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LEONARDO RÉ JORGE

REFORMULATION OF THE CONCEPT OF TROPHIC SPECIALIZATION AND ITS APPLICATION TO SPECIES AND COMMUNITIES

REFORMULAÇÃO DO CONCEITO DE ESPECIALIZAÇÃO TRÓFICA E SUA APLICAÇÃO A ESPÉCIES E COMUNIDADES

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Orientador: Prof. Dr. Thomas Michael Lewinsohn

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ABSTRACT

In this thesis we propose a development of the resource specialization concept to include aspects usually not taken into account, especially for trophic interactions: affinities among resource types, their availabilities and cooccurrence patterns with consumers. By incorporating recent advances in ecophylogenetics and null modelling, we propose new metrics for the specialization of species and communities and apply these advances to address particular questions. In the first chapter the general conceptual framework is presented and a metric for the specialization of species is proposed and exemplified. The second chapter, mostly methodological, expands this metric for communities and proposes a hierarchical partitioning of the variation in specialization among species and locations. The third chapter tests this toolset in showing the relationship between specialization and geographic distribution ranges of endophagous insects feeding in Asteraceae flowerheads in Brazil. Finally, the fourth chapter uses the same dataset to investigate the role of phylogenetic diversity of resources in determining the richness of insect herbivores using them.

RESUMO

Nesta tese propomos um desenvolvimento do conceito de especialização no uso de recursos, de forma a incluir aspectos pouco considerados, principalmente no contexto de interações: afinidades entre os tipos de recursos, sua disponibilidade e padrões de co-ocorrência. Por meio da incorporação de avanços recentes em estudos ecofilogenéticos e em modelos nulos, propomos novas métricas para a especialização de espécies e comunidades, e aplicamos esses avanços para responder a questões particulares. No primeiro capítulo, o arcabouço conceitual é apresentado de forma mais geral, e uma métrica para a especialização de espécies é proposta e exemplificada. O segundo capítulo, de natureza metodológica, estende essa métrica para comunidades e propõe uma partição da variação em especialização entre espécies e entre locais de forma hierarquizada. O terceiro capítulo investiga a capacidade destas ferramentas de evidenciar relações entre a especialização e a distribuição geográfica de espécies de insetos endófagos de capítulos de Asteraceae no Brasil. Por fim, o quarto capítulo utiliza o mesmo conjunto de dados para investigar o papel da diversidade filogenética de recursos na determinação da riqueza de insetos que os utilizam.

SUMÁRIO

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Introdução – O conceito de especialização ecológica

Especialização ecológica, a amplitude de recursos utilizada por uma espécie (Colwell & Futuyma 1971; Vázquez & Stevens 2004), está entre os conceitos mais importantes na teoria ecológica, sendo fundamental para a definição de nicho, e frequentemente utilizado como explicação para grandes padrões de ocorrência e diversidade de espécies, como a área de distribuição (Brown 1984), a coexistência de espécies (Chase & Leibold 2003) e o gradiente latitudinal de riqueza (Dyer *et al.* 2007; Schemske *et al.* 2009). Ainda assim, há grandes discrepâncias entre as definições de especialização utilizadas na literatura, a depender das perguntas ecológicas abordadas, do grupo de estudo, nível de organização de interesse e de diferentes tradições de pesquisa (Almeida-Neto *et al.* 2011; Bernays & Chapman 1994; Blüthgen *et al.* 2006; Colwell & Futuyma 1971; Prado & Lewinsohn 2004; Vázquez & Stevens 2004). O objetivo desta tese é examinar algumas das contradições nos conceitos de especialização correntemente empregados e contribuir para que este conceito seja utilizado de forma mais rigorosa, unificando aspectos da especialização presentes em diferentes tradições de pesquisa em um corpo conceitual comum, além de oferecer ferramentas para empregar esse conceito a espécies e comunidades. Nesta introdução, serão discutidos alguns dos aspectos do conceito de especialização presentes na literatura atual, de forma a explicitar essas contradições e discordâncias, para que em seguida um arcabouço conceitual unificado seja apresentado e aplicado nos capítulos subsequentes.

O foco da Introdução é elucidar o descompasso entre dois programas de pesquisa em ecologia, que apesar de terem objetos e questões semelhantes, vêm utilizando o conceito de especialização de forma aparentemente paralela: os estudos de interações, especialmente entre insetos e plantas ou redes tróficas por um lado (Bernays & Chapman 1994; Dyer *et al.*

2007; Jaenike 1990; Lewinsohn *et al.* 2005; Novotny 2006), e os estudos comportamentais de seleção de habitats ou de recursos (Colwell & Futuyma 1971; Manly *et al.* 1993), especialmente com vertebrados, por outro. Segue-se um panorama geral dos principais pressupostos e ferramentas utilizados nesses dois programas para abordar a especialização.

ESPECIALIZAÇÃO TRÓFICA EM ESTUDOS DE INTERAÇÕES

Desde os primórdios da história natural, listas de plantas utilizadas por espécies de herbívoros (assim como o contrário, listas de fauna associada a determinadas espécies de planta) têm sido compiladas (Beaver 1979; Lewinsohn *et al.* 2012; Southwood 1961; Strong *et al.* 1984), e generalizações e hipóteses formuladas a partir dessas informações. Esses estudos foram sendo aperfeiçoados à medida em que se passou a perceber que a heterogeneidade de dados compilados da literatura pode gerar relações e padrões espúrios, além de dificultar a elucidação dos processos que geram as diferenças de especialização em diferentes espécies. Esse avanço metodológico no desenho amostral culminou recentemente com o uso de redes complexas bipartidas para descrever as interações entre conjuntos de espécies que coexistem localmente.

É importante ressaltar, que, apesar de todo o avanço no rigor amostral e abrangência dos dados obtidos, o conceito de especialização progrediu muito pouco nesse programa de pesquisa. Especialização vem sendo entendida como o número de itens alimentares utilizados por determinada espécie de consumidor. Os trabalhos clássicos nessa área definem especialistas e generalistas como extremos de amplitude de dieta, em que especialistas se alimentam de uma ou poucas espécies de recurso, enquanto generalistas têm dietas amplas, alimentando-se de uma grande variedade de recursos. Mesmo em trabalhos que utilizam a abordagem de redes complexas, o grau (número de espécies com a qual determinado consumidor interage) ou a diversidade de recursos utilzada (utilizando o índice de Shannon ou similares) são as medidas de especialização preferidas e mais empregadas.

Tais classificações podem levar em conta um componente filogenético/taxonômico, ao considerar que o número de espécies de recurso deve ser ponderado pelo parentesco entre esses recursos. Assim, a maioria das classificações de especialização na dieta utiliza diferentes níveis taxonômicos, em que um consumidor monófago se alimenta de uma única espécie de planta (ou presa, ou hospedeiro), e quanto maior o nível taxonômico que engloba todos os recursos utilizados por uma espécie, maior sua amplitude de dieta (Almeida-Neto *et al.* 2011; Beaver 1979; Bernays & Chapman 1994). Note-se que essas definições raramente levam em consideração o conjunto de recursos disponível para a espécie focal, e a discussão da especialização em tais espécies é geralmente voltada à evolução e implicações da amplitude de dieta. Há aqui dois pressupostos implícitos: primeiro, que a disponibilidade de recursos não é relevante à amplitude de dieta; segundo, que o consumidor se alimenta de todos os recursos que é capaz de utilizar. Com o advento da cladística e das filogenias moleculares e sua subsequente popularização para uma grande quantidade de grupos de plantas e outros organismos, esses conceitos foram ampliados para levar em consideração o parentesco filogenético entre as espécies de recurso ao invés do nível taxonômico (Beccaloni & Symons 2000; Davis *et al.* 2013; Morse & Farrell 2005; Pellissier *et al.* 2012; Symons & Beccaloni 1999; Weiblen *et al.* 2006), mantendo, no entanto, os mesmos pressupostos e o entendimento da especialização como uma característica intrínseca das espécies independente de condicionantes ou restrições locais.

