Specific sequence 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 affects species' competitive ability and changes the outcome of competition. Contrary to expectations, order of arrival did not cause positive frequency dependent priority effects. Instead, coexistence was predicted when the inferior competitor (*Tetranychus urticae*) arrived first. In that case, *T. urticae* colonised the preferred feeding stratum (leaves) of *T. evansi* leading to spatial niche preemption, which equalised fitness and reduced niche differences, driving community assembly to a close-to-neutrality scenario. Our study demonstrates how the order of species arrival and the spatial context of competitive interactions can jointly determine whether species can coexist.

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). Inhibitory priority effects, when earlier arrival by one species inhibits the growth of the species arriving next, are expected to result in alternative stable states hampering coexistence (Chase 2003; Fukami 2015; Ke & Letten 2018). In turn, facilitative priority effects, when population growth is higher if individuals arrive after the settlement of a first species, do not always promote coexistence. Rather, the outcome depends on the interaction strength among species and on the environmental context in which they interact (Bulleri *et al.* 2016; Bimler *et al.* 2018). These effects have been less often observed 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 colonisers reduce the amount of resource available to late colonisers, and niche modification, in which the species arriving first modifies the environment, thereby inhibiting or facilitating later colonisation (Kardol *et al.* 2013; Vannette & Fukami 2014; Fukami 2015; Delory *et al.* 2019, 2021; Grainger *et al.* 2019). 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 colonisers by depleting space and light (Kardol *et al.* 2013). Niche modification was also detected in plants, as early colonisations modified the soil metabolome and inhibited population growth of forb, but not grass species arriving later (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 in 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.

86 Modern coexistence theory posits that the long-term persistence of competing species (i.e.,
87 species coexistence) can be attained by two non-mutually exclusive mechanisms: (i) equalising
88 mechanisms that reduce average fitness differences, and therefore, dominance between species
89 and (ii) stabilising mechanisms, which stabilise the interaction between competitors by
90 increasing the strength of intraspecific competition relative to interspecific competition
91 (Chesson 2000). Therefore, species will stably coexist if stabilising niche differences are larger
92 than differences in fitness between competitors. Otherwise the species with higher fitness will
93 eventually dominate the community (Chesson 2000; Barabás *et al.* 2018; Spaak & De Laender
94 2021). Under this framework, priority effects are strictly defined as positive frequency
95 dependence (i.e., via negative niche differences), leading to the dominance of the early-arriving
96 species (Ke & Letten 2018; Grainger *et al.* 2019; Spaak & De Laender 2021). Hence, species
97 cannot coexist unless there is spatial variability in the order of arrival. Although recent theory
98 offers predictions on the outcome of coexistence in systems with historical contingencies,
99 empirical tests are conspicuously lacking (but see Cardinaux *et al.* 2018; Grainger *et al.* 2019;
100 Song *et al.* 2020). Therefore, there is as yet scarce knowledge of which species traits interact
101 with historical contingencies to determine outcomes of interspecific interactions.
102 For herbivore communities, habitat use and dispersal capacity can affect resource use and
103 ultimately the spatial distribution of consumers. This may lead to niche preemption, as
104 herbivores generally have preferred plant strata and the first arriving species may monopolise
105 that resource (Grainger *et al.* 2018; Godinho *et al.* 2020a). Moreover, herbivores often induce
106 defences on the plants they colonise, which is expected to entail niche modification for species
107 arriving later (Erb *et al.* 2011; Moreira *et al.* 2015; Stam *et al.* 2017). For example, Hougen-
108 Eitzman & Karban (1995) showed that early colonisation of grape vine leaves by Willamette
109 mites negatively affected the growth of Pacific mites, probably due to systemic induction of
110 defences. Other herbivore species can instead down-regulate plant defences, improving the

111 performance of later colonisers (Sarmento *et al.* 2011a; Godinho *et al.* 2016), thereby
112 potentially causing facilitative priority effects. Overall, given the environmental heterogeneity
113 that herbivores experience (e.g., variation in leaf quality within and between plants), effects of
114 the order of arrival on species coexistence are expected to be prevalent in these systems
115 (Utsumi *et al.* 2010; Erb *et al.* 2011; Moreira *et al.* 2015; Stam *et al.* 2017, 2018; Godinho *et*
116 *al.* 2020a). Still, what type of competitive outcome we should expect is unclear. Indeed,
117 although the order of arrival is linked to priority effects, the interaction between the chronology
118 of community assembly and the impact of species on the environment (e.g. where they growth
119 and how they modify the habitat) can result in diverse outcomes, from competitive exclusion
120 to species coexistence. Applying modern coexistence theory to this open question can shed
121 light on the proximate mechanisms that allow for species to coexist under varied historical
122 contingencies.

