Underdispersion of anti-herbivore defence traits and phylogenetic structure of cerrado tree species at fine spatial scale

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Abstract

Question: Do species phylogeny and herbivory-related defence traits influence species co-occurrence in the cerrado? Are anti-herbivory defence traits under- or overdispersed in the quadrats? Do soil features mediate these patterns?

Location: A cerrado site in SE Brazil (21°58′05.3″ S, 47°52′10.1″ W).

Methods: We analysed 100 quadrats of 5 m × 5 m each, and sampled all woody individuals. For each species, we measured nine defence traits against herbivory and tested against null models whether (1) phylogenetic dissimilarities were under- or overdispersed, (2) trait species dissimilarities were under- or overdispersed, and (3) these spatial patterns were associated with soil nutrient content, considering the variation of sum of bases, organic matter, and aluminium.

Results: We found phylogenetic signals in two traits, and conservatism of traits as a whole was significant. Phylogenetic structure of communities was in general clustered. We found trait underdispersion for specific leaf area, water content, leaf toughness, and leaf nutritional quality. Specific leaf area was also overdispersed in quadrats. We did not find either under- or overdispersion related to soil features.

Conclusions: As phylogenetic and trait underdispersion were not associated with soil features, and fire and drought are not expected to change at study scale, some biotic interaction may be responsible for underdispersion. We postulated that insect herbivory, when representing a large constraint to trees in this environment, could lead to functional and phylogenetic underdispersion.

Introduction

The coevolution of herbivores and plants has been proposed as a major factor promoting the diversity of defence traits against herbivores (Becerra 1997, 2007). Closely related insects often feed on plants that share common traits to which they are adapted (Berenbaum 1983; Becerra 1997; Morais et al. 2011). Also, there is a high conservatism of functional traits in plant lineages (Ackerly 2003; Reich et al. 2003), including chemical and morphological characteristics that constrain herbivores (Ward & Spalding 1993; Futuyma & Mitter 1996). Consequently, herbivory is expected to limit the co-existence of related plants that share common defence traits (Becerra 2007; Gilbert & Webb 2007). Although strong herbivory may select for functional similar species, acting as an environmental filter (Harley 2003; Silva & Batalha 2011), most data in the literature suggest the opposite pattern. Herbivory usually generates spatial divergence, especially in narrow coevolved systems with specific plant–insect adaptations (Van Zandt & Agrawal 2004; Becerra 2007). As coevolutionary specialization increases and spatial scale decreases, plant co-occurring species tend to be more dissimilar (Becerra 2007). Thus, large differences in values of anti-herbivore traits – i.e. trait and phylogenetic overdispersion – are expected to occur at fine spatial scale.

Strategies against herbivory include nutritional quality, physical characteristics, toxicity, phenology, regrowth capacity and indirect defences, e.g. volatiles and branching

architecture (Agrawal & Fishbein 2006). The nutritional quality may influence the attack to plant tissues, since a high carbon–nitrogen ratio might decrease nitrogen acquisition by the herbivore, thus decreasing herbivore attack (Coley & Barone 1996; Agrawal & Fishbein 2006). Alternatively, some insects may compensate the low nutritive value of leaves, increasing consumption to acquire more nitrogen (Mattson 1980). In addition, these multiple defences may generate synergistic interactions, providing a greater level of defence than would be possible if traits were present independently (Berenbaum et al. 1991; Stapley 1998). Therefore, defence syndromes may also influence the spatial distribution of plant species at fine scales (Silva & Batalha 2011).

The cerrado, the largest savanna region in South America, is characterized by marked rainfall seasonality and experiences a pronounced dry season in the winter (Gottsberger & Silberbauer-Gottsberger 2006). The nutrient-poor, well-drained, acid soils, as well as recurrent fires, are additional environmental constraints for plant growth in this vegetation type (Gottsberger & Silberbauer-Gottsberger 2006). Accordingly, environmental filters may structure the distribution of plants at fine spatial scale, assembling species with small differences in values of functional traits, promoting phenotypic underdispersion (Silva & Batalha 2010; Willis et al. 2010). In this sense, environmental filters usually act as an ecological force opposite to what we expect for herbivory: whereas the former promotes spatial functional convergence, by assembling species from the regional pool with similar functional traits, the latter promotes spatial functional divergence, by selecting species with distinct anti-herbivore defences (Webb et al. 2002; Becerra 2007).