ESPECIALIZAÇÃO COMPORTAMENTAL OU DE HABITAT

Paralelamente, trabalhos comportamentais, de seleção de habitat e de recursos, realizados majoritariamente com vertebrados, definem a especialização como uma restrição da dieta/habitat de determinada espécie em relação ao conjunto de recursos disponível. Nesse caso a amplitude no uso de recursos é entendida de forma mais contingencial, sendo condicionada pelo contexto em que a espécie se encontra. Desde as primeiras propostas de medir especialização dessa forma, até abordagens mais recentes que utilizam modelos nulos, entende-se a especialização como uma característica ecológica dependente da comunidade em que a espécie está inserida. No entanto, por não considerar o parentesco ou similaridade dos recursos, esses conceitos de especialização deixam de revelar evidências quanto aos mecanismos envolvidos na seleção de recursos, já que simplesmente demonstrar que há restrições de uso em relação ao conjunto de recursos disponíveis, não permite investigar o que gera, ou gerou, essas restrições. Além disso, pela própria dificuldade de medir disponibilidade em larga escala, tais estudos dificilmente permitem generalizações.

Essa oposição entre as duas formas de conceituar especialização reflete, de certa maneira, a oposição entre nicho fundamental e nicho realizado (DeVictor *et al.* 2010). Enquanto os estudos de interações pretendem medir o nicho fundamental, estudos comportamentais focam o nicho realizado. No entanto, estudos de ambas tradições raramente se remetem a essas definições de nicho, medindo a especialização segundo seus conceitos estabelecidos sem esclarecer os pressupostos envolvidos. Além disso, apesar de ser interpretada como nicho fundamental, a amplitude de hospedeiros de insetos fitófagos (e parasitóides, parasitas, entre outros) é sempre medida de forma realizada, já que a dieta observada em condições naturais é registrada, em geral sem nenhuma forma de estimar a dieta potencial que não é utilizada devido a outros fatores, como competição ou falta de co-ocorrência espacial ou temporal. Os conceitos de especialização que levam em conta a disponibilidade, por outro lado, são explicitamente entendidos como especialização realizada, já a disponibilidade de recursos é necessariamente contexto-dependente.

Apresentar essas duas escolas como apoiadas em conceitos totalmente conflitantes é uma simplificação esquemática. Conceitos como a especialização local de insetos fitófagos de Fox & Morrow (1981) propõem que a especialização é contexto-dependente e pode variar entre comunidades e escalas. No entanto, por mais que essa seja uma ideia muito presente na literatura, tais ideias são normalmente interpretadas sem atenção às implicações de conceituar a especialização dessa forma, muitas vezes deduzindo especialização local sem avaliar diferentes escalas. Esse conjunto de contradições nos conceitos de especialização mais utilizados foram a base para a proposta de reformulação de que esta tese trata. A seguir, uma breve revisão de alguns dos avanços recentes que possibilitaram a conciliação de diferentes conceitos que levamos a efeito.

A PROPOSTA CONCEITUAL

O que propomos nessa tese é esclarecer e ampliar o conceito de especialização ecológica, de forma a incorporar, por um lado, avanços dos estudos de interações, especialmente a aplicação da teoria de redes complexas e o uso de filogenias dos recursos para considerar a afinidade dos recursos utilizados; por outro, também incorporar nessa abordagem avanços dos estudos comportamentais e de seleção de habitat, que dão grande importância à disponibilidade de recursos como fator determinante nos padrões de uso de recurso observados. Para isso, foi fundamental adaptar métodos e o corpo teórico desenvolvidos para tratar do parentesco de espécies em comunidades (Cavender-Bares *et al.* 2009; Emerson & Gillespie 2008; Kembel 2009; Webb *et al.* 2002). Essa área da ecologia de comunidades foi desenvolvida nos últimos 15 anos, e tem como objetivo utilizar os padrões de parentesco entre espécies que co-ocorrem para inferir processos ecológicos. Ela pode ser considerada o principal avanço conceitual em ecologia de comunidades desde a proposta da teoria neutra da biodiversidade (Hubbell 2001), e ao demonstrar um padrão de parentesco não aleatório em um grande número de comunidades em diferentes escalas, pode-se dizer que é uma das principais demonstrações de que as comunidades não são estruturadas apenas por dispersão, como proposto pela teoria neutra. Apesar disso, depois da euforia inicial com o novo ferramental, constatou-se que a ligação de padrões de parentesco com processos ecológicos, como competição ou filtros ambientais, não é biunívoca (Cavender-Bares *et al.* 2009). Isso estimulou novamente um grande desenvolvimento conceitual e metodológico, com diferentes propostas para incluir padrões de evolução de caracteres, ocorrência de espécies e variáveis ambientais em um único espectro para buscar inferir os processos estruturadores de comunidades (Leibold *et al.* 2010; Pavoine *et al.* 2011; Pillar & Duarte 2010).

De forma paralela a essa discussão, métricas de parentesco filogenético vêm sendo empregadas em estudos de interações, como discutido acima. Algumas dessas tentativas de incorporar parentesco apropriaram métodos desenvolvidos pela ecologia filogenética de comunidades (Weiblen *et al.* 2006). No entanto, esses métodos foram diretamente aplicados aos conjuntos de recursos utilizados por uma espécie, como se estes conjuntos fossem comunidades compostas a partir de um *pool* de recursos potenciais. O cerne da proposta aqui desenvolvida é adaptar esses métodos, de forma que incorporem processos como abundância de recursos e sua co-ocorrência com os consumidores, para criar um conceito e um método de medir especialização, incorporando tanto a similaridade dos recursos quanto sua disponibilidade. A partir daí, torna-se possível estudar os componentes da variação em especialização entre espécies e entre comunidades, de forma rigorosa e levando em conta fatores que, em geral, são ignorados por estudos em macroecologia e comparações entre redes de interação.

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Capítulo 1 – An integrated framework to improve the concept of resource specialization

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ABSTRACT

Resource specialization, though a fundamental component of ecological theory, is employed in disparate ways. Most definitions derive from simple counts of resource species. Building on recent advances in ecophylogenetics and null model analysis to propose a concept of specialization that comprises affinities among resources as well as their co-occurrence with consumers. In the Distance-based Specialization Index (DSI), specialization is measured as relatedness (phylogenetic or otherwise) of resources, scaled by the null expectation of random use of locally available resources. Thus, specialists use significantly clustered sets of resources, whereas generalists use over-dispersed resources. intermediate species are classed as indiscriminate consumers. The effectiveness of this approach was assessed with differentially restricted null models, applied to a dataset of 168 herbivorous insect species and their hosts. Incorporation of plant relatedness and relative abundance greatly improved specialization measures compared to taxon counts or simpler null models, which overestimate the fraction of specialists, a problem compounded by insufficient sampling effort. This framework disambiguates the concept of specialization with an explicit measure applicable to any mode of affinity among resource classes, and also linked to ecological and evolutionary processes. This will enable a more rigorous deployment of ecological specialization in empirical and theoretical studies.

INTRODUCTION

Ecological specialization, the diversity of resources used by a given species (Colwell & Futuyma 1971), is one of the most pervasive concepts in ecology. It is a fundamental element of niche theory that by way of resource use and overlap, extends to the functional organization of communities (Winemiller *et al.* 2001). Specialization features among preferred explanations for high tropical diversity (e.g. Lewinsohn & Roslin 2008) and species coexistence (Chase & Leibold 2003). However, ecological specialization is defined and evaluated in very disparate ways, which impairs the interpretation and comparison of theoretical and experimental results. With regard to specialization in resource use, a specialist/generalist dichotomy is often employed without any clear definition of these categories, either for potential or realized use (sensu DeVictor *et al.* 2010).