123 Here, we investigate the drivers of competitive outcomes by combining theoretical and
124 empirical tools to test the mechanisms through which order of arrival affects species
125 coexistence. We use as a model system the two closely-related competing herbivorous species,
126 the spider mites *Tetranychus urticae* and *T. evansi*. *Tetranychus evansi* generally outcompetes
127 *T. urticae* on tomato plants (Sarmento *et al.* 2011b; Orsucci *et al.* 2017; Alzate *et al.* 2020),
128 although both species are also commonly observed on the same location (Ferragut *et al.* 2013).
129 Niche modification is expected to be at play in this system, because the two species interact
130 with plant defences. Indeed, *T. evansi* suppresses plant defences (Sarmento *et al.* 2011a; Alba
131 *et al.* 2014), whereas most *T. urticae* populations induce them (Kant *et al.* 2008). This
132 asymmetrical niche modification is predicted to increase the probability of coexistence by
133 hampering growth of the stronger competitor and favouring growth of the inferior one, when
134 the later arrives on plants colonised by the other species. Moreover, niche preemption may
135 occur, as both *T. evansi* and *T. urticae* prefer the upper, more nutritious leaves of tomato plants,

136 where their performance is higher (Godinho *et al.* 2020a). Thus, early-arriving species could
137 occupy the preferred niche and displace the other species to lower, less optimal, plant strata.
138 We tested this by performing a series of multi-generational experiments where we varied order
139 of arrival and measured space use by the two competing species. To quantify the magnitude of
140 niche modification, we tested how these species modify the expression of genes associated
141 with induced defenses on tomato. We then applied modern coexistence theory to unravel the
142 conditions favouring coexistence or potentially leading to priority effects.
143

144 **Material and Methods**

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

146 *Tetranychus urticae* is a generalist herbivore that feeds on many economically important crops
147 (Helle & Sabelis 1985; Grbić *et al.* 2011; Sousa *et al.* 2019), whereas *T. evansi* is a solanaceous
148 specialist that has recently invaded Europe (Boubou *et al.* 2012). Both species colonise tomato
149 plants, although *T. urticae* may shift to other hosts if *T. evansi* is present (Ferragut *et al.* 2013).
150 All experiments were performed with outbred populations of *T. urticae* and *T. evansi*
151 spider mites, formed via controlled crosses among four *T. evansi* and three *T. urticae*
152 populations collected in different locations in Portugal (Godinho *et al.* 2020b). Populations
153 were maintained in boxes containing leaves detached from five-week-old tomato plants
154 (*Solanum lycopersicum*, *var* MoneyMaker), with their petiole in a small pot containing water
155 Twice a week, overexploited leaves were removed, and water and new tomato leaves were
156 added. Before infestation, tomato plants were kept in a separate climatic chamber and watered
157 three times per week. Mites and plants were kept under controlled conditions (25 ºC, 70%
158 humidity, 16 /8 L/D hours).
159 We created same-age cohorts of mated *T. urticae* and *T. evansi* females for each block. To
160 this aim, females were placed during 48h in petri dishes (14.5 cm diameter, with a layer of wet

cotton watered twice per week) and two freshly cut tomato leaves. One week later, another tomato leaf was added. 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 parameterise a mathematical model from which niche and average fitness differences can be quantified to then draw predictions of competitive outcomes. We assume that the population dynamics in our experiment can be described by a Beverton-Holt function (Levine & HilleRisLambers 2009; Godoy & Levine 2014):

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

Where $N_{i,t+1}$ is the number of individuals of species *i* in the next generation, λ_i the intrinsic growth rate of species *i* in absence of competitors, α_{ii} the intraspecific competitive interaction describing the per-capita effect of species *i* on itself, α_{ij} the interspecific competitive interactions describing the per-capita effect of species *j* on species *i*, and $N_{i,t}$, $N_{j,t}$ the number of individuals of species *i* and *j* in the current generation, respectively. We assume that spider mites do not have a dormant stage. Thus, λ_i represents the fraction of eggs that hatch and become females that reproduce in the next generation. One of the predictions of modern coexistence theory is that, for species to coexist, they must invade the resident species from rare. Because for our system equilibrium densities are difficult to attain within a time frame fast enough to study the impact of priority effects on species coexistence, we instead used experimental gradients of density and relative frequency to estimate intra and interspecific competitive interactions (the α's) and intrinsic growth rate (λ) for each species, an approach well established and validated by previous work (Godoy & Levine 2014; Matías *et al.* 2018; Song *et al.* 2020).