In spite of the scarcity of ungulates and other large herbivores in cerrado, herbivory by insects may be an important ecological factor. The cerrado contains a diverse and abundant community of herbivorous insects (Marquis et al. 2002). In communities where resources are not abundant, such as the cerrado (Gottsberger & Silberbauer-Gottsberger 2006), plants tend to concentrate their investments in defences against herbivory (Coley & Barone 1996; Fine et al. 2006), since they cannot replace damaged tissues as fast as in communities with abundant resources (Janzen 1974; Coley et al. 1985). Additionally, the cerrado vegetation seems to present a high specificity between herbivores and host plants (Marquis et al. 2002; Dyer et al. 2007). Diniz & Morais (1997) and Novotny et al. (2002), for example, found low faunal similarities among host plant genera and even within genus in the cerrado. Consequently, at community level, insect herbivores may represent a divergence pressure for cerrado plants, especially at distances smaller than 10 m (e.g. Coley & Barone 1996).

We assessed whether the species co-occurrences were correlated with anti-herbivore defence traits and with phylogenetic distances. We also assessed whether soil features could influence the observed patterns. Studies on plant–herbivore coevolution and its impact on plant defences have focused primarily on interactions that involve a small number of species or populations (Agrawal & Fishbein 2006; Becerra 2007). Here, we focused on all sampled tree species of the community, trying to assess whether the presence of anti-herbivore defence traits could create general patterns of spatial distribution of plant species. We addressed the following questions: do species phylogeny and herbivory-related defence traits influence species co-occurrence in the cerrado; are anti-herbivory defence traits under- or overdispersed in the quadrats; and are these patterns explained by soil features?

Methods

Study site and sampling

We surveyed a woodland cerrado site (21°58′05.3″ S, 47°52′10.1″ W) in São Carlos municipality, São Paulo State, SE Brazil. Regional climate is mesothermic, subtropical, with rainy summers and not severely dry winters (Cwa; Köppen 1931). The annual mean temperature is 21.3 °C, and the monthly mean precipitation is 131.3 mm. The soil is a dystrophic Oxisol, acidic (pH < 4), and with high concentrations of Al^{3+} (Dantas & Batalha 2011). Soil features sampled from the surface were analysed, and pH, organic matter, available P, total N and exchangeable K^+, Ca^{2+}, Mg^{2+} and Al^{3+} were determined. Also, sum of bases, cation exchange capacity, base saturation and aluminium saturation were calculated (Dantas & Batalha 2011). Among these, we considered only the soil features that presented variation at the study scale and could hence represent an environmental filter to species: sum of bases, organic matter, and aluminium concentration (Willis et al. 2010; Dantas & Batalha 2011). In the rainy season of 2008, we placed a grid with 100 contiguous quadrats of 5 m × 5 m, in which we sampled all woody individuals having a stem diameter at soil level equal to or more than 3 cm (SMA-SP. 1997). For species with more than ten individuals, we randomly picked ten individuals to measure anti-herbivore defence traits (Cornelissen et al. 2003). For species with less than ten individuals, we made an extra effort, looking for other individuals close to the grid, trying to reach ten individuals per species.

Defence trait data

We collected mature leaves, without symptoms of herbivore or pathogen attack, and measured the following

anti-herbivore leaf traits: specific leaf area, water content, latex content, toughness, trichome density, nutritional quality, and chemical defences (Agrawal & Fishbein 2006). Specific leaf area is positively related to mass-based maximum photosynthetic rate, or potential relative growth (Cornelissen et al. 2003). Lower values of specific leaf area tend to correspond with relatively high investments in leaf defences, particularly structural ones (Cornelissen et al. 2003), indicating slow growth and low palatability (Agrawal & Fishbein 2006). Low values of specific leaf area are also correlated to low herbivory rates (Neves et al. 2010). Water content is also related to palatability, and leaves with low water content are expected to be less attacked by herbivores than those with high water content (Agrawal & Fishbein 2006). To assess specific leaf area and water content, we collected two leaves per individual. We placed the leaves in a thermal box and weighed them while still fresh. We digitized the leaves to determine leaf area with ImageJ software (version 1.33, National Institutes of Health, Bethesda, MA, USA). After that, we oven-dried each leaf sample at 80 °C for 72 h, and then weighed the dry mass to obtain the specific leaf area (Cornelissen et al. 2003).

