

LETTER

Specific sequence of arrival promotes coexistence via spatial niche pre-emption by the weak competitor

Inês Fragata¹  | Raul Costa-Pereira²  | Mariya Kozak¹ | Agnieszka Majer³  | Oscar Godoy⁴  | Sara Magalhães¹ 

¹Centre for Ecology, Evolution and Environmental Changes, Department of Animal Biology, Faculty of Sciences University of Lisbon, Lisbon, Portugal

²Department of Animal Biology, Institute of Biology, Universidade Estadual de Campinas, Campinas, Brazil

³Population Ecology Lab, Faculty of Biology, Adam Mickiewicz University, Poznań, Poland

⁴Department of Biology, Instituto Universitario de Investigación Marina (INMAR), Universidad de Cádiz, Puerto Real, Spain

Correspondence

Inês Fragata, cE3c, Centre for Ecology, Evolution and Environmental changes, Faculdade de Ciências da Universidade de Lisboa, Edifício C2, Campo Grande 1749-016 Lisbon, Portugal.
Email: irfragata@fc.ul.pt

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Abstract

Historical contingency, such as the order of species arrival, can modify competitive outcomes via niche modification or pre-emption. 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 pre-emption, 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 may jointly determine whether species can coexist.

KEYWORDS

competitive ability, herbivores, modern coexistence theory, niche modification, order of arrival, priority effects, spatial segregation, *Tetranychus urticae* and *T. evansi*

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

Oscar Godoy: Co-last authorship

strength among species and on the environmental context in which they interact (Bimler et al., 2018; Bulleri et al., 2016). These effects have been less often observed in natural communities (Clay et al., 2019; Halliday et al., 2020; Queijeiro-Bolaños et al., 2017).

Two major mechanisms are predicted to cause priority effects: niche pre-emption, 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 (Delory et al., 2019, 2021; Fukami, 2015; Grainger et al., 2019; Kardol et al., 2013; Vannette & Fukami, 2014). Niche pre-emption 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 pre-emption and modification is not always possible (Boyle et al., 2021; Grainger et al., 2018), 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.

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) equalising mechanisms that reduce average fitness differences, and therefore, dominance between species and (ii) stabilising mechanisms, which stabilise the interaction between competitors by increasing the strength of intraspecific competition relative to interspecific competition (Chesson, 2000). Therefore, species will stably coexist if stabilising niche differences are larger than differences in fitness between competitors. Otherwise the species with higher fitness will eventually dominate the community (Barabás et al., 2018; Chesson, 2000; Spaak & De Laender, 2021). Under this framework, priority effects are strictly defined as positive frequency dependence (i.e. via negative niche differences), leading to the dominance of the early-arriving species (Grainger et al., 2019; Ke & Letten, 2018; Spaak & De Laender, 2021). Hence, species cannot coexist unless there is spatial variability in the order of arrival. Although 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 herbivore communities, habitat use and dispersal capacity can affect resource use and ultimately the

spatial distribution of consumers. This may lead to niche pre-emption, as herbivores generally have preferred plant strata and the first arriving species may monopolise that resource (Godinho et al., 2020a; Grainger et al., 2018). Moreover, herbivores often induce defences on the plants they colonise, which is expected to entail niche modification for species arriving later (Erb et al., 2011; Moreira et al., 2015; Stam et al., 2017). For example, Hougens-Eitzman and Karban (1995) showed that early colonisation of grape vine leaves by Willamette mites negatively affected the growth of Pacific mites, probably due to systemic induction of defences. Other herbivore species can instead down-regulate plant defences, improving the performance of later colonisers (Godinho et al., 2016; Sarmiento et al., 2011a), thereby potentially causing facilitative priority effects. Overall, given the environmental heterogeneity that herbivores experience (e.g. variation in leaf quality within and between plants), effects of the order of arrival on species coexistence are expected to be prevalent in these systems (Erb et al., 2011; Godinho et al., 2020a; Moreira et al., 2015; Stam et al., 2017, 2018; Utsumi et al., 2010). Still, what type of competitive outcome we should expect is unclear. Indeed, although the order of arrival is linked to priority effects, the interaction between the chronology of community assembly and the impact of species on the environment (e.g. where they grow and how they modify the habitat) can result in diverse outcomes, from competitive exclusion to species coexistence. Applying modern coexistence theory to this open question can shed light on the proximate mechanisms that allow for species to coexist under varied historical contingencies.