From the above mentioned 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 population growth of their own species more strongly than that of their competitors (α_{jj}, α_{ii}, are much greater than α_{ij}, α_{ji}), then niche overlap will be low, favouring coexistence. Alternatively, niche overlap will approach one, which hampers stable coexistence. Stabilising niche differences are thus 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. 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 stabilising 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 no coexistence is predicted, we can pinpoint if this is due to competitive exclusion (when fitness differences are larger than niche differences) or to priority effects, leading to alternative states when niche differences are negative. Negative niche

208 differences imply that each species limits the growth of the competitor more than their own
209 (Fukami & Nakajima 2011; Ke & Letten 2018).

210 We used maximum likelihood techniques to parameterise the population model following
211 a nested approach. That is, we first created a single model for which we estimate the intrinsic
212 growth rate in absence of competitors (λ), and then we used this information as prior for
213 subsequent more complex models that include intra and interspecific competitive interactions
214 (the α's) (Matías et al. 2018). λ values were considered fixed per species across empirical
215 treatments, but competition varied across treatments because mite species can differentially
216 disperse and modify leaf quality and availability (see the full details in the Supplementary
217 Material and Methods).

218

219 **Experiments**

220 To test the impact of order of arrival on coexistence, we performed a series of experiments in
221 which we either manipulated the order of arrival and relative frequency (i.e., relative initial
222 abundance with a constant density of 20 individuals), or the initial density of each of two
223 species of competing spider mites. Furthermore, to estimate the effect of order of arrival on
224 promoting niche preemption, we quantified leaf occupation for both species at the end of the
225 experiment. Finally, to estimate the effect of order of arrival on promoting niche modification,
226 we quantified induction of plant defences of both species.

227 In the first experiment, both species were introduced simultaneously using the following
228 proportions of *T. evansi* : *T. urticae*: 1:19; 10:10 and 19:1, along with the single-species
229 controls (20:0 and 0:20). To manipulate the order of arrival, we introduced (i)10 *T. evansi*
230 females 48h before 10 *T. urticae* females and vice versa and (ii) 19 *T. evansi* females 48h
231 before 1 *T. urticae* female and vice versa (Figure S1). The experiment was done in two blocks,
232 one week apart. Each block contained five boxes of each experimental treatment (nine

233 treatments, n=10), each with a pot filled with water and two freshly cut tomato leaves from
234 five-week-old tomato plants. Leaf pairs consisted of leaves 2 and 4 or 3 and 5 (leaf number is
235 inversely proportional to leaf age), to ensure that each box contained a younger and an older
236 leaf, since both species prefer younger leaves (Godinho *et al.* 2020a). Adult females were
237 distributed by the two leaves, following the treatments described above. After one generation
238 (circa 14 days), two more leaves were added to ensure enough resources for the second mite
239 generation. Boxes that initially received the leaf pair 2-4, received leaves 3-5 and vice versa.
240 After two generations, we counted the number of adult females of each species on each leaf.
241 Next, we estimated the growth rate of each species by counting the number of adult
242 females obtained from the progeny of a single *T. urticae* or *T. evansi* female ovipositing for
243 48h in two overlapping 18mm leaf disks (n=10). These disks were placed in square petri dishes
244 with a layer of wet cotton and were watered every two days. The number of adult females
245 produced was assessed after one generation.
246
247 Quantification of niche modification
248 To quantify the magnitude of niche modification induced by *T.urticae* and *T.evansi*, we
249 investigated how these two species modified the expression of genes associated with plant
250 defences. As controls, we quantified the expression of the same genes upon infestation with
251 spider mites from *T. urticae* Santpoort and *T.evansi* Viçosa populations, known to induce and
252 suppress tomato defences, respectively (Alba *et al.* 2014). Details of this experiment are given
253 in the Supplementary Material and Methods and Table S1.
254
255 **Data Analyses**
256 *Effect of order of arrival and initial frequency on species abundance*

257 To test the impact of order of arrival, frequency and their interaction on the proportion of adult
258 females of each species after two generations, we performed the following general linear mixed
259 model (lme4 package, Bates et al. 2015), using the binomial family:

260 $$(5)\ Y = \text{Treatment} + \text{Block} + \varepsilon$$

261 Where Y corresponds to the combination of two vectors with the number of *T. evansi* and
262 *T. urticae* females after two generations, Treatment (fixed factor) to the combination of
263 different orders of arrival and initial frequencies, Block (random factor) to whether the
264 experiment was performed on week one or two, and ε to the residual error. We then performed
265 *a priori* contrasts, using testInteractions from phia package (Rosario-Martinez 2015) as our
266 experimental design was not orthogonal. To compare the effect of different orders of arrival,
267 we performed contrasts between the treatments with same initial frequency but different orders
268 of arrival. To compare the effect of frequency, we performed contrasts between treatments with
269 same order of arrival but different initial frequencies. Contrasts were corrected for multiple
270 comparisons using FDR correction (Benjamini & Yekutieli 2001). To test whether the results
271 were biased by the order in which the leaf pairs were added to the boxes, we repeated these
272 statistical analyses separately for each leaf pair.

273

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

275 To test if coexistence outcomes could be explained by niche preemption, we compared
276 occupancy patterns of each species across the four leaves. For the single species treatment, we
277 tested if the number of females differed across leaves (model 6). For the double species
278 treatment, we tested if the order of arrival, initial frequency, or their interaction changed mite
279 distribution (model 7), by comparing it to the distribution of the single species treatment.

280 We applied the following binomial models, with Leaf and/or Treatment and their
281 interaction as fixed factors, for the control (model 6) and experimental (model 7) treatments:

$$(6)\ Y = Leaf + \varepsilon$$

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

where Y corresponds to the combination of two vectors with the number of *T. evansi* (or *T. urticae)* females on each leaf and the total number of individuals on each box that were not on that leaf. To test whether the results were biased by the order in which the leaf pairs were added to the boxes, we repeated these statistical analyses accounting for the preference of each species for each leaf pair. 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. We also tested in changes in order of arrival affected aggregation scores (see details in Supplementary Material and Methods).

All analyses were done using R (R Core Team 2021). To predict coexistence outcomes we used the package "cxr" (García-Callejas *et al.* 2020). 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/order_arrival_niche_preemption.

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 (contrasts between *T. evansi* arriving first vs. simultaneously: $\chi^2 = 44.252$, df = 1, p-value < 0.0001; or *T. urticae* arriving first vs. simultaneously: $\chi^2 = 375.860$, df = 1, p-value < 0.0001), and their initial frequency (contrasts between *T. evansi* starting at equal *vs.* higher frequency. *:* $\chi^2 = 784.335$, df = 1, p-value < 0.0001; or *T. urticae* starting at equal *vs*. higher frequency*:* $\chi^2 = 654.903$, df = 1, p-value < 0.0001). Specifically, the abundance of *T. evansi* females after

307 two generations was higher when this species arrived first or simultaneously with *T. urticae*,
308 independently of initial frequencies. However, the additional advantage provided by arriving
309 first was much larger in the equal frequency treatment (Table S2, Fig.1). The abundance of *T.*
310 *urticae* after two generations was also affected by initial frequency and order of arrival. Indeed,
311 the final number of *T. urticae* females was higher when this species arrived first and was at
312 high initial frequency, than in the equal frequency treatment (Table S2, Fig. 1). We observed
313 the same patterns when performing these analyses per leaf pair (Table S3). Overall, these
314 results confirm that *T. evansi* is a superior competitor as observed in previous studies
315 (Sarmento *et al.* 2011b; Ferragut *et al.* 2013; Alzate *et al.* 2020).
316
317 *Effect of order of arrival on coexistence*
318 The order of arrival modified the outcome of competition between the two species. *Tetranychus*
319 *evansi* (the superior competitor) is predicted to exclude *T. urticae* when it arrives first or at the
320 same time. Under this exclusion scenario, the rate of competitive exclusion is expected to be
321 faster when *T. evansi* arrives first due to a decrease in niche differences (Fig 2). The small
322 overlap between the lower confidence interval with the priority effects region suggests that
323 positive frequency dependence might also emerge in this system. Interestingly, coexistence
324 was only possible when *T. urticae* arrived first (Fig. 2). This outcome was due to small niche
325 and fitness differences among competitors, leading to a quasi-neutral scenario. Specifically,
326 when *T. urticae* arrived first, we observed similar strengths of intra- and interspecific
327 interactions among species (Fig S2A). Contrary to expectations and previous studies, the order
328 of arrival was not associated with positive frequency dependence leading to priority effects.
329 However, since the order of arrival modified the outcome of the interactions between *T. urticae*
330 and *T. evansi*, we can also interpret these results as priority effects (*sensu* (Chase 2003; Fukami
331 2015) allowing for coexistence between species in our system.