We also collected latex content, an important physical defence against herbivory (Agrawal & Fishbein 2006; Agrawal & Konno 2009). We measured latex content from ten different individuals of each species (Cornelissen et al. 2003). We sampled an intact leaf, cut its base in the field, and collected the exuding latex onto a filter paper. We oven-dried the filter paper at 75 °C for 24 h before and after the latex collection, to eliminate water, then weighed the filter paper to assess the latex content (Agrawal & Fishbein 2006). We measured leaf toughness with a force gauge penetrometer (dynamometer DFE 010, Chatillon, FL, USA). We used a conical tip to penetrate the leaf surface at each side of the midrib. For statistical analyses, we used the mean of these two measures. Leaf toughness is related to nutritional and defence components (Agrawal & Fishbein 2006) and is considered the main defence traits against herbivore activity (Coley & Barone 1996). Trichomes are also important physical defences against herbivory. In five leaves per species, we counted the trichomes on upper and lower sides of a leaf disc of 28 mm², under a dissecting microscope (Agrawal & Fishbein 2006). Total leaf carbon (C) and nitrogen (N) concentrations were measured from five replicates from each species to assess the C/N ratio. The C and N concentrations in samples were determined at the Laboratory of Stable Isotopes of the University of São Paulo.

We determined presence of chemical compounds on leaves following procedures described in Falkenberg et al. (2003). We determined presence of alkaloids, terpenoids and tannins – chemicals frequently found in Brazilian plants that work as defences against herbivores (Coley & Barone 1996; Lima 2000). We used a series of three assays, Mayer, Dragendorff, and Wagner reactions, to determine presence of alkaloids, considering positive the samples that reacted to at least two assay methods (Falkenberg et al. 2003). We used Liebermann-Burchard and Salkowisk reactions to test the presence of terpenoids, and a ferric chloride reaction to determine presence of tannins (Falkenberg et al. 2003). All defence traits were related to chewing insects, since, in tropical plant communities, folivorous insects are the most important consumers, and chewing insects constitute 75% or more of the annual leaf consumption (Coley & Barone 1996). We calculated the mean of each trait per species and standardized all variables to zero mean and unit variance.

Phylogenetic data and analysis

We initially constructed a phylogenetic tree using Phylomatic software, a phylogenetic database and a toolkit to assemble phylogenetic trees (version 2, Molecular Ecology Notes, Blackwell Publishing, US). The tree topology was based on the current Phylomatic tree (tree R20091110). We assigned branch lengths to the phylogenetic tree by spacing undated nodes evenly between dated nodes (Wikström et al. 2001) in the trees with the Branch Length Adjustment averaging algorithm of the Phylocom software (version 4.1, Bioinformatics, Oxford University, UK).

Testing the assumption of phylogenetic conservatism is of major importance and is a requisite in phylogenetic analyses (Losos 2008). Hence, we investigated whether the functional traits were conserved or convergent in the phylogeny of the species. We analysed the variance of the phylogenetically independent contrasts (PICs) to test which traits presented phylogenetic signals. Phylogenetic signal may be defined as the tendency of closely related species to resemble each other (Blomberg et al. 2003). If the evolution of some trait is conserved in the phylogeny, divergences will be small and related species will be similar to each other (Blomberg et al. 2003). To test the significance of the phylogenetic signal, we compared the observed variance of the PICs with pseudo-variances generated by randomization of the trait values in the phylogenetic tree (Blomberg et al. 2003). We also calculated the strength of the phylogenetic signal (K) as a departure from the expected with a Brownian motion model of trait evolution (Blomberg et al. 2003).

We estimated an index of phylogenetic community structure for each quadrat, the nearest taxon relatedness index (NTI; Webb 2000; Kraft et al. 2007; Kraft & Ackerly 2010). NTI is based on the phylogenetic distance of the most closely related co-occurring taxa (mean nearest taxon distance, MNTD). The significance of NTI for a given

quadrat was assessed by comparing the observed MNTD to a null distribution of MNTD measured on 1000 null communities. We created null communities for a given quadrat by randomly drawing an equal number of species from the phylogeny. Positive values of NTI indicate that the taxa are more related than expected by chance, i.e. the taxa are phylogenetically underdispersed, whereas negative values indicate that taxa are less related than expected, i.e. the taxa are phylogenetically overdispersed (Kraft & Ackerly 2010). We assessed the significance of the analyses to our community with a two-tailed Wilcoxon signed ranks test, testing the hypothesis that the observed ranks of MNTD were equally distributed about the null expectation (Kraft & Ackerly 2010). As a result, we were able to detect phylogenetic under- and overdispersion in our community data.

