause priority effects: niche preemption, in which early colonizers reduce the amount of resource available to late colonizers, and niche modification, in which the species arriving first modifies the environment, thereby inhibiting or facilitating later colonization (Kardol *et al.* 2013; Vannette & Fukami 2014; Fukami 2015; Delory *et al.* 2019, 2021; Grainger *et al.* 2019). For example, niche preemption in plant communities was found to be strong in environments with high nutrient supply, as early arriving plants grew quickly and prevented growth of later colonizers by depleting space and light (Kardol *et al.* 2013). Also, previous colonization by different plant communities modified the soil metabolome and inhibited population growth of forb, but not grass species, via decreased root foraging (Delory *et al.* 2021). Although distinguishing among niche preemption and modification is not always possible (Grainger *et al.* 2018; Boyle *et al.* 2021), recent advances from coexistence theory can serve as a powerful approach to better understand the importance of historical contingencies for species coexistence. Yet the combination of these theoretical tools has seldom been applied in empirical settings.

Modern coexistence theory posits that the long-term persistence of competing species (i.e., species coexistence) can be attained by two non-mutually exclusive mechanisms: (i) equalizing mechanisms that reduce average fitness differences, and therefore, dominance between species and (ii) stabilising mechanisms, which tend to stabilise the interaction between competitors by increasing the strength of intraspecific competition relative to interspecific competition (Chesson 2000). Therefore, species will stably coexist if the stabilising niche differences, leading to negative frequency dependence, are larger than differences in fitness between competitors. Otherwise the species with higher fitness will eventually dominate the community (Chesson 2000; Barabás *et al.* 2018; Spaak & De Laender 2021). Under this framework, priority effects are strictly defined as the case in which the population dynamics of interacting species are governed by positive frequency dependence (i.e., via negative niche differences), which leads to the dominance of the early-arriving species (Ke & Letten 2018; Grainger *et al.* 2019; Spaak & De Laender 2021). Hence, species cannot coexist locally unless there is spatial variability in the order of arrival. However, despite the fact that recent theory offers predictions on the outcome of coexistence in systems with historical contingencies, empirical tests are conspicuously lacking (but see Cardinaux *et al.* 2018; Grainger *et al.* 2019; Song *et al.* 2020). Therefore, there is as yet scarce knowledge of which species traits interact with historical contingencies to determine outcomes of interspecific interactions.

For the herbivore communities, such traits can be the spatial distribution of consumers associated with resource use. Indeed, niche preemption may arise in this system, as herbivores generally have preferred plant strata and the first arriving species may monopolize that resource (Grainger *et al.* 2018; Godinho *et al.* 2020a). Moreover, herbivores often induce defences on the plants they colonize, which is expected to entail niche modification for species arriving later (Erb *et al.* 2011; Moreira *et al.* 2015; Stam *et al.* 2017). Additionally, some other herbivore species are known to down-regulate plant defences, improving the performance of later

86 colonizers (Sarmento *et al.* 2011a; Godinho *et al.* 2016), thereby potentially causing facilitative
87 priority effects. Overall, given the spatial heterogeneity of the environment that herbivores
88 experience (e.g., variation in leaf quality within and between plants), effects of the order of
89 arrival on species coexistence are expected to be prevalent in herbivore communities (Utsumi
90 *et al.* 2010; Erb *et al.* 2011; Moreira *et al.* 2015; Stam *et al.* 2017, 2018; Godinho *et al.* 2020a).
91 But what type of competitive outcome we should expect is unclear. Order of arrival has been
92 a traditional link to priority effects, yet the interaction of the chronology of community
93 assembly with the type of impact of species have on the environment (e.g. where they growth
94 and how they modify the habitat) can result in diverse outcomes, from competitive exclusion
95 to species coexistence. Applying the modern coexistence framework to this open question can
96 shed light on the proximate mechanisms that allow for species to coexist under varied historical
97 contingencies.

98 Here shed new light on the drivers of competitive outcomes by combining theoretical and
99 empirical tools to experimentally investigate the mechanisms through which order of arrival
100 affects species coexistence. We use as a model system, the two closely-related competing
101 herbivorous species, the spider mites *Tetranychus urticae* and *T. evansi* (Alzate *et al.* 2020).
102 *Tetranychus evansi* generally outcompetes *T. urticae* on tomato plants (Sarmento *et al.* 2011b;
103 Orsucci *et al.* 2017; Alzate *et al.* 2020), although both species are also commonly observed to
104 co-occur on the same location (Ferragut *et al.* 2013). Niche modification is expected to be at
105 play in this system, because the two species interact with plant defences, albeit differently: *T.*
106 *urticae* induces tomato defences, whereas *T. evansi* suppresses them (Sarmento *et al.* 2011a;
107 Alba *et al.* 2015). This asymmetrical niche modification is predicted to facilitate coexistence
108 by hampering growth of the stronger competitor and favouring growth of the inferior one, when
109 they arrive on plants colonized by the other species. Moreover, niche preemption may occur,
110 as both *T. evansi* and *T. urticae* prefer the upper, more nutritious leaves of tomato plants, where

111 their performance is higher (Godinho *et al.* 2020a). Thus, early-arriving species could occupy
112 the preferred niche and displace the other species to lower, less optimal, plant strata. We tested
113 this, by performing a series of multi-generational experiments where we varied order of arrival
114 and measured space use by the two competing species. We then applied modern coexistence
115 theory framework to unravel the conditions favouring coexistence or potentially leading to
116 priority effects.
117

118 **Material and Methods**

119 **Model system, species characteristics, and maintenance of experimental populations**

120 *Tetranychus urticae* is a generalist herbivore that feeds on many economically important crops
121 (Helle & Sabelis 1985; Grbić *et al.* 2011; Sousa *et al.* 2019), whereas *T. evansi* is a solanaceous
122 specialist that has recently invaded Europe from South America (Boubou *et al.* 2012). Both
123 species colonize tomato plants, although *T. urticae* may shift to other hosts if *T. evansi* is
124 present (Ferragut *et al.* 2013).