332

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

334 When *T. evansi* was alone, it reached higher abundances on leaves 3 and 4 (Table S4A, Fig
335 3B), whereas *T. urticae* was less abundant on leaf 2 in comparison to all others (Table S4A,
336 Fig 3D). Fewer *T. evansi* females were found on leaf 4 when *T. urticae* arrived first, and on
337 leaf 3 when *T. urticae* started with higher frequency and both species arrived at the same time
338 (Fig 3, Fig S3A, Table S4B). When *T. evansi* arrived first or started at higher frequency, we
339 observed fewer changes on its own leaf occupancy (Fig S4A). The distribution of *T. urticae*
340 showed a slight shift when it arrived first, with a reduction on the prevalence of leaves 2 and 5
341 and slightly higher occupation of leaves 3 and 4 (Fig. S3B, Table S4B). When *T. evansi* started
342 at high frequency, there was also a shift in *T. urticae* distribution, with a lower occupancy of
343 leaves 2 and 5 (Fig S3B). We observed similar shifts in leaf occupation when performing the
344 analyses accounting for the order in which each leaf pair was added (Fig S4, Table S5).

345 Spatial aggregation significantly differed among treatments ($\chi^2 = 18.186$, df = 6, p-value
346 = 0.01279), being higher in treatments with similar initial densities (cf. Fig S5 with Fig 1, Table
347 S5). We observed a significant difference in C-score, with higher aggregation when both
348 species arrived at the same time and had equal frequency, and a lower aggregation when both
349 species arrived at the same time and *T. evansi* started at higher frequency (Table S6). Order of
350 arrival did not change the C-score (Fig. S5, Table S6).

351

352 *Quantification of niche modification*

353 Plants infested by *T. urticae* or *T. evansi* populations showed patterns of gene expression
354 similar to those of Viçosa, the suppression control, and significant differences with Santpoort,
355 the induction control (Fig. S6; Table S7). We thus conclude that, both populations suppress
356 plant defences.

357

358 **Discussion**

359 This study shows that order of arrival interacts with competitive ability to determine the
360 probability of coexistence between congeneric species that share common resources. When
361 both species arrive at the same time or the superior competitor (*Tetranychus evansi*) arrived
362 first, *T. urticae* was predicted to be excluded. Coexistence was only predicted when the inferior
363 competitor (*T. urticae*) was the first species colonising the habitat. Analyses of leaf occupancy
364 show that these competition outcomes are linked to a spatial niche preemption process in which
365 *T. evansi* was displaced from its preferred food stratum when *T. urticae* arrived first. As a result
366 of this complex interaction between order of arrival, species competitive ability, and spatial
367 occupancy, we observed a particular configuration that allows species coexistence: both
368 species equalised their fitness differences to the extent that they can coexist despite small niche
369 differences. These multiple lines of evidence challenge the common understanding of the
370 inhibitory role of niche preemption in coexistence between species.

371 We found that *T. evansi* had higher competitive ability and growth rate, and often excluded
372 *T. urticae* (Fig 1, 2). This is in line with laboratory observations showing that *T. evansi*
373 outcompeted *T. urticae* on tomato plants (Sarmento *et al.* 2011b; Alzate *et al.* 2020, but see
374 Orsucci *et al.* 2017) and with field observations showing a shift in host use in *T. urticae* upon
375 invasion by *T. evansi* (Ferragut et al. 2013). Still, these two species can co-occur in the field in
376 the same plant species (Ferragut *et al.* 2013; Orsucci *et al.* 2017; Zélé *et al.* 2018). The
377 advantage created by the earlier arrival of *T. urticae*, and associated reduction in interspecific
378 competition by *T. evansi*, could be one of the possible mechanisms fostering their coexistence.
379 Indeed, *T. urticae* can withstand colder temperatures than *T. evansi* (Gotoh *et al.* 2010;
380 Khodayari *et al.* 2013; Riahi *et al.* 2013; White *et al.* 2018), hence it is expected to arrive first

381 in the season. Field surveys that sample both species in the same location across seasons are
382 needed to further explore this hypothesis.

383 Historical contingencies emerging from order of arrival can happen through two main
384 mechanisms: niche modification or niche preemption (Fukami 2015). In our system, niche
385 modification may arise via interactions between spider mites and plant defences. However, we
386 observe that both species suppress plant defenses. If suppression would affect species
387 performance, we would expect higher production of offspring when the competitor arrives first.
388 We did not observe this, suggesting that this mechanism of niche modification does not affect
389 the outcome of competition in this system.