In this paper, we build on recent advances in ecophylogenetics and null model analysis to propose an explicit concept of specialization that comprises affinities among resources as well as their co-occurrence with consumers. This concept should be useful at the theoretical level, by meshing with evolutionary and ecological mechanisms to explain various levels of specialization and their effects, but also operationally by providing a consistent and replicable way of measuring and comparing levels of specialization. Our approach is applicable to any system in which there are different resource categories whose similarities can be assessed. Thus, it bears directly on consumer-resource as well as mutualistic interactive systems; in both cases, interactions comprise pairings of lineages, species or their equivalents. In fact, most studies of ecological specialization focus on one of these modes of species interactions. Henceforth we will refer particularly to plant-herbivore interactions, which also include the data set on which we demonstrate the proposed concept. However, it should be noted that the same approach can also be employed for non-trophic resources, such as habitat categories, as long as their similarities can be gauged in an objective way.

Most species tend to consume a small fraction of the kinds of resources available (Strong *et al.* 1984; Bernays & Chapman 1994; Thompson 2005; Barrett & Heil 2012); in this sense, virtually every species would be a specialist. Hence, in commonplace use (e.g. Scriber 1973; Beaver 1979; Kitahara & Fujii 1994), categories of specialization are established arbitrarily for a given number or range of host taxonomic units (species or higher levels) used by a consumer species. In most cases, operational definitions for levels of specialization are simple counts of resource classes; in network representations of ecological interactions, they appear as the number of links (L), its average per species (L/S) and its distribution over some or all species in a community (Dunne 2006). This approach is also often applied in other kinds of interaction, such as pollination networks (Ollerton *et al.* 2007) or entire food webs (Dunne 2006).

The taxonomic scaling of resources used makes sense because most consumers feed on related species, due to the phylogenetic conservation of traits that enable or restrain consumption, such as chemical defences or cues (Rasmann & Agrawal 2011). With the increasing availability of phylogenies, host phylogenetic relatedness is being incorporated in newer measures of host range, though these are not yet widely used (Symons & Beccaloni 1999; Beccaloni & Symons 2000; Morse & Farrell 2005; Pellissier *et al.* 2012; Davis *et al.* 2013). Further progress was made by Weiblen *et al.* (2006) who employed measures of phylogenetic community structure to assess herbivore specialization relative to the entire set of host plant species in the system studied. However, their null model does not take into account differences in host abundance nor in the frequency of herbivore-plant interactions, so that any plant is equally likely to interact with any herbivore.

The selective use of resources among the potential range available to each consumer species is a key feature of ecological specialization (Colwell & Futuyma 1971; Manly *et al.* 1993). We propose to integrate the phylogeny of resource species with the co-occurrence between consumer and consumed species groups in our concept of specialization. Other, non-phylogenetic criteria, e.g., functional (Junker *et al.* 2013) or habitat/spatial (Chazdon *et al.* 2011), can also be used to gauge affinities among potential and used resources, but such alternatives are not pursued here.

In the following sections, we formalize this concept of specialization and outline a null modelling approach, in which host range is measured as phylogenetic relatedness and compared with the null expectation of random use of the plants with which the herbivore co-occurs. A progression of null models correspond to increasingly constrained ecological processes. This framework is then applied to a large database of endophagous flowerhead herbivores of Asteraceae in Brazil, in which we can examine its behaviour and results at different spatial scales.

DEFINING AND EVALUATING SPECIALIZATION

We defina a specialist as a species that selects a subset of the resources available, so that the components of the used subset are more related (or similar) than expected by chance. Conversely, a generalist selects resources less related (or similar) than expected by chance (Fig. 1). The inclusion of relatedness or similarity in the concept of specialization builds on the premise that, the more similar a given set of resources, the more likely that shared adaptations will enable their use with equivalent costs. When the resources are species, this similarity can be approximated by phylogenetic relatedness, and in this case the problem is similar to the non-independence of species in the comparative method (Felsenstein 1985). We address this issue by measuring the phylogenetic scope of a consumer’s diet. For this purpose we employ a widely adopted metric for relatedness among species in communities: the mean pairwise phylogenetic distance – MPD (Webb *et al.* 2002). The MPD metric is the mean phylogenetic distance between all pairs of species in a given subset of the species pool in question. When applied to the list of resources used by a species, it is the mean phylogenetic distance between each pair of resource components in a diet. MPD is fairly independent from species richness and therefore also from sampling intensity, contrary to phylogenetic diversity (Faith 1992), which tends to be highly correlated with species richness (Clarke & Warwick 2001; and also in our dataset, see Fig. S1), MPD also reflects more properly the idea of relatedness within the resource use of a given group of species.

To evaluate if observed diet breadths deviate from expectation according to the phylogenetic relatedness of potential resources, we propose null models that sample the pool of available resources. Observed diet breadths are then standardized by the null model to gauge how clustered or dispersed they are. As in any null model approach, a crucial step is the definition of the sampling pool, which is the potential resource range for the consumer species. We propose that when studying a given set of consumer species, the full list of resource species recorded for that set of consumers be considered the resource pool. In this way, one can compare different levels of specialization for a given set of consumers, without overestimating specialization by including resources outside the known range for the group of species being studied. A less restrictive choice would also encompass similar or related available resources (e.g., in the same taxon or phylogenetic branch) whose use was not observed in that study but is known from other areas.

After defining the potential resource species pool, the next step is assigning different weights for the resource species based on the availability of each resource class for different consumers. Our initial null model assigns equal probability to all resource kinds, and is equivalent to shuffling the resources' phylogeny while maintaining its richness (number of resource species) for each consumer. In a second, improved model, resources are also weighted by their abundance or commonness; in this way, the use of a single phylogenetically unique resource represents a stronger specialization signal, the scarcer that resource is (Feinsinger *et al.* 1981; Manly *et al.* 1993). Finally, a more comprehensive model also includes co-occurrence between resources and consumers. In this complete null model, sampling of the resources in the potential pool is weighted differentially for each consumer species, both by the resources' regional commonness (frequency of collection) and by the frequency of the consumer species in the different localities, so that only resources that actually co-occur with the consumer are selected, and resources are represented in proportion to the abundance of each consumer in the localities where it occurs (Fig. 1).

As many resource items as the observed frequency of the consumer (fixing the observed interaction frequency of each consumer species) are sampled and the null expectation for MPD is calculated. The observed MPD for the consumer is then standardized by the null model's mean and standard deviation, producing a Z-score. This index is equivalent to the net relatedness index (NRI) (Webb *et al.* 2002; Weiblen *et al.* 2006) widely used in community ecology, and has a similar interpretation. However, the null model we employ is more restricted, and represents potential resources more accurately than the unweighted sampling of the list of potential resources. Note that by incorporating resource-consumer co-occurrences, this null model is not directly comparable to the models in current use for phylogenetic community structure (Kembel 2009). We name this Z-score transform of the MPD the Distance-based Specialization Index (DSI).

To assess the contribution of different components of this null model and investigate the biases created by excluding them from specialization assessments, we also calculated specialization by using the abovementioned less-restricted models: 1) a weighted richness index (WRI) using the same null model, but employing resource richness (e.g. number of host species) instead of MPD as the measure of diet breadth. In this case, the number of resources each consumer feeds upon is also standardized by the resampling results, but the corresponding specialization index leaves out resource relatedness. It is equivalent to calculating DSI assuming all species are equally related to each other (i.e., a star phylogeny). 2) A simple species list-based phylogenetic specialization index (DSI_S) with unweighted sampling of all potential resource species, regardless of their commonness or their local co-occurrence with consumers. In this model, only the number of resource species recorded for each consumer is maintained and that number is sampled without replacement from the entire resource list to produce the null model. This index is equivalent to the NRI as applied to herbivore host ranges by Weiblen et al. (2006) and is the same as null model 3 in Kembel (2009). 3) A phylogenetic specialization index that incorporates commonness or abundance of resources but not their observed co-occurrence with consumers (DSI_A), which can be used when no local interaction data are available. Here, the entire set of resources is weighted by resource commonness, and then sampled for each consumer species for their resource frequency in samples. This model follows the same rationale as Kembel's (2009), null model 5; however, the latter if adapted to interaction studies would use interaction frequencies instead of resource availabilities as used in our model.