Here, we investigate the drivers of competitive outcomes by combining theoretical and empirical tools to test the mechanisms through which order of arrival affects species coexistence. We use as a model system the two closely-related competing herbivorous species, the spider mites *Tetranychus urticae* and *T. evansi*. *Tetranychus evansi* generally outcompetes *T. urticae* on tomato plants (Alzate et al., 2020; Orsucci et al., 2017; Sarmiento et al., 2011b), although both species are also commonly observed on the same location (Ferragut et al., 2013). Niche modification is expected to be at play in this system because the two species interact with plant defences. Indeed, *T. evansi* suppresses plant defences (Alba et al., 2014; Sarmiento et al., 2011a), whereas most *T. urticae* populations induce them (Kant et al., 2008). This asymmetrical niche modification is predicted to increase the probability of coexistence by hampering growth of the stronger competitor and favouring that of the inferior one, when the later arrives on plants colonised by the other species. Moreover, niche pre-emption may occur, as both *T. evansi* and *T. urticae* prefer the upper, more nutritious leaves of tomato plants, where their performance is higher (Godinho et al., 2020a). Thus, early-arriving species could occupy the preferred niche and displace the other species to lower,

less optimal, plant strata. We tested this by performing a series of multi-generational experiments where we varied order of arrival and measured space use by the two competing species. To quantify the magnitude of niche modification, we tested how these species modify the expression of genes associated with induced defences on tomato. We then applied modern coexistence theory to unravel the conditions favouring coexistence or potentially leading to priority effects.

MATERIAL AND METHODS

Model system, species characteristics and maintenance of experimental populations

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

All experiments were performed with outbred populations of *T. urticae* and *T. evansi* spider mites, formed via controlled crosses among four *T. evansi* and three *T. urticae* populations collected in different locations in Portugal (Godinho et al., 2020b). Populations were maintained in boxes containing leaves detached from five-week-old tomato plants (*Solanum lycopersicum*, var MoneyMaker), with their petiole in a small pot containing water. Twice a week, overexploited leaves were removed, then water and new tomato leaves were added. Before infestation, tomato plants were kept in a separate climatic chamber and watered three times per week. Mites and plants were kept under controlled conditions (25°C, 70% humidity, 16/8 L/D hours).

We created same-age cohorts of mated *T. urticae* and *T. evansi* females for each block. To this aim, females were placed during 48 h 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 (Godoy & Levine, 2014; Levine & HilleRisLambers, 2009):

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

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).

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

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 $1-\rho$.

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

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

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):

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

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

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

Experiments

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

In the first experiment, both species were introduced simultaneously using the following proportions of *T. evansi*: *T. urticae*: 1:19; 10:10 and 19:1, along with the single-species controls (20:0 and 0:20). To manipulate the order of arrival, we introduced (i) 10 *T. evansi* females 48h before 10 *T. urticae* females and vice versa and (ii) 19 *T. evansi* females 48h before 1 *T. urticae* female and vice versa (Figure S1). The experiment was done in two blocks, one week apart. Each block contained five boxes of each experimental treatment (nine treatments, $n = 10$), each with a pot filled with water and two freshly cut tomato leaves from 5-week-old tomato plants. Leaf pairs consisted of leaves 2 and 4 or 3 and 5 (leaf number is inversely proportional to leaf age), to ensure that each box contained a younger and an older leaf, since both

species prefer younger leaves (Godinho et al., 2020a). Adult females were distributed by the two leaves, following the treatments described above. After one generation (circa 14 days), two more leaves were added to ensure enough resources for the second mite generation. Boxes that initially received the leaf pair 2–4, received leaves 3–5 and vice versa. After two generations, we counted the number of adult females of each species on each leaf.