390 Niche preemption can occur through monopolisation of nutrients or space, which can be
391 particularly important among competitors with similar requirements (Grainger *et al.* 2018;
392 Holditch & Smith 2020). In our study, we observed a shift in the leaf occupancy pattern of *T.*
393 *evansi* females when *T. urticae* arrived first. This displacement of *T. evansi* from the preferred
394 food stratum (i.e., younger, more nutritious leaves) by early-arriving *T. urticae* can explain the
395 decreased performance of the superior competitor. Thus, our results indicate that variation in
396 species performance driven by habitat quality heterogeneity (Orians *et al.* 2000; Orians & Jones
397 2001) combines with order of arrival to generate niche preemption, providing a mechanism for
398 the two herbivores to coexist.

399 Order of arrival is a major determinant of community assembly across diverse taxa, from
400 microbes to plants (Chase 2003; Erb *et al.* 2011; Kardol *et al.* 2013; Stam *et al.* 2017; Grainger
401 *et al.* 2018, 2019; Clay *et al.* 2019, 2020; Halliday *et al.* 2020). Most of these studies show that
402 early colonisers inhibit growth and decrease performance of late arriving species, especially in
403 those that occupy very similar niches (Fargione *et al.* 2003; Vannette & Fukami 2014; Delory
404 *et al.* 2019, 2021; Grainger *et al.* 2019), although very few concern herbivorous species
405 competing for the same niche (e.g. Grainger *et al.* 2018; Holditch & Smith 2020). Other studies

found that order of arrival does not affect community assembly (e.g. Delory *et al.* 2021) or that initial colonisers facilitate later colonisation of other species (e.g. Queijeiro-Bolaños *et al.* 2017; Delory *et al.* 2019). Here, we show that coexistence is promoted by niche preemption because early colonisation by the inferior competitor leads to increased intraspecific competition for the superior competitor and reduced interspecific competition for itself. . As a result, both species can coexist under a quasi-neutral scenario because this equalising effect on fitness differences is enough to fit within the constraints of small niche differences. Our study adds a novel perspective to the growing body of evidence that historical contingencies shape ecological communities, by showing that the probability of coexistence of two competing herbivores changes due to an interaction between order of arrival and species competitive ability.

Priority effects were recently incorporated into modern coexistence theory (Ke & Letten 2018; Spaak & De Laender 2021), but empirical tests quantifying the effects of order of arrival on species coexistence remain very rare. In another study, Grainger *et al.* (2019) documented that positive frequency dependence, due to strong priority effects, arose from changes in order or arrival in yeast species feeding on floral nectars. In contrast, our results show that order of arrival did not lead to alternative states caused by priority effects under positive frequency dependence. Rather, we predicted either competitive exclusion when *T. evansi* arrived first because it excluded *T. urticae* or coexistence when *T. urticae* arrived first. Overall, these results suggest that in this system deterministic expectations, stemming from theory, can be strongly influenced by small stochastic events, such as changes in order of arrival, because it affects the timing of dispersal across and within host plants.

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, in this framework, priority effects are only caused

431 by positive frequency dependence (i.e., population growth rate is higher as individuals become
432 relatively more abundant) (Fukami 2015; Song *et al.* 2020). Including other types of
433 interactions and outcomes into modern coexistence framework is fundamental to improve our
434 ability to understand how species coexist (Spaak *et al.* 2021). Here we show that order of arrival
435 can lead to coexistence via niche preemption by the inferior competitor. Thus, our results show
436 that changes in the order of arrival can produce a wide range of competitive outcomes from
437 coexistence to competitive exclusion due to positive and negative frequency dependence.
438 Therefore, it is urgent that ecologists widen the scope of the multiple outcomes that historical
439 contingency can produce on species coexistence.