125 All experiments were performed with outbred populations of *T. urticae* and *T. evansi*
126 spider mites, formed via controlled crosses among four *T. evansi* and three *T. urticae*
127 populations collected in different locations in Portugal (Godinho *et al.* 2020b). Before the
128 experiment, spider mite populations were maintained in boxes containing leaves detached from
129 five-week-old tomato plants (*Solanum lycopersicum*, *var* MoneyMaker), with their petiole in
130 a small pot containing water, under controlled conditions (25 ºC, 68% of humidity, photoperiod
131 of 16 hours light/8 hours dark). Twice a week, overexploited leaves were removed, and water
132 and new tomato leaves were added. Before infestation, tomato plants were kept in a climatic
133 chamber at 25ºC, with a photoperiod of 16 hours light/8 hours dark with 75% humidity and
134 watered three times per week.

To ensure that females used in the experiments were of similar age, we created cohorts of mated *T. urticae* and *T. evansi* females for each block. To this aim, females were placed during 48h in petri dishes (14.5 cm diameter) with a layer of wet cotton and two freshly cut tomato leaves. One week later, another tomato leaf was added. Petri dishes were watered twice per week. In the experiment, we used females with 13-15 days of age.

Theoretical approach for predicting competitive outcomes: quantifying niche and fitness differences

Data collected in the experiments were used to parameterize a mathematical model from which niche and average fitness differences can be quantified as well as the predictions of competitive outcomes. We assume that the population dynamics in our experiment can be described by a Beverton-Holt function (Hart *et al.* 2018):

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

Where $N_{i,t+1}$ is the number of individuals of species i in the next generation, λ_i the growth rate of species i in absence of competitors, α_{ii} the intraspecific competitive ability, α_{ij} the interspecific competitive ability and $N_{i,t}$, $N_{j,t}$ the number of individuals of species i and j in the current generation, respectively. In this model, we assume that spider mites do not have a dormant stage. This means that λ_i represents only the fraction of eggs that hatch and become female individuals that grow and reproduce in the next generation.

From this model, niche overlap (ρ) is defined as follows (see details in Chesson 2012; Godoy & Levine 2014.

$$(2)\ \rho = \sqrt{\frac{\alpha_{ij}}{\alpha_{jj}} \frac{\alpha_{ji}}{\alpha_{ii}}}$$

This formula reflects the average degree to which species limit individuals of their own species relative to heterospecific competitors. If species limit individuals of their own species more strongly than competitors (α_{jj}, α_{ii}, are much greater than α_{ij}, α_{ji}), then niche overlap will be low, favouring coexistence. Alternatively, if species limit individuals of their own species and those of their competitor equally, niche overlap equals one, hampering stable coexistence. With ρ defining niche overlap between a pair of species, stabilising niche differences are expressed as 1-ρ.

Average fitness differences ($\frac{\kappa_j}{\kappa_i}$) (Chesson 2012; Godoy & Levine 2014) are defined as:

$$(3)\ \frac{\kappa_j}{\kappa_i} = \frac{\lambda_j - 1}{\lambda_i - 1} * \frac{\sqrt{\alpha_{ij} * \alpha_{ii}}}{\sqrt{\alpha_{ji} * \alpha_{jj}}}$$

The greater the ratio, ($\frac{\kappa_j}{\kappa_i}$), the greater the fitness advantage of species *j* over *i*. If this ratio is one, species are equivalent competitors. With niche overlap and average fitness defined in eqn. 2 and 3 respectively, we can determine the coexistence condition. Specifically, coexistence requires both species to invade when rare (Chesson 2012), which is satisfied when (Godoy & Levine 2014):

$$(4)\quad \rho < \frac{\kappa_j}{\kappa_i} < \frac{1}{\rho}$$

Stable coexistence is possible whenever species have either large niche differences (corresponding to small niche overlap) that overcome large average fitness differences, or at the other extreme, via an a close-to-neutral scenario (Scheffer *et al.* 2018), where, even with weak niche differences, small fitness differences stabilise the interaction between competitors. If competitors are not predicted to coexist, we can pinpoint if this is due to competitive exclusion (when fitness differences are larger than niche differences) or due to priority effects, leading to alternative states when niche differences are negative (Fukami & Nakajima 2011;