Trait under- and overdispersion

We followed the methods proposed in Kraft & Ackerly (2010) to test for under- and overdispersed distribution of defence traits. The metrics are able to distinguish the combined habitat filtering and species interaction assembly model from the random model; the signal of both assembly processes could be detected individually (Kraft & Ackerly 2010). We used the range of continuous traits as a measure of sensitive to underdispersion, nominal traits were not used in this analysis (Cornwell et al. 2006; Kraft et al. 2008) and the standard deviation of successive neighbour distances along trait axes, divided by the range (SDNDr; Ingram & Shurin 2009) as a metric sensitive to overdispersion. SDNDr metric shifts the focus from testing for minimum spacing to a focus on how regularly spaced species are across a given range of trait values (Kraft & Ackerly 2010). This metric is the most powerful of the statistics used to detect functional overdispersion and the most resistant to producing non-random values when analysing simulated communities (Kraft & Ackerly 2010).

In each quadrat, we compared the observed values of range and SDNDr to a null expectation generated by creating 1000 random communities of equal richness. We considered a given quadrat significantly non-random if the observed metric fell into the extreme 5% of the null distribution for the quadrat (Kraft & Ackerly 2010). We also assigned the significance for each metric with a plot Wilcoxon signed-ranks test, with a null hypothesis that the observed values of each anti-herbivore defence trait across all quadrats, relative to their respective null distributions, were evenly distributed around the null expectation (Kraft et al. 2008; Kraft & Ackerly 2010). To test for under- and overdispersion of traits, we did not considered chemical defences, because they were binary values, which could not be processed in these tests. We carried out the analyses with picante and ade4 packages in the R environment (version 2009, R Foundation for Statistical Computing, Vienna, AT).

Community structure considering soil features

We used a method proposed by Pillar et al. (2009) originally to elucidate trait-convergence and trait-divergence assembly patterns along ecological gradients. Trait convergence, analogous to functional underdispersion, may result from environmental filtering (Pillar et al. 2010). Trait divergence, or functional divergence, may be the result of competition or biotic interactions. This analysis tested whether the distribution of anti-herbivore defence traits across the quadrats differed significantly from random expectation, weighting the trait occurrence, and species composition considering trait similarities, phylogenetic similarities, and environmental variables (Pillar et al. 2010). First, we constructed four matrices: (1) a matrix with species abundances in each quadrat; (2) a phylogenetic pair-wise similarities matrix of these species; (3) a trait matrix describing the species, with anti-herbivore defence traits; and (4) an environment matrix with three soil features for each quadrat (Pillar et al. 2010). To reveal trait convergence, we multiplied the trait matrix by the abundance matrix, and to reveal trait divergence, we multiplied the abundance matrix by a matrix U of fuzzy sets derived from the similarity matrix of species based on the traits (Pillar et al. 2009). We used all traits sampled, i.e. the selected combination of optimal traits to reveal both patterns of convergence and divergence (Pillar et al. 2010). Also, we analysed data with each trait separately.

We assessed the phylogenetic signal at species level by matrix correlation between species phylogenetic similarities and species trait similarities. We assessed the strength of the association between community distances based on their phylogenetic structure and environmental distances with a matrix correlation. We assessed the phylogenetic signal related to trait convergence, trait divergence and both of them, by matrix correlation between the phylogenetic structure and trait structure (Pillar et al. 2010). Finally, we tested three correlation matrices between traits and environment: related to trait convergence, related to trait divergence, and related to both trait convergence and divergence (Pillar et al. 2009, 2010).

We did 1000 iterations to test the significance of all phylogenetic signals and of the correlations between both trait convergence and divergence and soil features. These tests were carried out with SYNCSA software (http://ecoqua.ecologia.ufrgs.br/ecoqua/SYNCSA.html) and with SYNCSA package in the R environment (version 2009).