Next, we estimated the growth rate of each species by counting the number of adult females obtained from the progeny of a single *T. urticae* or *T. evansi* female ovipositing for 48h in two overlapping 18mm leaf disks ($n = 10$). These disks were placed in square petri dishes with a layer of wet cotton and were watered every two days. The number of adult females produced was assessed after one generation.

Quantification of niche modification

To quantify the magnitude of niche modification induced by *T. urticae* and *T. evansi*, we investigated how these two species modified the expression of genes associated with plant defences. As controls, we quantified the expression of the same genes upon infestation with spider mites from *T. urticae* Santpoort and *T. evansi* Viçosa populations, known to induce and suppress tomato defences, respectively (Alba et al., 2014). Details of this experiment are given in the Supplementary Material and Methods and Table S1.

Data analyses

Effect of order of arrival and initial frequency on species abundance

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

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

where Y corresponds to the combination of two vectors with the number of *T. evansi* and *T. urticae* females after two generations, Treatment (fixed factor) to the combination of different orders of arrival and initial frequencies, Block (random factor) to whether the experiment was performed on week one or two, and ε to the residual error. We then performed *a priori* contrasts, using testInteractions fromphia package (Rosario-Martinez, 2015). As our experimental design was not orthogonal, to compare the effect of different orders of arrival, we performed contrasts between the treatments with same initial frequency but different orders of arrival. To compare the effect of frequency, we performed contrasts between treatments with same

order of arrival but different initial frequencies. Contrasts were corrected for multiple comparisons using FDR correction (Benjamini & Yekutieli, 2001). 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 separately for each leaf pair.

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

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

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

$$Y = \text{Leaf} + \epsilon \quad (6)$$

$$Y = \text{Leaf} + \text{Treatment} + \text{Leaf} \times \text{Treatment} + \epsilon \quad (7)$$

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 if 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 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 (Table S2, Figure 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 S2, Figure 1). We observed the same patterns when performing these analyses per leaf pair (Table S3). Overall, these results confirm that *T. evansi* is a superior competitor as observed in previous studies (Alzate et al., 2020; Ferragut et al., 2013; Sarmiento et al., 2011b).

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) is predicted to exclude *T. urticae* when it arrives first or at the same time. Under this exclusion scenario, the rate of competitive exclusion is expected to be faster when *T. evansi* arrives first due to a decrease in

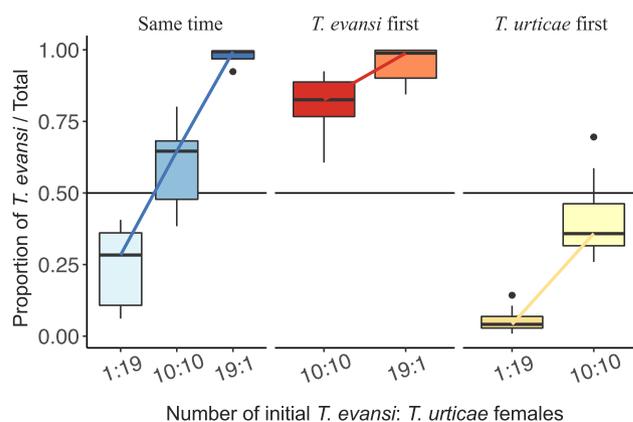


FIGURE 1 Proportion of spider mites *Tetranychus evansi* females (y-axis) depending on initial frequency (number of initial females *T. evansi*: *Tetranychus urticae*, x-axis) and order of arrival (same time vs. *T. evansi* or *T. urticae* arriving 48 h before its competitor) after two generations. *T. 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