181 Ke & Letten 2018). We used data from the fully parameterized models (see below) to generate
182 these predictions.
183
184 **Experiments**
185 We performed a series of experiments in which we either manipulated the order of arrival and
186 relative frequency (i.e., relative initial abundance with a constant density of 20 individuals), or
187 the initial density of each of the two species.
188 In the first experiment, both species were introduced simultaneously using the following
189 proportions of *T. evansi* / *T. urticae*: 1:19; 10:10 and 19:1, along with the single-species
190 controls (20:0 and 0:20). To manipulate the order of arrival, we introduced (i)10 *T. evansi*
191 females 48h before 10 *T. urticae* females and vice versa and (ii) 19 *T. evansi* females 48h
192 before 1 *T. urticae* female and vice versa. The experiment was done in two blocks, one week
193 apart. Each block contained five boxes of each experimental treatment (nine treatments, with
194 ten boxes per treatment), each with a pot filled with water and two freshly cut tomato leaves
195 from five-week-old tomato plants. Leaf pairs consisted of leaves 2 and 4 or 3 and 5 (leaf
196 number indicates leaf age: leaves 2 and 5 correspond to the oldest and youngest leaves,
197 respectively). This was done to ensure that each box contained a younger and an older leaf,
198 since both species have a preference for younger (upper) leaves (Godinho *et al.* 2020a). Adult
199 females were distributed by the two leaves, following the treatments described above. After
200 one generation (circa 14 days), two more leaves were added to ensure enough resources for the
201 second mite generation. Boxes that initially received the leaf pair 2-4, received leaves 3-5 and
202 vice versa. After two generations, we counted the number of adult females of each species per
203 leaflet and leaf.
204 In the second experiment we also estimated the growth rate of each species by counting
205 the number of adult females obtained from the progeny of a single *T. urticae* or *T. evansi* female

206 ovipositing for 48h in two overlapping 18mm leaf disks, replicated 18 times. These disks were
207 placed in square petri dishes with a layer of wet cotton and were watered every two days. The
208 number of adult females produced was assessed after one generation.
209
210 **Data Analyses**
211 *Effect of order of arrival and initial frequency on species abundance*
212 To test the impact of order of arrival and frequency and their interaction on the proportion of
213 adult females of each species after two generations, we performed the following general linear
214 mixed model (lme4 package, Bates et al. 2015), using the binomial family:
215 (5) Y= Frequency + Order + Frequency x Order + Block + ε
216 Where Y corresponds to the combination of two vectors with the number of *T. evansi* and
217 *T. urticae* females after two generations, Frequency (fixed factor) to the initial ratio between
218 the two species, Order (fixed factor) to the order of arrival, Block (random factor) to whether
219 the experiment was performed on week one or two, and ε to the residual error. Additionally,
220 we ran the same model as above, but merging Frequency, Order and their interaction in a single
221 factor (Treatment, 7 levels). This allowed performing *a priori* contrasts to compare between
222 different orders of arrival and frequencies for each species, since our experimental design was
223 not orthogonal. To compare the effect of different orders of arrival, we performed contrasts
224 between the treatments with same initial frequency but different orders of arrival. To compare
225 the effect of frequency, we performed contrasts between treatments with same order of arrival
226 but different initial frequencies. Contrasts were done using testInteractions from phia package
227 (Rosario-Martinez 2015) and were then corrected for multiple comparisons using FDR
228 correction (Benjamini & Yekutieli 2001).
229
230 *Effects of order of arrival on coexistence*

231 We used a maximum likelihood approach to estimate the growth rate in absence of competitors
232 (λ) and the intra and interspecific competitive interactions (the α's) for each species. To ensure
233 model convergence, we took a nested perspective in which the results of the first model were
234 used as priors for the subsequent models which increased in complexity (Matías et al. 2018).
235 That is, estimates obtained from model 6A were used as priors for model 6B, and those
236 obtained from model 6B were used as priors for model 6C.

237 $$6A)\ N_{t+1} = \lambda * N_t$$

238 $$6B)\ N_{i,t+1} = \frac{\lambda * N_t}{(1+\alpha * N_t)}$$

239 $$6C)\ N_{i,t+1} = \frac{\lambda_i * N_{i,t}}{(1+\alpha_{ii} * N_{i,t} + \alpha_{ij} * N_{j,t})}$$

240 The initial model (6A) considers only the intrinsic growth rate in the absence of
241 interactions (λ). This model was parameterized using estimates from the experiment with
242 single *T. urticae* or *T. evansi* female. Model 6B adds an α parameter, which accounts for the
243 overall effect of competition, and finally, model 6C separates this overall competitive effect
244 into intra and interspecific components.

245

246 *Effect of order or arrival and initial frequency on leaf occupancy and aggregation*

247 To test if coexistence vs. exclusion outcomes could be explained by niche preemption due to
248 changes in leaf occupancies, we compared occupancy patterns of each species across the four
249 leaves. For the single species treatment, we tested if the number of females differed across
250 leaves (model 7). For the double species treatment, we tested if the order of arrival or initial
251 frequency, or their interaction changed mite distribution across leaves (model 8). For that, we
252 compared the number of females in each leaf to the distribution of the single species treatment.

253 We applied the following binomial models, with Leaf and/or Treatment and their
254 interaction as fixed factors, for the control (model 7) and experimental (model 8) treatments:

$$(7)\ Y = Leaf + \varepsilon$$

$$(8)\ Y = Leaf + Treatment + Leaf \times Treatment + \varepsilon$$

where Y corresponds to the combination of two vectors with the number of *T. evansi* (or *T. urticae)* females on each leaf per box and the total number of individuals on each box that were not on that leaf. For the double treatment, a posteriori contrasts were done between each treatment and the corresponding single species treatment. The initial fitting with Block as a random factor, indicated no variance in this factor, thus we fitted only fixed factors.