Results

We sampled 2062 individuals, comprising 61 species and 29 families (Fig. 1). The mean ± SD of the density of individuals in each quadrat was 20.62 ± 7.04. We found 9.66 ± 2.19 species per quadrat, and the most abundant species was *Myrsine umbellata* Mart., with 567 individuals in the study system. Other common species were *Vochysia tucanorum* Mart. (168 individuals), *Myrcia guianensis* (Aubl.) DC. (131 individuals), *Miconia albicans* (Sw.) Triana (125 individuals) and *Piptocarpha rotundifolia* (Less.) Baker (103 individuals). These five more abundant species corresponded to 53.39% of the total abundance within the plots. Species trait defence data are available in Appendix S1.

Phylogenetic analyses

Phylogenetically independent contrasts showed two traits with significant phylogenetic signal, after Bonferroni correction: trichome density and nutritional quality, at low strength ($K < 1$; Table 1). Other traits were not
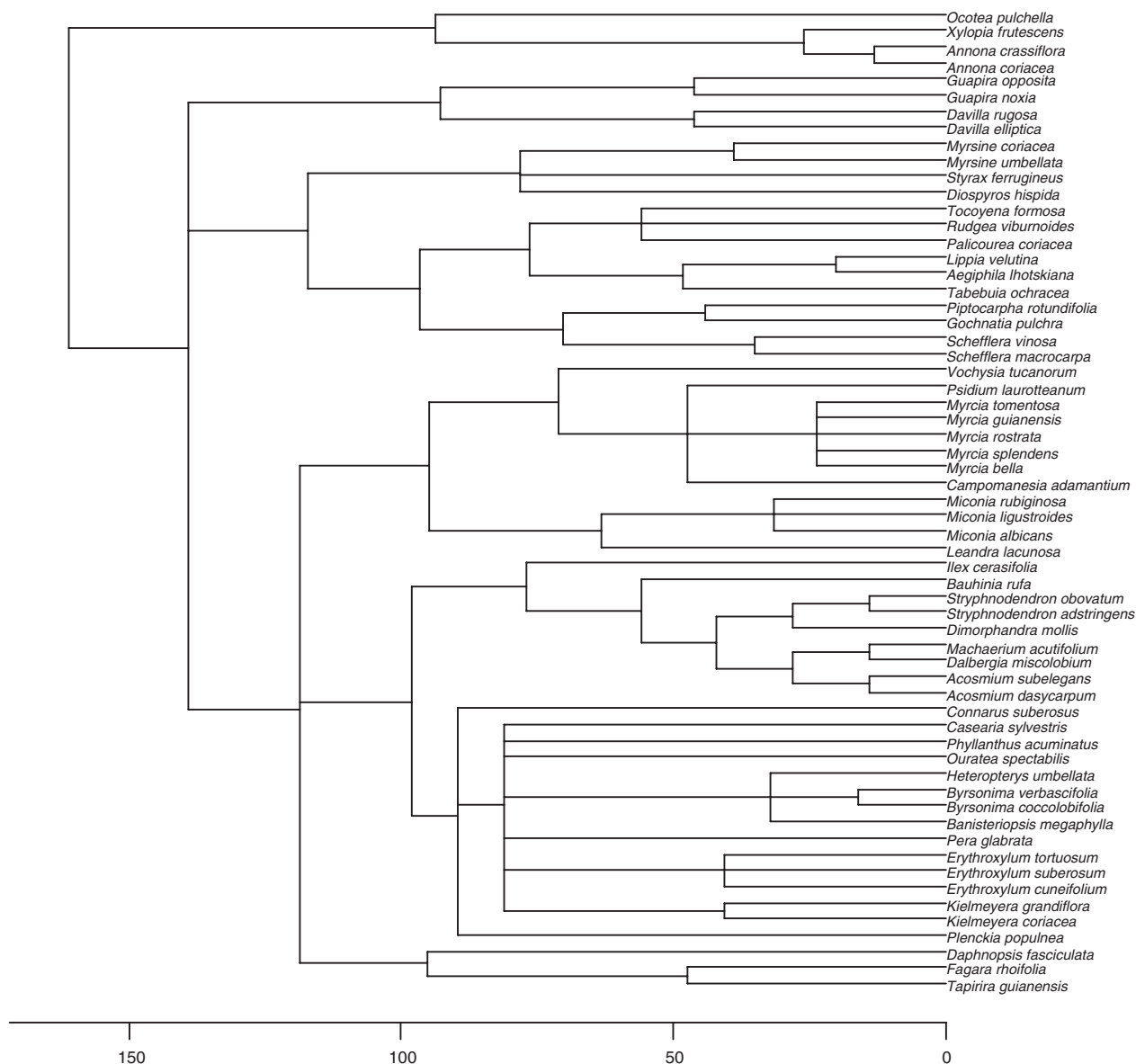

Fig. 1. Phylogenetic tree of 61 woody species in a southern cerrado site (21°58′05.3″ S, 47°52′10.1″ W). The relationship among species was based on the current Phylomatic tree (tree R20091110, with ages from Wikström et al. 2001).