niche differences (Figure 2). The small overlap between the lower confidence interval with the priority effects region suggests that positive frequency dependence might also emerge in this system. Interestingly, coexistence was only possible when *T. urticae* arrived first (Figure 2). This outcome was due to small niche and fitness differences among competitors, leading to a quasi-neutral scenario. Specifically, when *T. urticae* arrived first, we observed similar strengths of intra- and interspecific interactions among species (Figure S2a). Contrary to expectations and previous studies, the order of arrival was not associated with positive frequency dependence leading to priority effects. However, since the order of arrival modified the outcome of the interactions between *T. urticae* and *T. evansi*, we can also interpret these results as priority effects (*sensu* (Chase, 2003; Fukami, 2015) allowing for coexistence between species in our system.

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

When *T. evansi* was alone, it reached higher abundances on leaves 3 and 4 (Table S4a, Figure 3b), whereas *T. urticae* was less abundant on leaf 2 in comparison with all others (Table S4a, Figure 3d). Fewer *T. evansi* females

were found on leaf 4 when *T. urticae* arrived first, and on leaf 3 when *T. urticae* started with higher frequency and both species arrived at the same time (Figure 3, Figure S3a, Table S4b). When *T. evansi* arrived first or started at higher frequency, we observed fewer changes on its own leaf occupancy (Figure S4a). The distribution of *T. urticae* showed a slight shift when it arrived first, with a reduction on the prevalence on leaves 2 and 5 and slightly higher occupation of leaves 3 and 4 (Figure S3b, Table S4b). When *T. evansi* started at high frequency, there was also a shift in *T. urticae* distribution, with a lower occupancy of leaves 2 and 5 (Figure S3b). We observed similar shifts in leaf occupation when performing the analyses accounting for the order in which each leaf pair was added (Figure S4, Table S5).

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

Quantification of niche modification

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

DISCUSSION

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

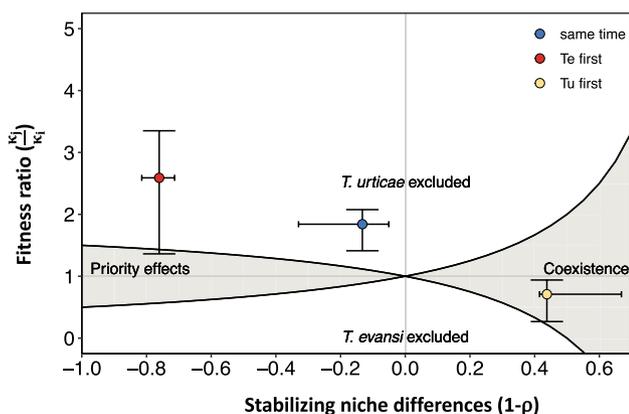


FIGURE 2 Relationship between average fitness differences ($\frac{K_i}{K_j}$, y-axis) and stabilising niche differences ($1-p$, x-axis) for different orders of arrival (*Tetranychus evansi* first – red, same time – blue, *Tetranychus urticae* first – yellow). Plotting average fitness differences against niche differences allows mapping different competitive outcomes predicted by modern coexistence theory. The coexistence condition (Equation 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*