Both DSI_S and DSI_A are calculated in the same way as DSI. Additionally to analysing these continuous metrics, we also used them to classify species in the widely used categories of specialists and generalist. Species whose index values exceed 1.96 (i.e. in the upper 2.5% of the fitted normal distribution) are considered specialists; conversely, values below -1.96

indicate generalists, which feed on resources less related than expected by the available pool. The remaining species with an index between these cutoff values, are classed as indiscriminate feeders. Note that the latter category includes scarce species for which statistical power is too low to detect a deviation from the null expectation, together with well-represented species that are true indiscriminate feeders (Fig. 1).

This framework can be applied at a single scale, which can be a local assembly or encompass a larger spatial extent. However, if data span multiple scales, the analysis can be enriched by including the frequency of interacting species in local communities within the studied region. A species can be more specialized locally than in its entire geographical range (a local specialist, Fox & Morrow 1981). In the framework we propose, a local specialist should have significantly higher values for local DSI than for its regional DSI. To inspect local patterns taking regional differences among species into account, we measured local specialization as the difference between the local and regional DSI for the species. This Local Distance-based Specialization Index (DSI-L) is interpreted in the same manner as DSI, so that a species is a local specialist when this value exceeds 1.96. In this case local co-occurrence is not applicable, and DSI_A becomes identical to DSI. All models were built in the R statistical environment (R Core Team 2013), using functions from the ape package (Paradis *et al.* 2004) to deal with phylogenies and the picante package (Kembel *et al.* 2010) to measure relatedness of resources. The models built to measure DSI are available with a worked example in file S5.

MATERIAL AND METHODS

Sampling methods and database

We used a large dataset on endophagous herbivore insects that breed in Asteraceae flowerheads, reared out from plants collected over eight years in four regions in Brazil – montane grasslands in the Espinhaço and Mantiqueira ranges, *Cerrado* vegetation in São Paulo state and coastal lower to montane grasslands in southern Brazil. In these regions, spanning 15º latitude, 26 localities were sampled (Table S1), most of them more than once and in different seasons. We followed the plant sampling and insect rearing procedures described in Prado *et al*. (2002): flowerheads in different developmental stages of all flowering Asteraceae species in each locality were collected and kept in plastic vials covered with a mesh cap to await adult emergence.

The sampling unit in the dataset is a population of a given Asteraceae species in a site, and each entry is a recorded interaction, for which we have both plant and herbivore identity, location (with exact geographic coordinates in most cases), number of reared individuals and total weight of flowerheads. The dataset comprises 3309 interactions among insects from two orders: Diptera (especially Tephritidae and Agromyzidae) and Lepidoptera (Tortricidae, Pterophoridae, Pyralidae, Gelechiidae, and three other occasional families); and Asteraceae belonging to 372 species and 83 genera; other reared herbivorous groups were more difficult to identify or separate into morphospecies. We selected the four insect families that are taxonomically better resolved at the species level: Tephritidae (TE, 106 species), Tortricidae (TO, 32), Agromyzidae (AG, 20) and Pterophoridae (PT, 10). This subset of the database comprises 2690 interactions and 337 plant species from 66 genera.

Analysis

To apply our analytical framework, we built a hybrid tree for all plant genera in our dataset (Fig. S2). This was produced by combining the information from a composite tree of the whole Asteraceae family (Funk *et al.* 2009) for most of the genera, with the taxonomy as a surrogate for phylogenetic relationships of nodes for which no information was available. When even the taxonomy was unable to provide relationships, unresolved nodes were left as polytomies. Based on this tree, we calculated MPD both for the herbivore species' observed diets and in the four null models as described above. In all cases we ran each of the different null models for each herbivore species with 1000 repetitions, calculating the indices separately for each.

We also measured specialization in local communities of the four localities in the Espinhaço Range with the most samples and the highest richness of plants and herbivores. We ran the null model for these local communities also with 1000 repetitions. For each locality we measured DSI-L for the occurring species, classifying their level of local specialization according to the criteria proposed above. All analyses were performed in the R statistical environment (R Core Team 2013).

RESULTS

DSI was highly variable among the 168 herbivore species, with a strong bias towards specialization. When classifying these herbivores as specialists (DSI > 1.96), generalists (DSI < -1.96) or indiscriminate feeders (-1.96 < DSI < 1.96), there are marked differences among families in the proportion of species assigned to each specialization category (Table 1). As a continuous specialization metric, DSI showed substantial disagreement with three host range measures in current use: S, the number of host-plant species, MPD among hosts and *d'* (Blüthgen *et al.* 2006) (Fig. 2). DSI was correlated with the number of host species (Fig. 2a, rho=0.34, $p < 0.001$) but, contrary to expectation, the correlation was positive. The relationship with MPD was negative as expected (DSI, similarly to NRI, increases with phylogenetic clustering, contrary to MPD which is a distance metric). Nonetheless, , DSI values were highly variable in herbivorous species feeding on closely related plants (Fig. 2b, rho=-0.55, $p < 0.001$). The specialization metric *d'*, proposed by Blüthgen et al. (2006) showed no relationship with DSI (Fig. 2c, rho = 0.04, p = 0.67). Even when adjusted for potential host plants, specialization as evaluated against less restricted null models was biased in relation to DSI (Fig. 3, Table 2). The WRI index, which ignores phylogenetic relatedness, showed higher levels of specialization, especially in Tortricidae and Agromyzidae (Fig.2a,b). Similarly, DSI_S, which gives equal probability to all potential host species, also estimated higher specialization than DSI, although to a lesser degree. The degree of overestimation was not constant among herbivore groups; it was weaker for Tephritidae compared to the other families (Fig. 3c,d, 14% species of Tephritidae misclassified as specialists, against 30% for Tortricidae and 42% for Agromyzidae). On the other hand, DSI_A, whose model considers host commonness but not co-occurrence, gave very similar estimates to the full model, with almost no incongruence among them (Fig. 3e,f).

When applying these models at the local scale, local specialization was very uncommon. With the exception of two species of *Trupanea* (Tephritidae) in Grão Mogol, all other species were not significantly more specialized locally than regionally (Fig. 4). These two species bred solely on *Trixis* in this locality, feeding on other genera elsewhere. Most species in different localities show less specialization at the local than the regional level, with many differences in specialization between localities for species occurring in more than one site.

DISCUSSION

We developed a null modeling framework to define and measure specialization phylogenetically, taking the pool of potential resources, their commonness and co-occurrence between resources and consumers all into account. By applying this framework, the concept of specialization is disambiguated and its measure becomes more explicit and comparable. Our example data set is illustrative in this regard, because it concerns a herbivore guild whose members, as far as known, are entirely restricted to a single plant family (Asteraceae); therefore, in accordance with conventional standards, all of them should be considered highly specialized (c.f. Scriber 1973; Beaver 1979). According to our DSI index, host range is highly overestimated by established approaches; specialization was highly variable within this guild and local specialization was very rare. These results enable us to reappraise our understanding of this particular system in the light of the proposed approach, and furthermore to examine new paths of investigation of specialization in various kinds of interactive assemblages, both antagonistic and mutualistic.