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

453

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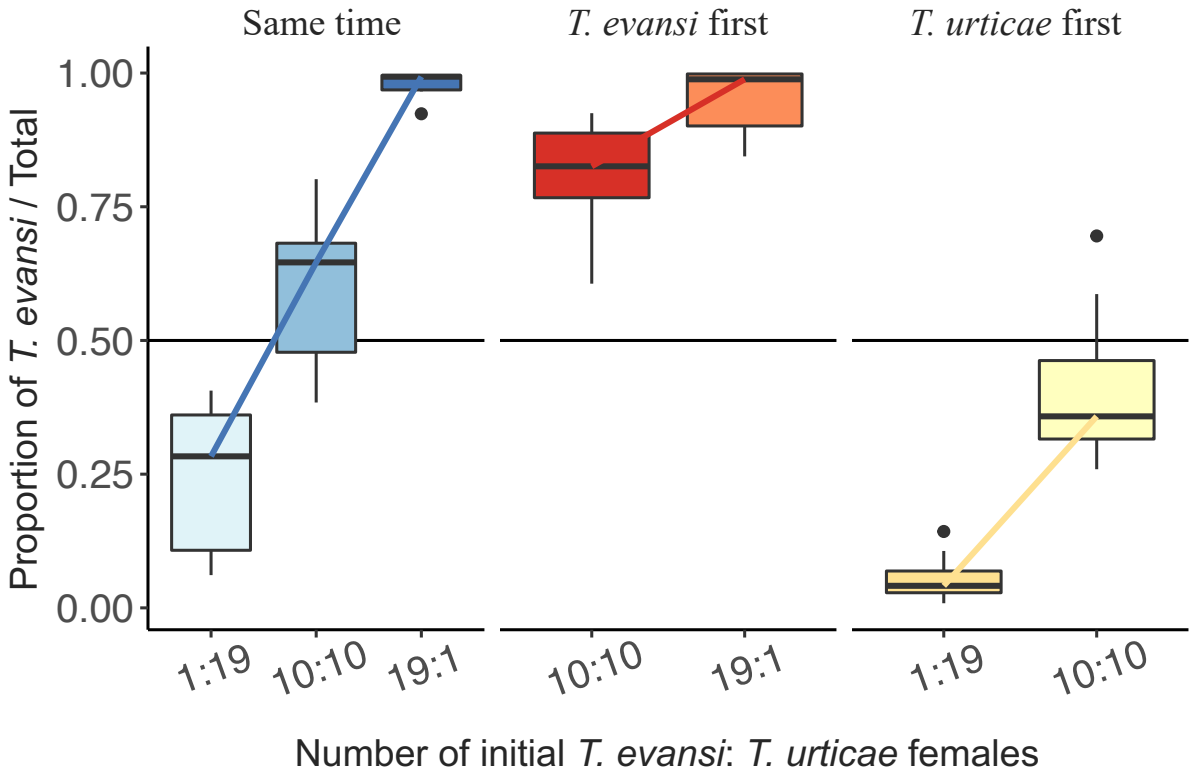
Figure 1 – Proportion of spider mites *Tetranychus evansi* females (y-axis) depending on initial frequency (number of initial females *T. evansi*: *T. urticae*, x-axis) and order of arrival (same time vs. *T. evansi* or *T. urticae* arriving 48h before its competitor) after two generations. *Tetranychus 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 (Table S2B). Initial frequency also impacts the final ratio, with a stronger effect when *T. urticae* arrives first or at the same time than *T. evansi* (Table S2B). 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. The coexistence condition (eq. 4) and its symmetrical for each competing species, 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 S3 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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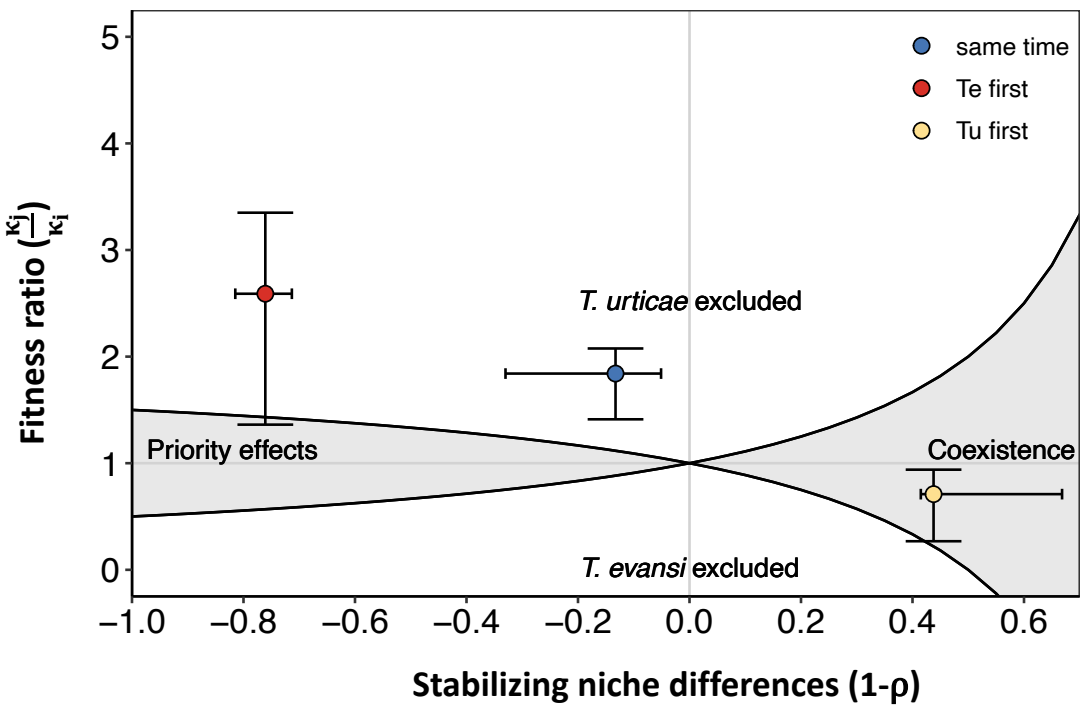