Table 1. Phylogenetic signals of defence traits. Phylogenetic signals measured from the variance of phylogenetically independent contrasts (PICs) in tree species of a southern cerrado site (21°58′05.3″ S, 47°52′10.1″ W). Test for phylogenetic signal in each trait sampled: specific leaf area ($cm^2 \cdot g^{-1}$); water content ($mg \cdot cm^{-2}$); toughness (N); trichome density (cm^{-2}); latex content (mg); C:N (carbon:nitrogen ratio); and presence of alkaloids, terpenoids, and tannins.

	Observed PICs	Random PICs	P	K
Specific leaf area	0.0166	0.0224	0.064	0.4659
Water	0.0160	0.0222	0.032	0.4709
Toughness	0.0217	0.0222	0.510	0.3561
Trichomes	0.0137	0.0225	**0.001**	0.5595
Latex	0.0089	0.0220	0.016	0.8500
C:N	0.0143	0.0222	**0.002**	0.5324
Alkaloids	0.0141	0.0219	0.349	0.5335
Terpenoids	0.0194	0.0221	0.172	0.4111
Tannins	0.0262	0.0223	0.738	0.2891

The significance level was adjusted by Bonferroni correction to $\alpha' = 0.006$. Bold type indicates significant results. K is the strength of the phylogenetic signal.

Table 3. Permutation tests done to test the significance of (1) the phylogenetic signal at species pool level, (2) phylogenetic signal related to soil features, (3) phylogenetic structure related to trait convergence, (4) phylogenetic structure related to trait divergence and (5) phylogenetic signal related to trait convergence and trait divergence. Also, (6) the correlation of trait convergence, (7) trait divergence and (8) both trait convergence and divergence related to soil features in a southern cerrado site (21°58′05.3″ S, 47°52′10.1″ W), after 1000 randomizations (Pillar et al. 2009, 2010). For these analyses, we used all traits sampled: specific leaf area ($cm^2 \cdot g^{-1}$), water content ($mg \cdot cm^{-2}$), toughness (N), trichome density (cm^{-2}), latex content (mg), C:N (carbon:nitrogen ratio) and the presence of alkaloids, terpenoids and tannins.

	P
(1) Phylogenetic signal	**0.021**
(2) Phylogenetic structure: soil features	0.516
(3) Phylogenetic structure: trait convergence	0.157
(4) Phylogenetic structure: trait divergence	0.141
(5) Phylogenetic structure: trait convergence and divergence	**0.047**
(6) Trait convergence: soil features	0.632
(7) Trait divergence: soil features	0.557
(8) Trait convergence and divergence: soil features	0.595

Significant values at $\alpha = 0.05$ are in bold.

conserved in the phylogeny (Table 1). Assessing phylogenetic signal by matrix correlation showed traits not conserved in the phylogeny, although specific leaf area, trichome density, presence of terpenoids and tannins presented marginally significant values ($0.05 < P < 0.10$; Table 2). However, when considering species of the whole community, phylogenetic signal was significant (Table 3). Also, phylogenetic structure showed a significant phylogenetic signal at community level when considering trait convergence and divergence in the analysis, but no phylogenetic signal when convergence and divergence were accounted separately ($P > 0.05$; Table 3). No phylogenetic structure was observed related to soil environmental variables (Table 2). Also, phylogenetic analyses obtained with the nearest taxon relatedness index (NTI) showed that the general structure of the community was clustered ($V = 3520$, $P < 0.001$).

Trait under- and overdispersion

We found an overdispersion pattern within plots along functional axes only for specific leaf area ($P < 0.001$; Table 4), but all other traits were non-significant for overdispersion. Moreover, trait ranges within plots were significantly reduced relative to the null expectation for specific leaf area, water content, toughness and nutritional quality (all $P < 0.001$; Table 4), indicating that these traits were underdispersed in the quadrats. When we included soil variables in the analysis, we did not find evidence of either trait convergence or divergence among communities, indicating that soil features do not represent a driver for community structuring at the plot scale (all $P > 0.05$; Tables 2, 3).