Specialization in flowerhead endophages

The null modelling approach we employed clarifies the patterns observed in the Asteraceae-flowerhead endophage assemblage, first, by disentangling the effect of host commonness and actual co-occurrence on observed host ranges; second and most importantly, it tests explicitly the effect of phylogenetic constraints on the host ranges of phytophagous insects. The phylogenetic specialization index (DSI) showed some unexpected results in this herbivore guild. By comparing the specialization evaluated under a fully restricted model (DSI) with simpler models that exclude some of the processes at play in host-plant selection, we were able to show that the detection of specialization is strongly sensitive to the phylogenetic relatedness among plant species (excluded from WRI) and their geographical commonness (excluded from DSI_S). Conversely, in our dataset the co-occurrence of hosts with herbivores (excluded from DSI_A), had no substantial effect, so that values of DSI and DSI_A were largely similar.

The contrast of DSI and WRI shows that the widespread use of the number of host species as a measure of specialization produces frequent overestimates. In fact, though most herbivores do feed on a smaller number of plants than would be expected by chance, for about 20% of the studied herbivorous species their hosts are not more closely related than expected by chance. If phylogeny is indeed a good surrogate for the plant traits that mediate host-plant selection (Rasmann & Agrawal 2011), herbivores feeding on small but phylogenetically random host groupings are limited by other factors that constrain the set of potential host-plants for each species. For this to happen, availability must not be phylogenetically structured; for example, it might reflect seasonal or microhabitat mismatches. In systems where the assumption that phylogenetic similarity is a key mediator of resource selection does not hold, the contrast between WRI and DSI may serve as a test of the importance of resource similarity in the resource selection process.

The second alternative model, DSI_S, incorporates another widely employed assumption, that herbivores are equally likely to feed upon all potential hosts regardless of their commonness and degree of co-occurrence. In our data set, many herbivore species feeding on several genera are nonetheless considered specialists according to DSI_S. This overestimation of specialization occurs because rare unrelated plant species strongly raise the average phylogenetic distance among available plants, inflating the null expectation for the host ranges. Moreover, this model is unable to assess specialization for apparent monophages, reducing the number of species for which a comparison is possible.

In the last comparison, which isolates the effect of host-herbivore co-occurrence in the complete model, there was a surprisingly high congruence between DSI_A and DSI. With only six divergences, there is also no signal of bias in the estimation, as there were both over- and underestimates of specialization, with very symmetric deviations (Fig. 3e,f). This indicates that for the majority of insect species most of the phylogenetic diversity (though not the species diversity) in the regional set of hosts is available to the insects in any locality.

The larger proportion of specialists among the Tephritidae compared to the other families (especially Tortricidae and Agromyzidae) indicates that within the specialized guild of endophagous flowerhead herbivores there are some strong discrepancies. As shown in Fig. 2, even for similar numbers of host species or unstandardized MPD, Tephritidae tended to have higher DSI values than the other families. Note that some of the simpler null models do not show such marked differences. WRI and DSI_S overestimate specialization to a much higher degree for Tortricidae and Agromyzidae than Tephritidae (11% and 14% species misclassified as specialists for Tephritidae vs. 39% and 30% for Tortricidae and 50% and 43% for Agromyzidae – Table S2) when compared to DSI. This shows that the exclusion of phylogenetic relatedness and/or resource commonness can hide important differences in resource use by different lineages of herbivores.

The variation of specialization within species ranges and its scale dependency was proposed by Fox and Morrow (1981) and has been widely applied to date. According to our framework, in the test data local specialization was unexpectedly scarce, being restricted to only two related species in the same locality. However, these two species correspond precisely to the expectation for a local specialist: they use several unrelated hosts in their entire range, but feed selectively on a single genus in one locality. Thus, on the local scale our criterion for specialization is much stricter than simpler criteria in common usage.

Perspectives and conclusions

The incorporation of relatedness among host plants to assess and contrast host ranges was an important step in the comprehension of insect-plant associations. However, only recently this is becoming feasible with the gradual availability of phylogenies for major groups and the development of metrics for relatedness of subsets of a species pool (Webb *et al.* 2002; Weiblen *et al.* 2006). By integrating this metric with statistical models that take plant commonness (geographical spread and/or local abundance) and spatial co-occurrence into account, we were able to define and measure specialization in phytophagous insect communities in a more rigorous and theoretically sounder way. We should note that here we use DSI as a comparison standard, not because we consieder it an intrinsically “true” measure of specialization, but because it is more explicit and comprehensive, with higher power to detect differences and hence more useful than other measures of specialization.

We designed different models to reflect distinct combinations of evolutionary and ecological processes. Comparison of these models reveals that, with the incorporation of phylogenetic information, the concept of specialization becomes much more effective than the simple count of host species to differentiate degrees of specialization. Most importantly, it is clear that measuring either relatedness or the number of host species without reference to the set of potentially available resources has a pronounced effect on the measurement of specialization. Most previous endeavours to incorporate phylogenetic relatedness in measures of specialization did not take the resource pool into account (e.g. Morse & Farrell 2005; Pellissier *et al.* 2012), even when, for instance, independent data for plant communities were available.

A similar problem besets studies that investigate the role of phylogeny in structuring interactions without direct reference to specialization (Ives & Godfray 2006; Rafferty & Ives 2013). By using linear mixed models with a phylogenetic component in the errors, these approaches disregard species abundances and co-occurrences; moreover, they cannot be employed when a consumer phylogeny is not available. In these cases, our framework could improve the comprehension of the processes at work, and is likely to change the inferred patterns of specialization. An improvement is also to be expected in studies that measured specialization by the number of species or other taxonomic levels (e.g. Aizen *et al.* 2012), as in this case both sources of bias in the estimation of specialization apply. Even when the resource pool was included in phylogenetic measures of specialization (Weiblen *et al.* 2006; Vamosi *et al.* 2014), the exclusion of resource commonness and of frequency of consumers may still affect the estimates of specialization, as we showed in our dataset. Likewise, previous attempts to consider species frequencies and abundances (Novotny *et al.* 2004) did exclude relatedness and in turn did not compare the observed patterns with random expectations.

Other specialization metrics that do not measure relatedness have been applied in interaction network studies, such as Blüthgen *et al.*'s (2006) d'. In this case, local resource availability is taken into account; nonetheless, these methods use only frequencies of interaction within the network, regardless of sampling intensity, resource commonness or the absolute number of observations of the interactions. As shown in the analysis of our dataset, the exclusion of these factors can overestimate specialization of scarce species. By using the number of interactions of each species as a surrogate for its availability, host usage cannot be decoupled from abundance; in this sense, the metric of Blüthgen *et al*. (2006) deviates from the classical concepts of resource availability and selection (Colwell & Futuyma 1971; Manly *et al.* 1993). The absence of relationship between DSI and d' (Fig. 2c), fuels the current discussion on the inadequacy of using interaction frequencies as a proxy for abundance (Vizentin-Bugoni *et al.* 2014), reinforced by the fact that d' also showed a very weak relationship with WRI (Fig. S3, rho = 0.20, p = 0.02). As d’ and WRI both disregard phylogenetic relatedness of resources and use a similar rationale for resource diversity and availability, the key difference between them is the use of interaction frequencies for d' and resource commonness for WRI.

Our framework has the advantage of being quantitative, because DSI is a continuous measure. Since, by consensus, specialists and generalists are extremes of a specialization gradient, this might obviate the need for classifying species into specialization categories. All comparisons of specialization both among and within species would then be quantitative, with a continuous metric applied to spatial and temporal gradients or different ecological settings. Nevertheless, the specialist/generalist dichotomy continues to thrive in the recent literature, both theoretical and empirical (McCann 2012; Börschig *et al.* 2013). In the Web of Science© database, a combined search for specialists and generalists returned over 1,000 publications. Given that the dichotomy will continue to be used, a firmer basis for this classification is certainly useful.