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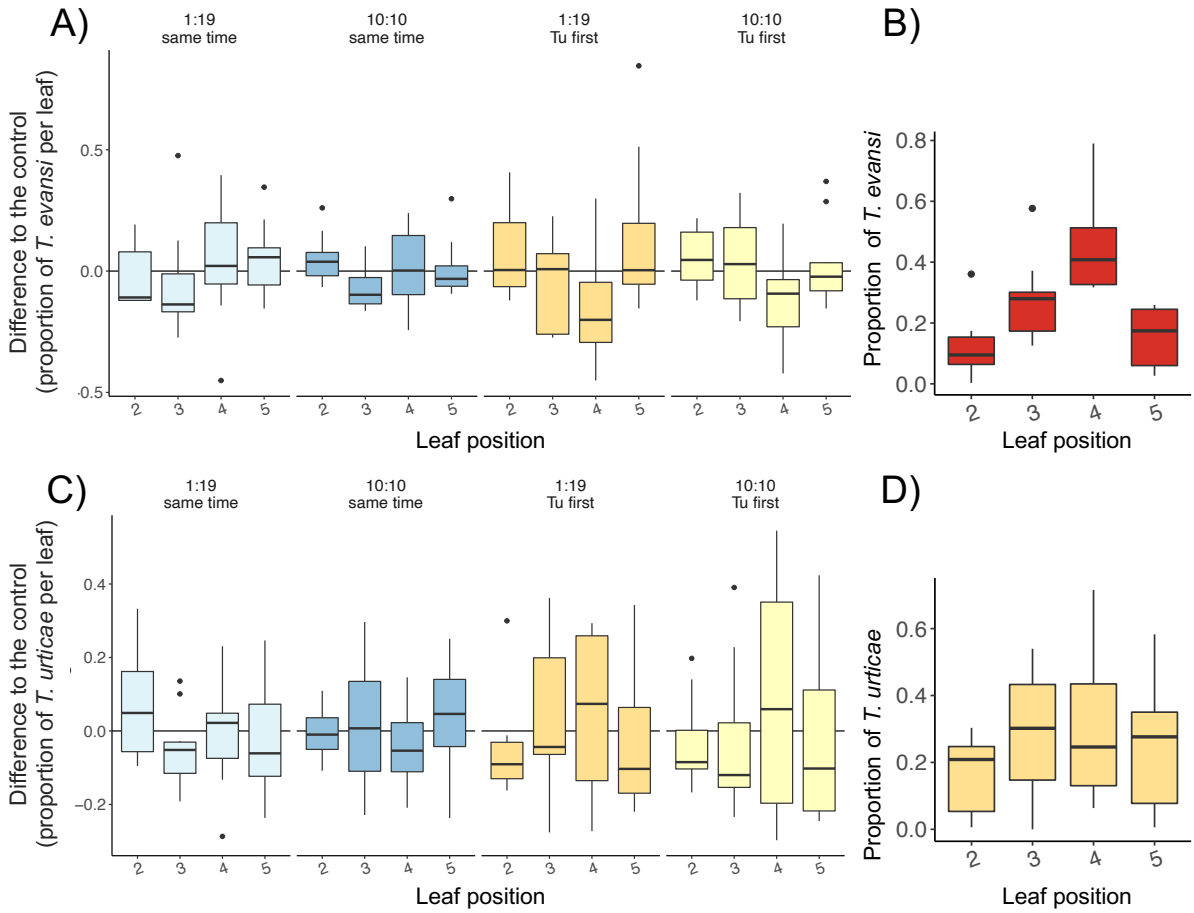

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