Table 2. Permutation tests done to test the significance of (1) the phylogenetic signal, (2) phylogenetic signal related to soil features, (3) phylogenetic structure related to trait convergence, (4) phylogenetic structure related to trait divergence and (5) phylogenetic signal related to trait convergence and trait divergence. Also, (6) the correlation of trait convergence, (7) trait divergence and (8) both trait convergence and divergence related to soil features in a southern cerrado site (21°58′05.3″ S, 47°52′10.1″ W), after 1000 randomizations (Pillar et al. 2009, 2010). Each trait were tested separately: specific leaf area ($cm^2 \cdot g^{-1}$); water content ($mg \cdot cm^{-2}$); toughness (N); trichome density (cm^{-2}); latex content (mg); C:N (carbon:nitrogen ratio); and the presence of alkaloids, terpenoids, and tannins.

	SLA	Water	Tough	Tric	Latex	C:N	Alk	Ter	Tan
(1) Phylogenetic signal	0.07	0.19	0.26	0.08	0.79	0.31	0.32	0.08	**0.05**
(2) Phylogenetic structure: soil features	0.77	0.78	0.76	0.79	0.79	0.77	0.79	0.76	0.80
(3) Phylogenetic structure: trait convergence	0.49	**0.03**	0.09	0.46	0.09	0.46	0.18	0.58	0.95
(4) Phylogenetic structure: trait divergence	0.78	0.16	0.62	0.79	0.88	**0.02**	1	1	1
(5) Phylogenetic structure: trait convergence and divergence	0.58	0.23	0.92	0.58	0.14	0.57	0.60	0.48	0.38
(6) Trait convergence: soil features	0.68	0.48	0.62	0.68	0.13	0.47	0.62	0.50	0.43
(7) Trait divergence: soil features	0.20	0.11	0.99	0.22	0.54	0.93	0.54	0.77	0.54
(8) Trait convergence and divergence: soil features	0.56	0.23	0.93	0.6	0.15	0.59	0.63	0.49	0.43

Significant values at $\alpha = 0.05$ are in bold.

Table 4. Trait-based tests for community assembly processes in a southern cerrado site (21°58′05.3″ S, 47°52′10.1″ W). P values are reported for a Wilcoxon signed-rank test of the hypothesis that the observed distribution of observed traits is lower than the null expectation. Anti-herbivore defence traits are: specific leaf area ($cm^2·g^{-1}$), water content ($mg·cm^{-2}$), toughness (N), trichome density (cm^{-2}), latex content (mg), and C:N (carbon:nitrogen ratio).

Trait	Underdispersion	Overdispersion
Specific leaf area	**P < 0.001**	**P < 0.001**
Water	**P < 0.001**	P = 0.99
Toughness	**P < 0.001**	P = 0.09
Trichomes	P = 0.99	P = 0.99
Latex	P = 0.99	–
C:N	**P < 0.001**	P = 0.97

Bold type indicates P < 0.05. Latex test could not be done for overdispersion due insufficient data to run the analyses. Only two species presented latex in our community. Also, chemical defences were not considered due to their binary data structure.

Discussion

We investigated the influence of anti-herbivore defence traits and phylogeny on patterns of co-occurrence of tree species in cerrado. We expected to find co-occurring species with different defence traits, due to the decrease of conspecific co-occurring plants caused by herbivory at fine spatial scale (Coley & Barone 1996; Becerra 2007). However, our results indicated that only one anti-herbivore defence trait was overdispersed within the plots, suggesting that herbivory did not limit co-occurrence of similar plant species. Moreover, most anti-herbivore defence traits had their range reduced, i.e. they presented an underdispersed pattern. This underdispersed phylogenetic structure may indicate the presence of an ecological process assembling functional and phylogenetic similar species.