Since *T. evansi* suppresses defences locally, it is expected that *T. urticae* aggregates with it (Sato *et al.* 2016). To test if aggregation changed with order of arrival or initial frequency, we calculated the Checkerboard score (C-score) (Gotelli & Rhode 2002) per replicate. The C-score quantifies species co-occurrence, measuring the extent to which they segregate or aggregate across environments (Gotelli & Rhode 2002). The bipartite package (Dormann *et al.* 2008) normalizes the C-score between 0 (no aggregation) and 1 (aggregation), allowing comparisons between treatments. To calculate the C-score per leaf, we created a presence-absence matrix per leaflet and leaf for each box. We then applied the following general linear mixed model to test for differences in aggregation between treatments:

$$(9)\ Y = Treatment + Block + \varepsilon$$

Where Y is the computed C-score and Treatment is a fixed factor. Contrasts were performed between initial frequency and order of arrival, applying FDR correction for multiple comparisons, as described above.

All analyses were done using R (R Core Team 2021) using package "cxr" (García-Callejas *et al.* 2020), and plots were done using ggplot2 (Wickham 2016) and cowplot (Wilke 2020) packages. Data and scripts are available in the github repository: https://github.com/irfragata/priority_effects.

Results

Effect of order of arrival and initial frequency on species abundance

The number of individuals of each species on tomato plants were affected by the order of arrival (χ^2 = 298.93, df = 2, p-value < 0.0001), their initial frequency (χ^2 = 568.12, df = 2, p-value < 0.0001), and the interaction among these factors (χ^2 = 188.61, df = 2, p-value < 0.0001). Specifically, the abundance of *T. evansi* females after two generations was higher when this species arrived first or simultaneously with *T. urticae*, independently of initial frequencies. However, the additional advantage provided by arriving first was much larger in the equal frequency treatment, as contrasts comparing order of arrival were highly significant for equal frequencies (10:10), but not for the high *T. evansi* frequency (19:1) (Table S1, Fig.1). The abundance of *T. urticae* after two generations was also affected by initial frequency and order of arrival. Indeed, the final number of *T. urticae* females was higher when this species arrived first and was at high initial frequency, than in the equal frequency treatment (Table S1, Fig. 1). Overall, these results confirm that *T. evansi* is a superior competitor as expected from previous field and laboratory observations (Sarmento *et al.* 2011b; Ferragut *et al.* 2013; Alzate *et al.* 2020).

Effect of order of arrival on coexistence

The order of arrival modified the outcome of competition between the two species. *Tetranychus evansi* (the superior competitor) excluded *T. urticae* when it arrived first or at the same time. Under this exclusion scenario, the rate of competitive exclusion is expected to be faster when *T. evansi* arrived first due to an increase in fitness differences and due to a decrease in niche differences (Fig 2). Coexistence was only possible when *T. urticae* arrived first (Fig. 2). This outcome was due to small fitness and niche differences among competitors, leading to a quasi-neutral scenario. Specifically, when *T. urticae* arrived first, we observe almost similar

305 strengths of intra- and interspecific interactions among species (Fig S1A), combined with an
306 increase in the intrinsic growth rate of *T. urticae* (in comparison to other orders of arrival, Fig
307 S1B). Contrary to expectations and previous studies, order of arrival was not associated with
308 priority effects.
309
310 *Effect of order of arrival and initial frequency on leaf occupancy and aggregation*
311 To explore whether changes in coexistence outcomes were driven by a shift in leaf occupancy,
312 we tested how initial frequency and order of arrival affected the proportion of females of the
313 two species on each leaf (Fig 3 A, C, Fig S2) vs. the occupancy in the single species treatments
314 (Fig 3B, D). When *T. evansi* was alone, it reached a consistently higher abundances on leaves
315 3 and 4 (Table S2A, Fig 3B), whereas *T. urticae* was less abundant on leaf 2 (the oldest leaf)
316 in comparison to all others (Table S2A, Fig 3D). In treatments where *T. urticae* arrived first,
317 significantly fewer *T. evansi* females were found on leaf 4 (Fig 3, Fig S2A, Table S2B). This
318 pattern was observed for leaf 3 when *T. urticae* started with higher frequency and both species
319 arrived at the same time. When *T. evansi* arrived first or started at higher frequency, we
320 observed fewer changes on its own leaf occupancy (Fig S2A). The distribution of *T. urticae*
321 showed a slight shift when it arrived first, with a reduction on the prevalence of leaves 2 and 5
322 and slightly higher occupation of leaves 3 and 4 (Fig. S2B, Table S2B). When *T. evansi* started
323 at high frequency, there was also a shift in *T. urticae* distribution, with a lower occupancy of
324 leaves 2 and 5 (Fig S2B).
325 Spatial aggregation significantly differed among treatments ($\chi^2 = 18.186$, df = 6, p-value
326 = 0.01279), being higher in treatments with similar initial densities (cf. Fig S3 with Fig 1, Table
327 S3). We observed a significant difference in C-score (the degree of spatial aggregation) with
328 higher aggregation when both species arrived at the same time and had equal frequency, and a