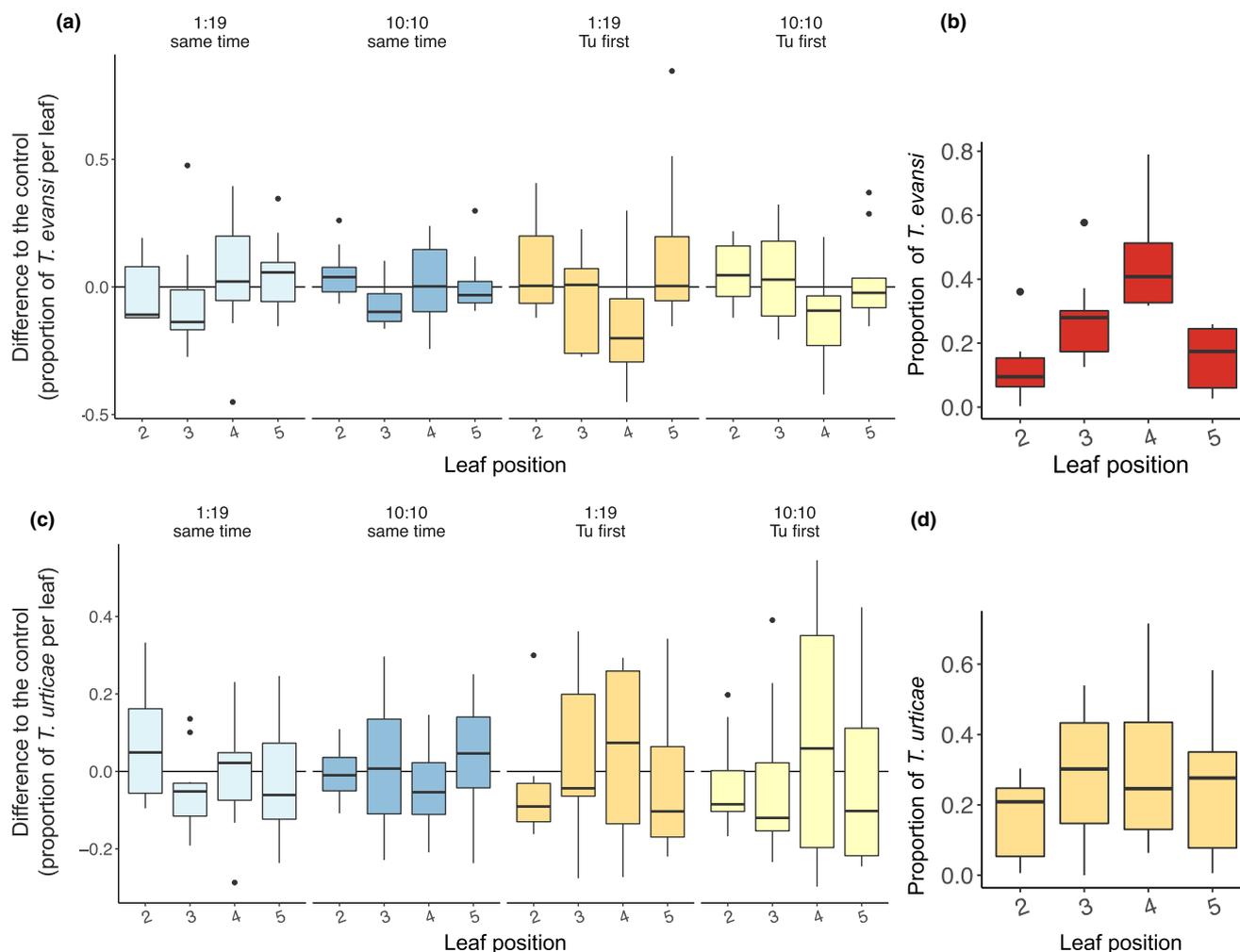


FIGURE 3 Differences between expected and observed leaf occupancy for *Tetranychus evansi* (a) and *Tetranychus urticae* (c) for a subset of the experimental treatments (when *T. urticae* arrived first or at the same time as *T. evansi*, note that Figure S3 includes all treatments); leaf occupancy for *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 treatments with both species, 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 contrast, *T. urticae* shows a slight increase in occupancy of leaf 4 and a slight decrease in occupancy of leaves 2 and 5 when it arrives first

common understanding of the inhibitory role of niche pre-emption in coexistence between species.