Several environmental filters in cerrado, such as drought, fire, and nutrient-poor soils (Gottsberger & Silberbauer-Gottsberger 2006), may influence the co-occurrence of plant species, even at fine spatial scale (Silva & Batalha 2009, 2010; Willis et al.2010). In our study, some anti-herbivory defence traits – specific leaf area, water content, toughness and nutritional quality – varied less within the plots than expected, which suggests the effect of environmental filtering (Cornelissen et al. 2003; Pais & Varanda 2003; Neves et al. 2010). Also, the phylogenetic pattern of co-occurring species depends on the evolutionary history of species traits, the interaction between herbivores and plants, and the environmental filters (Webb et al. 2002; Becerra 2007). Although fire and drought are expected to be constant within the quadrats, some soil features influenced the plant species distribution and could have generated the observed underdispersed pattern observed (Willis et al. 2010; Dantas & Batalha 2011). However, our analysis indicated that trait and phylogenetic underdispersion were not correlated to soil features. Thus, trait underdispersion was not the result of environmental filters that were evaluated, but may be related to biotic interactions.

Environmental heterogeneity also encompasses biotic variables, such as neighbouring plant communities and herbivory (Agrawal et al. 2006; Hakes & Cronin 2011). Convergence of traits within communities may also be caused by biotic effects, as facilitation and competition among plants or strong herbivory (Cavieres et al. 2002; Harley 2003; Mayfield & Levine 2010). The biotic environment was found to be essential in influencing spatial distribution of plant defences, even overlapping with the abiotic environment (Hakes & Cronin 2011). Density-dependent processes, such as herbivory, may interact with abiotic conditions, reinforcing or diminishing habitat filtering (Cavender-Bares et al. 2009). Although very few studies about herbivory on whole communities have been carried out, herbivory leading to underdispersed defence traits has previously been suggested (Harley 2003; Cavender-Bares et al. 2009). Furthermore, herbivory by ungulates influenced co-occurrence of plants through selective consumption (Gómez-Aparicio et al. 2008). If biotic effects are very strong, most species probably present convergence of functional traits, as in highly grazed habitats or in environments with competition for light (Mayfield & Levine 2010). In cerrado, leaf-cutter ants remove about 13–17% of the annual leaf production, representing a large biotic stress (Costa et al. 2008). Thus, herbivory may constitute a strong pressure in these communities, leading to trait underdispersion at fine spatial scales.

Despite the paucity of ungulates and other large herbivores, insect herbivory may be an important filter in determining species co-occurrence in cerrado. Although some studies have been carried out (Costa et al. 2008), quantification of the impact of herbivores in savannas is very rare (Marquis et al. 2002; Costa et al. 2008). Therefore, it would be relevant to assess herbivory rates in cerrado vegetation to test the generality of underdispersion of traits and phylogeny among cerrado woody vegetation. Moreover, it is important to include herbaceous species, which may contribute to elucidate the role of environmental filtering and species interactions in species assembly (Loiola et al. 2010). Another open question is related to other plant life stages and the changes that may occur in anti-herbivore defence traits during ontogenetic development, in relation to herbivore pressure (Boege & Marquis 2005).

Only specific leaf area was both under- and overdispersed within plots in this cerrado site. The metrics we used are able to distinguish these effects, and the presence of habitat filtering does not erase the competition signal (Kraft & Ackerly 2010). In our data, we found an

underdispersion of specific leaf area in some quadrats and overdispersion in others. Specific leaf area responds to many environmental factors and affects resistance and tolerance to herbivory (Cornelissen et al. 2003; Agrawal & Fishbein 2006; Hakes & Cronin 2011). Thus, the variability could be a consequence of several factors, but the environmental variables did not explain variation in traits. For example, Hakes & Cronin (2011) found specific leaf area was strongly correlated with canopy cover and neighbour stem density. Biotic interactions, such as neighbouring plant variables, may be more important to spatial distribution of defence traits than the abiotic environment, e.g. soil features (Hakes & Cronin 2011). Therefore, herbivory may be displacing species with similar specific leaf area from neighbourhoods because of associational susceptibility (Hakes & Cronin 2011).

The role that herbivory played in trait structure varied across different traits. Although only two traits were conserved with low strength within the lineages, the association of all traits showed a significant phylogenetic signal. This conservatism of defence traits, associated with an underdispersion of most traits, is in accordance with the predictions of phylogenetic underdispersion in communities. Therefore, we suggest that herbivory must be responsible for the underdispersion in phylogenetic structure and defence traits. In the cerrado site we studied, herbivory seems to act as a biotic filter, clustering most anti-herbivore defence traits.

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Supporting Information

Additional supporting information may be found in the online version of this article:

Appendix S1. Mean values and standard deviation to anti-herbivore traits sampled for each species.

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