329 lower aggregation when both species arrived at the same time and *T. evansi* started at higher
330 frequency (Table S3). Order of arrival did not change the C-score (Fig. S3, Table S3).
331
332 **Discussion**
333 This study shows that order of arrival interacts with the competitive ability to determine the
334 probability of coexistence between congeneric species that share common resources such as
335 food and/or space. When both species arrive at the same time or the superior competitor
336 (*Tetranychus evansi*) arrived first, *T. urticae* was predicted to be excluded. Coexistence was
337 only predicted when the inferior competitor (*T. urticae*) was the first species colonizing the
338 habitat. Analyses of leaf occupancy show that these competition outcomes are linked to a
339 spatial niche preemption process in which *T. evansi* was displaced from its preferred food
340 stratum when *T. urticae* arrived first. As a result of this complex interaction between order of
341 arrival, species competitive ability, and spatial occupancy, we observed a particular
342 configuration that allows species coexistence: both species increased niche differences and
343 reduced fitness differences to the extent that they can coexist despite small niche differences.
344 These multiple lines of evidence challenge current paradigms on the direct connection between
345 order of arrival and priority effects.
346 We found that *T. evansi* had higher competitive ability and growth rate, and often excluded
347 *T. urticae* (Fig 1, 2). This is in line with laboratory observations showing that *T. evansi*
348 outcompeted *T. urticae* on tomato plants (Sarmento *et al.* 2011b; Alzate *et al.* 2020, but see
349 Orsucci *et al.* 2017) and with field observations showing a reduction in the prevalence and a
350 shift in host use in *T. urticae* upon invasion by *T. evansi* (Ferragut et al. (2013). Still, these two
351 species can co-occur in the field in the same plant species (Ferragut *et al.* 2013; Orsucci *et al.*
352 2017; Zélé *et al.* 2018). The advantage created by the earlier arrival of *T. urticae*, and associated
353 reduction in interspecific competition by *T. evansi*, could be one of the possible mechanisms

354 fostering coexistence of the two species in nature. Indeed, *T. urticae* can withstand colder
355 temperatures than *T. evansi* (Gotoh *et al.* 2010; Khodayari *et al.* 2013; Riahi *et al.* 2013; White
356 *et al.* 2018), hence it is expected to arrive first in the season. Field surveys that sample both
357 species in the same location across seasons are needed to further explore this hypothesis.

358 Historical contingencies emerging from order of arrival can happen through two main
359 mechanisms: niche modification or niche preemption (Fukami 2015). In our system, niche
360 modification may arise via interactions between spider mites and plant defences. As *T. evansi*
361 suppresses plant defences, *T. urticae* could benefit from an early colonization from its
362 competitor (Alba *et al.* 2015; de Oliveira *et al.* 2016, 2017; Godinho *et al.* 2020a). However,
363 we observe competitive exclusion when *T. evansi* arrives first, suggesting that if *T. urticae*
364 gained this benefit, it was not enough to outcompete *T. evansi*. In turn, niche preemption can
365 occur through monopolization of nutrients or space, which can be particularly important in
366 intraguild competitive interactions (Grainger *et al.* 2018; Holditch & Smith 2020). In this study,
367 resource depletion could not explain the differences we observe in competitive outcomes, since
368 order of arrival did not have a large impact on the growth rate of both species. However, we
369 observed a shift in the leaf occupancy pattern of *T. evansi* females when *T. urticae* arrived first.
370 This displacement of *T. evansi* from the preferred food stratum (i.e., younger, more nutritious
371 leaves) by early-arriving *T. urticae* can explain the decreased competitive ability of the superior
372 competitor. Thus, our results indicate that variation in species performance driven by habitat
373 quality heterogeneity (Orians *et al.* 2000; Orians & Jones 2001) combines with order of arrival
374 to generate niche preemption, providing a mechanism for the two herbivores to coexist.

375 Order of arrival is a major determinant of community assembly across diverse taxa, from
376 microbes to plants (Chase 2003; Erb *et al.* 2011; Kardol *et al.* 2013; Stam *et al.* 2017; Grainger
377 *et al.* 2018, 2019; Clay *et al.* 2019, 2020; Halliday *et al.* 2020). Most of these studies show that
378 early colonizers inhibit growth and decrease performance of late arriving species, especially in

379 those that occupy very similar niches (Fargione *et al.* 2003; Vannette & Fukami 2014; Delory
380 *et al.* 2019, 2021; Grainger *et al.* 2019), although very few concern herbivorous species
381 competing for the same niche (e.g. Grainger *et al.* 2018; Holditch & Smith 2020). Nevertheless,
382 other studies show that order of arrival does not always affect community assembly (e.g.
383 Delory *et al.* 2021) or that initial colonizers may facilitate later colonization of other species
384 (e.g. Queijeiro-Bolaños *et al.* 2017; Delory *et al.* 2019). Here, we show that coexistence is
385 promoted by early colonization by the inferior competitor species, which reduces the
386 competitive ability of the superior competitor and increases the fitness of the inferior
387 competitor. Through fully parameterized models from modern coexistence theory, we found
388 that niche preemption increases niche differences and strongly reduces fitness differences,
389 leading to an equalising effect that allows species to coexist. Our study adds a novel perspective
390 to the growing body of evidence that historical contingencies shape ecological communities,
391 by showing that the probability of coexistence of two competing herbivores changes due to an
392 interaction between order of arrival and species competitive ability.