As our specialization measure is compared to a null model, one can place observed values in the tails of the corresponding distribution. The cut-off points we employ to assign species to specialization categories follow the common practice of using a normal distribution for reference. Though it is as arbitrary as any division of a continuous variable into discrete classes, this approach has the advantage of being familiar and replicable, improving the quality of comparisons within and among studies. Other alternative statistics are applicable, for instance the proportion of the null distribution exceeding the observed value for each species. We chose to use a Z-score to maintain comparability with currently used phylogenetic structure metrics (NRI and similar). Moreover, values of DSI measured as a proportion are forced to vary between 0 (all null model iterations above observed MPD) and 1 (all null model iterations below observed MPD). These limits are dependent on the number of iterations used, and for our data both measures are nearly identical for species whose DSI measured as a proportion is between 0 and 1 (Fig. S4). Furthermore, when the proportion of values above and below the observed MPD are used to classify consumers into specialists or generalists, only three species out of the 168 change category when compared with a Z-score. Even when considering only the 127 species that are not singletons and hence for which a classification is possible, only 2.4% of the species have mismatched classifications among both metrics.

By defining three categories of specialization, we highlight that so-called generalists comprise two very different kinds of resource use: indiscriminate use, which is the usual meaning of generalist, and over-dispersed use of resources. This disambiguation opens up the possibility of investigating which processes cause each of these kinds of generalism. Also, by categorizing undersampled species as indiscriminate feeders we improve the assessment of specialization, given that most studies would classify these species as highly specialized. A further advancement in this direction would require a quantitative method to sort indiscriminate feeders from undersampled species, in a similar manner to the criterion proposed by Chazdon *et al*. (2011) for a classification in a simple two-habitat situation.

The importance of adequate regional species pools to test for phylogenetic structure has already been advocated in the context of community structure (Cavender-Bares *et al.* 2009), and this is also crucial for specialization measures, such as DSI_A and DSI, that evaluate usage with respect to the set of available resources (see also Forister *et al.* 2012). To measure specialization in interactive assemblages, the spatial scale, extent of the study, and the effective range of usable resources are all choices to be considered carefully, preferably when designing the study.

Local specialization has become a popular concept, very often unrelated from the scale dependency of host-plant selection as proposed by Fox and Morrow (1981). Many studies discussing local specialization neither test for specialization at different spatial scales, nor consider differences in availability of host-plants among localities as an alternative explanation for local patterns of host use. The framework we propose has an explicit measure of local specialization accounting for these neglected factors, allowing to test for scale dependency in specialization. Under these explicit restrictions, local specialization was shown to be very unusual in our data set. It should now be tested whether this is an idiosyncrasy of our system or if it is a broader pattern that remains hidden by ignoring differences in host availability, or by other concepts of specialization used in most studies.

Generalists in the proposed sense were also rare in our test study. Significant phylogenetic overdispersion of used resources could have different causes. If spatial differentiation of host use is involved, we might expect regional generalists to be local specialists, which did not occur in our dataset; if so, they might be an extreme case of a geographic mosaic of interactions (Thompson 2005). However, for a more economical explanation, generalists are born candidates to be investigated whether they are not in fact unresolved cryptic species or host races. On a finer scale, populations may comprise individuals whose use of distinct resources is due to phylopatry or idiosyncratic preference (e. g. grasshoppers, Ben Halima *et al.* 1985). Larger-bodied organisms often combine unrelated resources to complement nutritional requirements or to avoid intoxication, and these could appear as generalists in community surveys (Belovsky 1984).

Our approach can be applied more broadly, as for instance, to any other kind of bipartite interaction, such as mutualistic networks of pollination and seed dispersal (Aizen *et al.* 2012). As long as there is knowledge about local resource communities and interaction networks, allied with a phylogeny for the resource group, this specialization metric can be applied. When resources and consumers, or mutualistic partners, are two sets of species, specialization becomes a two-way phenomenon that can be evaluated for any set with regard to the other (Bascompte *et al.* 2006).

Restrictions in other aspects of the resource range can be equally measured with this approach, by using different metrics instead of phylogenetic relatedness. For example, diversity of any functional trait (as in Junker *et al.* 2013) can be measured and compared to the null expectation. It can also be applied for specialization beyond the interaction context, if the resources or even habitats for which specialization is measured can be characterized and classified in a hierarchical manner; for instance, the habitats in a landscape whose attributes can be used to produce a similarity matrix and a cluster analysis. Habitat selection studies would benefit from this approach when there are many habitat classes to be selected, as the problem is simplified and tests are more powerful by habitat clustering instead of proportion of habitats available(Davies *et al.* 2004).

Other methodological advances in this framework are possible. Ideally, a highly resolved phylogeny with stem lengths is recommended. However, given that such trees are hard to produce, an important further step would incorporate uncertainties in phylogenetic knowledge into null models (Huelsenbeck *et al.* 2000). Another fairly straightforward addition to these models would be the detectability of both consumers and resources in a hierarchical model when measuring co-occurrences, instead of assuming there is no sampling error (Dorazio *et* *al.* 2006). Further on, a logical next step is the derivation of a specialization measure for the community level, integrating the information from co-occurring species into a single measure that can be compared among communities and related to biotic and abiotic variables in macroecological or metacommunity studies. By highlighting neglected aspects in the measurement of ecological specialization and proposing an integrated framework to apply them, we expect that our contribution will enable a more rigorous application of one of the most important ecological concepts in empirical and theoretical studies.

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Table 1: Classification of host range of 168 species of Asteraceae flowerhead endophagous insects belonging to four families according to the null modelling approach proposed in this study.

Family	Specialists	Indiscriminate	Generalists	Singletons
Agromyzidae	2	12	0	6
Pterophoridae	4	3	0	3
Tephritidae	62	21	0	23
Tortricidae	7	15	1	9

Table 2: Congruence between the classification of Asteraceae flowerhead endophagous insects as specialists, generalists and indiscriminate feeders according to the most restricted model proposed in this study (DSI) and three simpler models, that do not incorporate all host pool attributes. DSI takes into account phylogenetic relatedness and abundance of hosts and also plants and herbivore co-occurrence. WRI is equivalent to DSI without relatedness, measuring numbers of host plants instead. DSI_S measures relatedness but considers neither abundance nor co-occurrence. DSI_A is similar to DSI without plant-herbivore co-occurrence. Values in the diagonal (in bold), are congruences between the models. Values below the diagonal are overestimates of specialization from the simpler models, while values above the diagonal are underestimates.

DSI		WRI	
	Specialist	Indiscriminate	Generalist
Specialist	**72**	3	0
Indiscriminate	25	**26**	0
Generalist	0	1	**0**

DSI		DSI_S	
	Specialist	Indiscriminate	Generalist
Specialist	**65**	0	0
Indiscriminate	24	**24**	0
Generalist	0	1	**0**

DSI		DSI_A	
	Specialist	Indiscriminate	Generalist
Specialist	**71**	4	0
Indiscriminate	2	**49**	0
Generalist	0	0	**1**