We found that *T. evansi* had higher competitive ability and growth rate, and often excluded *T. urticae* (Figures 1, 2). This is in line with laboratory observations showing that *T. evansi* outcompeted *T. urticae* on tomato plants (Sarmiento et al., 2011b; Alzate et al., 2020, but see Orsucci et al., 2017) and with field observations showing a shift in host use in *T. urticae* upon invasion by *T. evansi* (Ferragut et al., 2013). Still, these two species can co-occur in the field in the same plant species (Ferragut et al., 2013; Orsucci et al., 2017; Zélé et al., 2018). The advantage created by the earlier arrival of *T. urticae*, and associated reduction in interspecific competition by *T. evansi*, could be one of the possible mechanisms fostering their

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

Historical contingencies emerging from order of arrival can happen through two main mechanisms: niche modification or niche pre-emption (Fukami, 2015). In our system, niche modification may arise via interactions between spider mites and plant defences. However, we observe that both species suppress plant defences. If suppression would affect species performance, we would expect higher production of offspring when the competitor arrives first. We did not observe this, suggesting that

this mechanism of niche modification does not affect the outcome of competition in this system.

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

Order of arrival is a major determinant of community assembly across diverse taxa, from microbes to plants (Chase, 2003; Clay et al., 2019, 2020; Erb et al., 2011; Grainger et al., 2018, 2019; Halliday et al., 2020; Kardol et al., 2013; Stam et al., 2017). Most of these studies show that early colonisers inhibit growth and decrease performance of late arriving species, especially in those that occupy very similar niches (Delory et al., 2019, 2021; Fargione et al., 2003; Grainger et al., 2019; Vannette & Fukami, 2014), although very few concern herbivorous species 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. Delory et al., 2019; Queijeiro-Bolaños et al., 2017). Here, we show that coexistence is promoted by niche pre-emption 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 of 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 they affect 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 by positive frequency dependence (i.e. population growth rate is higher as individuals become relatively more abundant; Fukami, 2015; Song et al., 2020). Including other types of interactions and outcomes into modern coexistence framework is fundamental to improve our ability to understand how species coexist (Spaak et al., 2021). Here we show that order of arrival can lead to coexistence via niche pre-emption by the inferior competitor. Thus, our results show that changes in the 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 emphasise the inhibitory nature of niche pre-emption (Delory et al., 2019; Fargione et al., 2003; Fukami, 2015; Vieira et al., 2018), with the early arriving species outcompeting the other. However, recent theory suggests that, in a resource competition model of two species, niche pre-emption 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 pre-emption by the weaker competitor promotes coexistence. This striking change in the outcome of competitive interactions emerges mostly due to a decrease in niche overlap, shifting niche differences from negative to positive. This suggests that even small differences in order of arrival can be sufficient for the monopolisation 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.

COMPETING INTERESTS

Authors declare no competing interests.

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AUTHORSHIP STATEMENT

IF, OG and SM designed the experiment, IF, AM, MK and RCP collected data, IF and OG performed modelling work and analysed data. IF and SM wrote the first draft of the manuscript, and all authors contributed substantially to revisions.

PEER REVIEW

The peer review history for this article is available at <https://publons.com/publon/10.1111/ele.14021>.

OPEN RESEARCH BADGES



This article has earned an Open Data badge for making publicly available the digitally-shareable data necessary to reproduce the reported results. The data is available at: https://github.com/irfragata/priority_effects.

DATA AVAILABILITY STATEMENT

Data and scripts for data analyses are available at <https://doi.org/10.6084/m9.figshare.19593481.v1> (data set) and <https://doi.org/10.6084/m9.figshare.19593493.v1> (scripts).

ORCID

Inês Fragata <https://orcid.org/0000-0001-6865-1510>

Raul Costa-Pereira <https://orcid.org/0000-0003-2370-5866>

Agnieszka Majer <https://orcid.org/0000-0002-2425-7885>

Oscar Godoy <https://orcid.org/0000-0003-4988-6626>

Sara Magalhães <https://orcid.org/0000-0002-8609-7768>

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