393 Priority effects were recently incorporated into modern coexistence theory (Ke & Letten
394 2018; Spaak & De Laender 2021), but to our knowledge, this is one of the very few studies
395 that empirically tested the impact of changes in order of arrival on species coexistence so far,
396 using this theoretical framework. Grainger *et al.* (2019) observed that positive frequency
397 dependence, due to strong priority effects, arose from changes in order or arrival in yeast
398 species feeding on floral nectars. In contrast, our results show that order of arrival did not lead
399 to priority effects caused by positive frequency dependent alternative states. Instead, when *T.*
400 *evansi* arrived first, it excluded *T. urticae* due to a large increase in fitness differences and a
401 reduction in stabilising niche differences. However, when *T. urticae*, arrived first, coexistence
402 was predicted, via an increase in niche differences and a decrease in fitness differences, making
403 the competition between the two species more neutral. These results suggests that, in the event

of a small environmental perturbation that produces changes in niche or fitness differences, the two studied species might not be able to coexist, compared to a scenario in which species coexist via strong niche differences. Framing priority effects in the modern coexistence theory (Ke & Letten 2018) is undoubtedly an important step to mechanistically understand how order of arrival affects community assembly processes. However, here we show that order of arrival does not always affect competitive outcomes via priority effects; instead, it can lead to coexistence via niche preemption by the inferior competitor. Thus, our results show that order of arrival can produce a wide range of competitive outcomes from coexistence to competitive exclusion due to positive and negative frequency dependence. Therefore, it is urgent that ecologists widen the scope of the multiple outcomes that historical contingency can produce on species coexistence.

Most empirical and theoretical studies emphasize the inhibitory nature of niche preemption (Fargione *et al.* 2003; Fukami 2015; Vieira *et al.* 2018; Delory *et al.* 2019), with the early arriving species outcompeting the other. However, recent theory suggests that, in a resource competition model of two species, niche preemption by the inferior competitor could facilitate coexistence under a trade-off between order of arrival and the resource levels of zero net growth (R*) (Qi et al. 2021). Our study is, to the best of our knowledge, the first empirical study showing that niche preemption by the weaker competitor facilitates coexistence. This striking change in the outcome of competitive interactions emerge mostly due to a decrease in fitness differences coupled with the decrease in niche overlap. This suggests that even small differences in order of arrival can be sufficient for the monopolization of a resources in plant-herbivore interactions, which may suffice to allow coexistence between competitor species. Therefore, our results demonstrate how small temporal differences percolate into small spatial heterogeneities, fostering coexistence and the maintenance of diversity.

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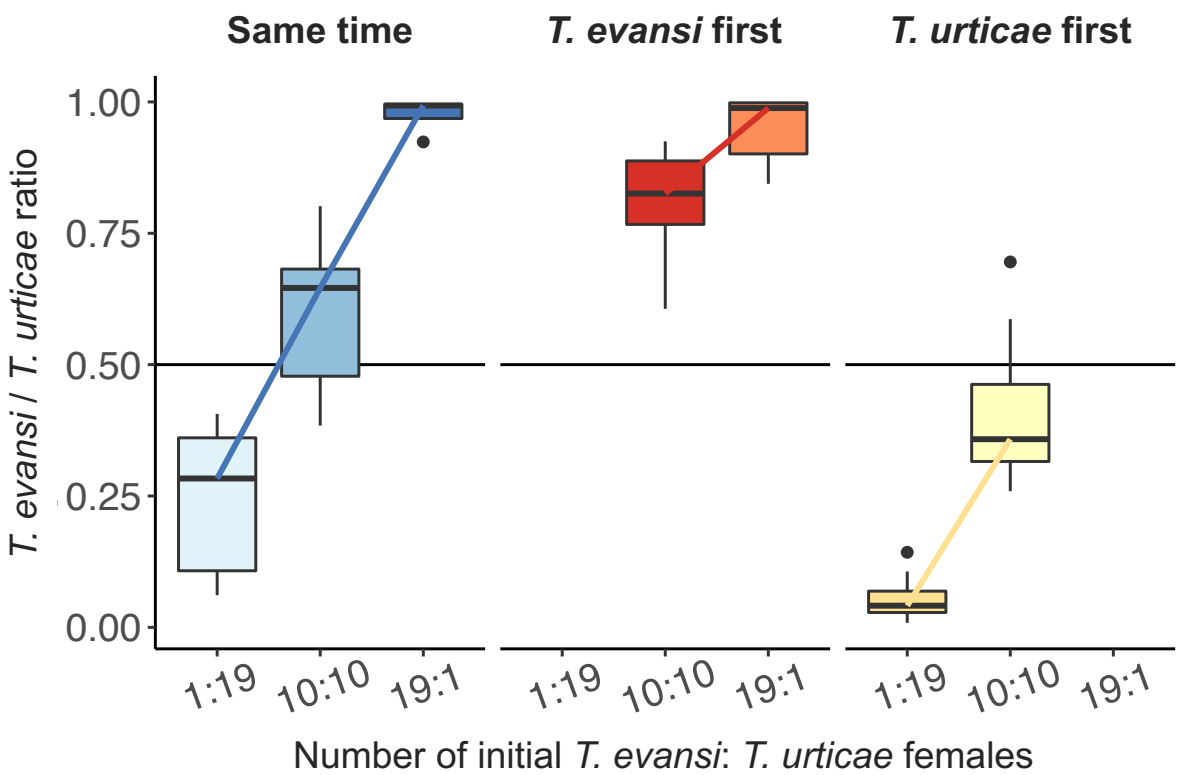
Figure 1 – Proportion of spider mites *Tetranychus evansi* females (y-axis) depending on initial frequency (number of initial females *T. evansi*: *T. urticae*, x-axis) and order of arrival (same time vs. *T. evansi* or *T. urticae* arriving 48h before its competitor) after two generations. *Tetranychus evansi* is the better competitor overall (ratio above 0.5), unless *T. urticae* arrives first or is at higher initial frequency. A posteriori contrasts show a strong effect of order of arrival in the proportion of females of the two species (Suppl. Table 1B). Initial frequency also impacts the final ratio, with a stronger effect when *T. urticae* arrives first or at the same time than *T. evansi* (Suppl. Table 1B). Boxplots represent median and quartiles of the 10 boxes within treatment.