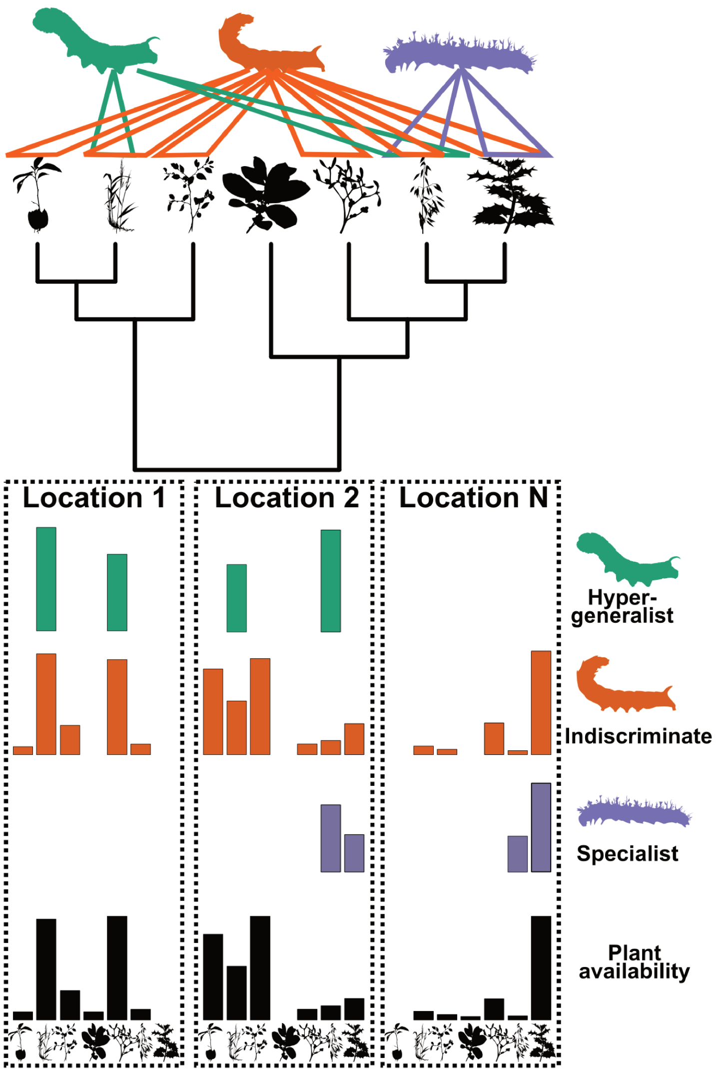


Fig. 1: A schematic representation of the factors considered in the specialization framework we propose. Resource use is assessed, and the similarity among used resources is calculated by means of the Mean Pairwise Distance (MPD) calculated from a phylogeny or similarity dendrogram of the whole resource pool. This observed MPD is then compared to a null distribution obtained from sampling resources available for each consumer, taking both abundances and co-occurrence into account. Species that feed in an over-dispersed set of resources (green) are generalists, while species using clustered resources (purple) are specialists. When resources are used according to their availability (orange) consumers are considered indiscriminate feeders.

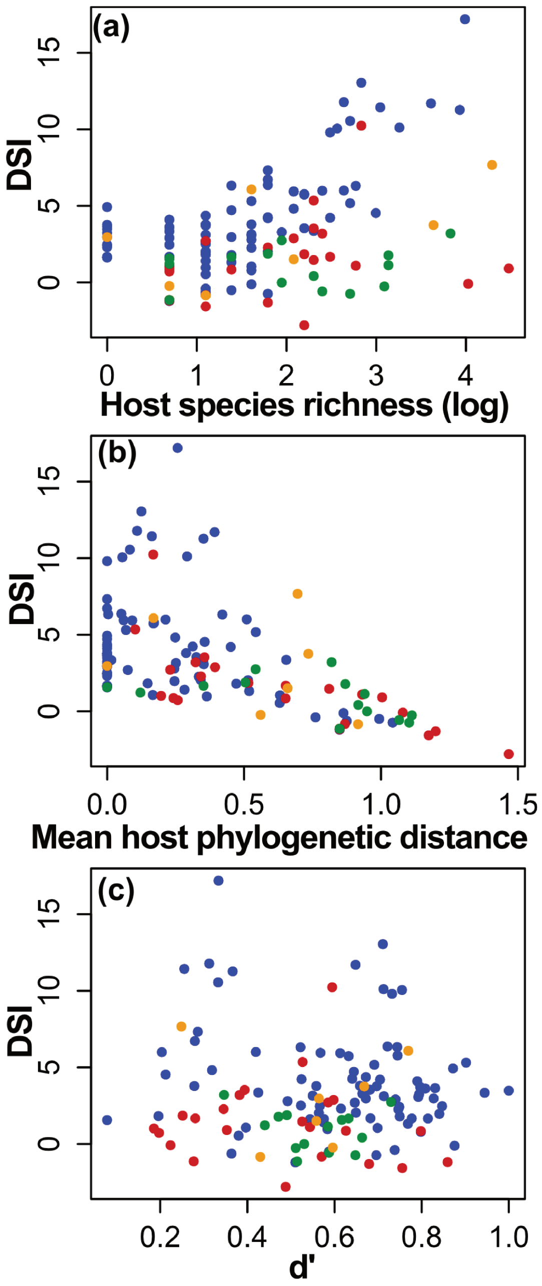
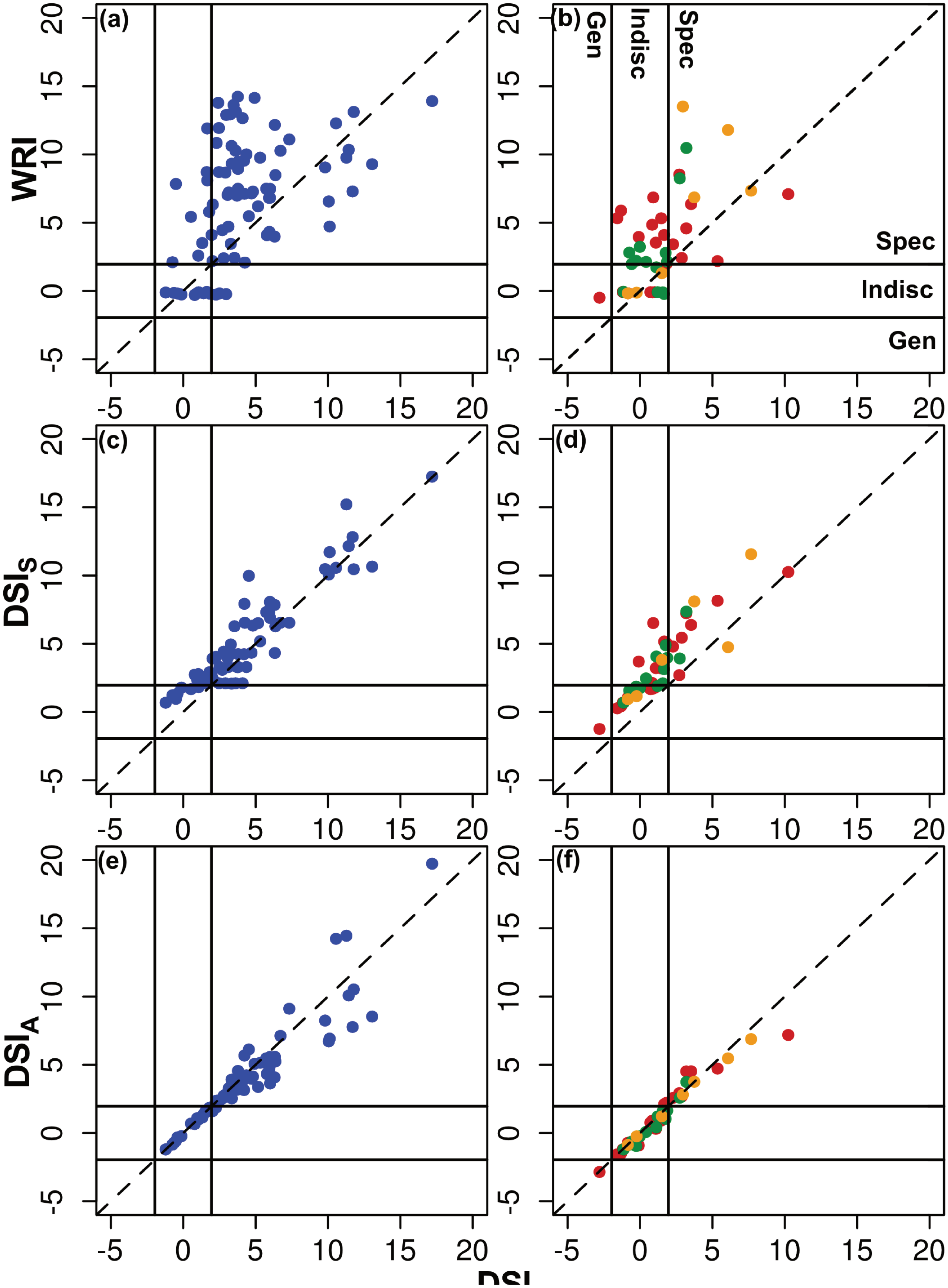