Figure 2 – Relationship between average fitness differences ($\frac{\kappa_j}{\kappa_i}$, y-axis) and stabilising niche differences (1- ρ, x-axis) for different orders of arrival (*Tetranychus evansi* first – red, same time – blue, *T. urticae* first – yellow). Plotting average fitness differences against niche differences allows mapping different competitive outcomes predicted by modern coexistence theory (Chesson 2000; Ke & Letten 2018; Spaak & De Laender 2021). The coexistence condition (eq. 4) and its inverse, represented by the two solid black lines, allow defining the space in which species can coexist due to negative frequency dependence or enter alternative stable states due to positive frequency dependence, whenever niche differences are greater or smaller than zero respectively. Otherwise, the species with higher fitness will exclude the other. In our case, the only scenario in which species are predicted to coexist is when *T. urticae* arrives first (yellow). Error bars for each outcome indicate the 95% confidence interval from the maximum likelihood estimates. For the other two cases, it is predicted that the superior competitor *T. evansi* will exclude *T. urticae*.

Figure 3 – Differences between expected and observed leaf occupancy for *Tetranychus evansi* (A) and *T. urticae* (C) for a subset of the experimental treatments (when *T. urticae* arrived first or at the same time as *T. evansi*, note that Figure S2 includes all treatments); leaf occupancy for *T. evansi* (B) and *T. urticae* (D) in the control, single species, treatments. Leaf 2 corresponds to the oldest leaf and leaf 5 to the youngest. For each box, we calculated the ratio of females occupying each leaf in relation to the total number of females present. For the experimental treatments we calculated the difference between this ratio and the average ratio for the control treatments. Thus, positive values indicate that there are more females on that leaf than expected based on the single-species treatment and negative values indicate the reverse pattern. Overall, we see that. *T. evansi* reduces occupancy on leaf 4 when *T. urticae* arrives first and on leaf 3 when the two species arrive at the same time. In contrasts, *T. urticae* shows a slight increase in occupancy of leaf 4 when it arrives first and a slight decrease in occupancy of leaves 2 and 5.



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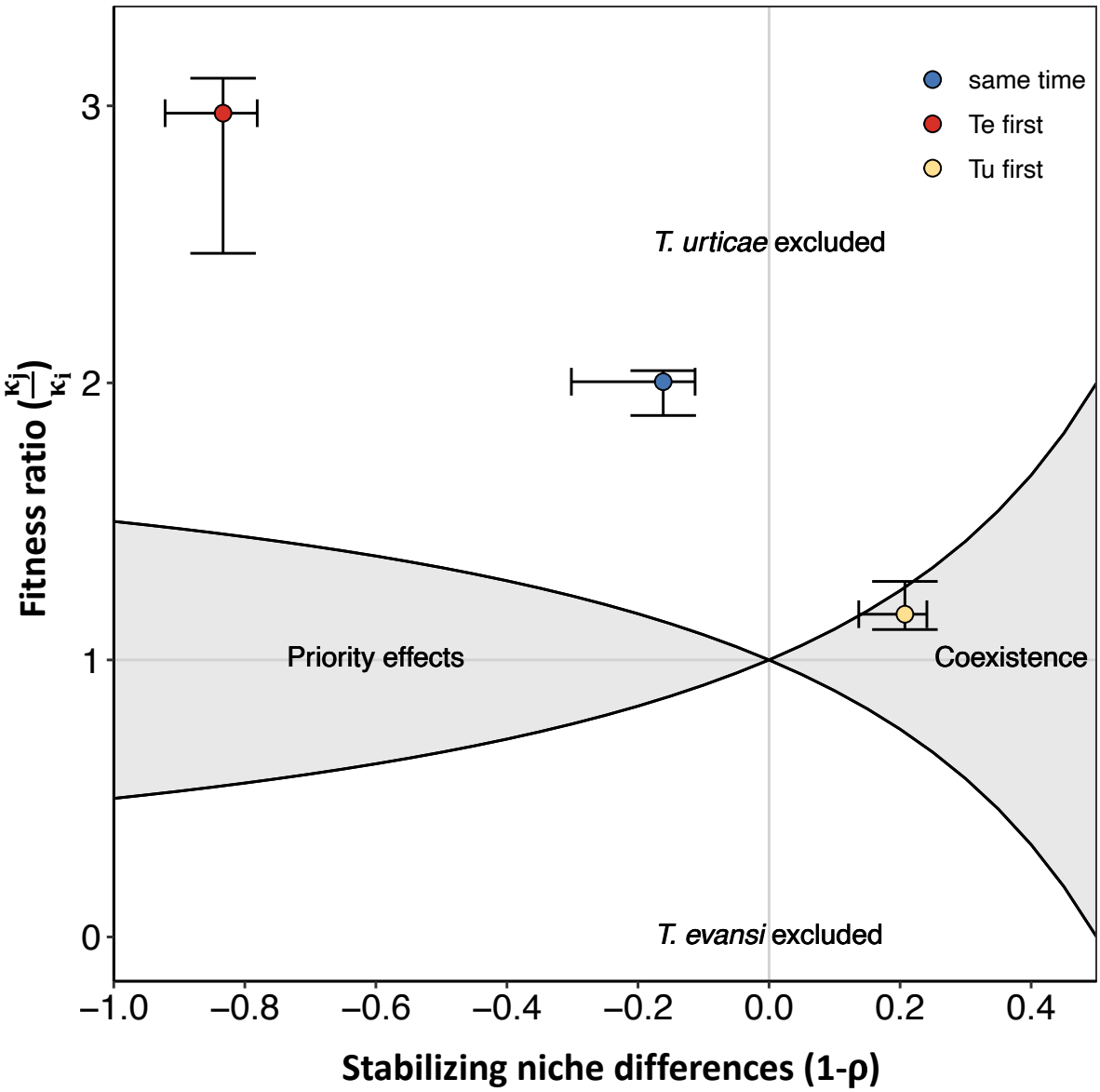

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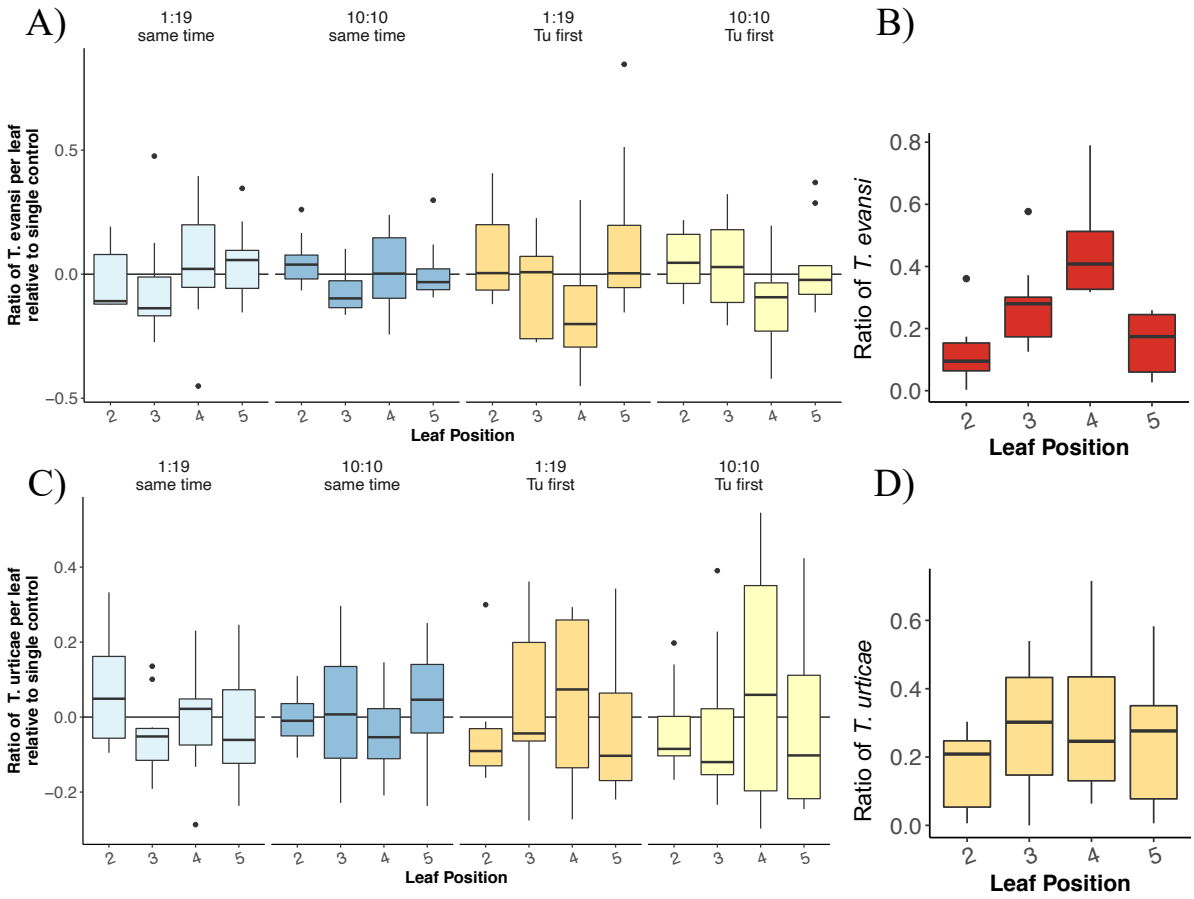

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