Fig. 2: Relationship between the specialization metric proposed in this study (DSI), and three measures of host range widely employed in the literature. (a): Host plant richness, (b): Mean phylogenetic distance among the host plants, (c): Blüthgen et al's (2006) *d'*, a metric of interaction diversity weighted for interaction frequency of the resources. Different colours represent species belonging to the four herbivore families included in the study: Agromyzidae (green), Pterophoridae (orange), Tephritidae (blue) and Tortricidae (red).


(a)
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WRI
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Fig. 3: Specialization in four families of herbivores feeding on Asteraceae flowerheads in Brazil, according to four different null models. In all figures, the most restricted model, measuring phylogenetic relatedness and accounting for plant commonness and co-occurrence with herbivores (DSI, abcissa) is matched to simpler models: a similarly restricted model with host species numbers instead of relatedness (a,b; WRI), a model based on relatedness in simple species lists (c,d; DSI_S) and a phylogeny-based model including plant commonness but not co-occurrence with herbivores (e,f; DSI_A). Left figures (a,c,e) are for Tephritidae and right (b,d,f) are for Agromyzidae (green), Tortricidae (red) and Pterophoridae (orange). Both WRI and the DSI measures are Z-deviates, with higher values representing higher specialization. The continuous lines represent the boundaries for considering specialists (values above 1.96) or generalists (values below -1.96); intermediate species, generalists s.l., are better designated as indiscriminate feeders. The dashed lines represent equal degrees of specialization in both models.

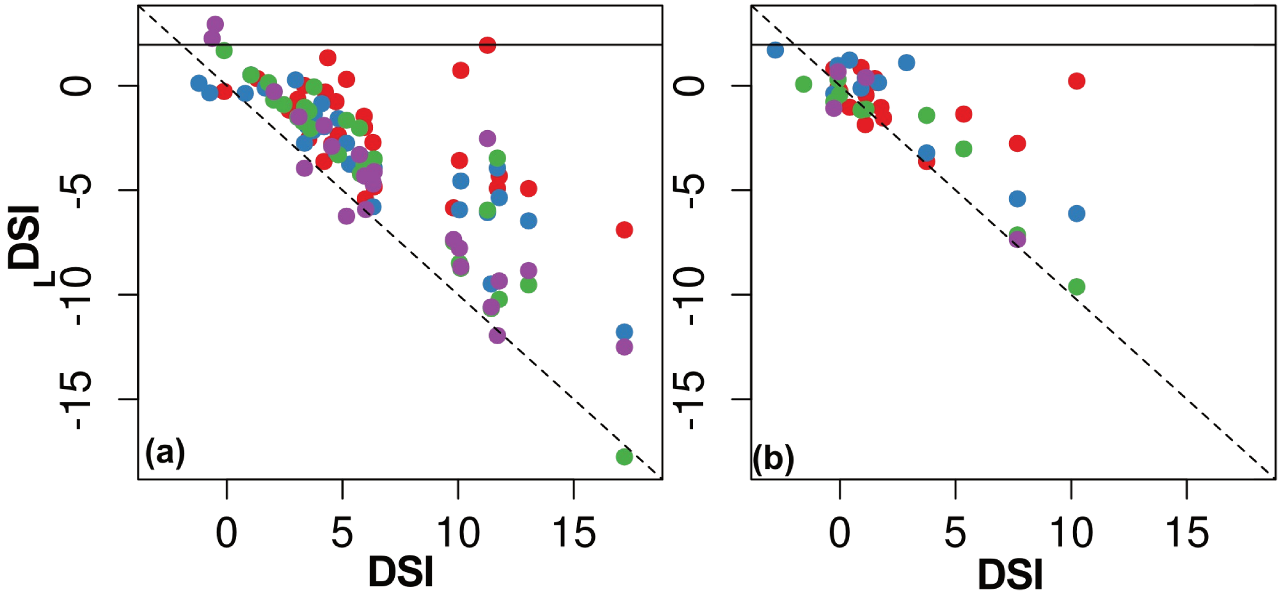

Fig 4: Relationship between regional specialization and the change in specialization at local scale (DSI-L) in four families of herbivores feeding on Asteraceae flowerheads in four localitiies at the Espinhaço Mountain Range in Brazil: Diamantina (red), Serra do Cabral (blue), Serra do Cipó (green) and Grão Mogol (purple). Negative values represent lower local than regional specialization, while species with higher local than regional specialization have positive values, being significant local specialists with values above the continuous line. The dashed line represents a DSI of 0 at the local level without taking regional DSI into account. A: Tephritidae, B: Agromyzidae, Tortricidae and Pterophoridae.

Table S1: List of locations used in this study, with the geographic region they belong to and the average Latitude and Longitude of each one of them.

State	Locality	Region	Latitude	Longitude
SP	Agudos	Cerrado	22°27,93'	48°54,97'
RS	Aparados	Sul	29°4,29'	50°4,95'
SP	Águas Sta. Bárbara	Cerrado	22°48,82'	49°13,76'
SP	Assis	Cerrado	22°45,58'	49°54'
SP	Bauru	Cerrado	22°20,82'	49°0,49'
SC	Bom Jardim da Serra	Sul	28°17,62'	49°39,42'
MG	Cabral	Espinhaço	17°42,43'	44°13,33'
MG	Serra do Cipó	Espinhaço	19°14,97'	43°33,25'
SP	Campos do Jordão	Mantiqueira	22°38,94'	45°34,2'
MG	Diamantina	Espinhaço	18°12,42'	43°44,03'
MG	Grão Mogol	Espinhaço	16°34,22'	42°55,46'
RS	Guaíba	Sul	30°10,84'	51°23,52'
MG	Ibitipoca	Mantiqueira	21°42,31'	43°53,49'
RJ	Itatiaia	Mantiqueira	22°22,32'	44°43,1'
SP	Itirapina	Cerrado	22°14,6'	47°50,44'
SC	Lajes	Sul	27°51,91'	50°10,71'
SC	Mafra	Sul	26°8,51'	49°48,09'
SP	Marinópolis	Cerrado	22°15,14'	51°6,56'
SC	Matos Costa	Sul	26°32,76'	51°1,78'
SP	Mogi-Guaçu	Cerrado	22°15,78'	47°16,21'
MG	Ouro Branco	Espinhaço	20°30,27'	43°39,2'
SP	Pedregulho	Cerrado	20°12,05'	47°22,8'
SP	Passa Quatro	Mantiqueira	22°24,9'	45°3,13'
SC	Sul Litoral	Sul	29°24,55'	50°0,29'
SP	Santa Rita do Passa Quatro	Cerrado	20°30,39'	46°18,68'
MG	Visconde de Mauá	Mantiqueira	22°17,04'	44°31,83'

Table S2: Congruence between the classification of four families of Asteraceae flowerhead endophagous insects as specialists, generalists and indiscriminate feeders according to different null models. DSI is restricted by plant relatedness and commonness and includes co-occurrence between plants and herbivores. WRI is similar, but does not include relatedness, measuring number of host plants instead. $\mathrm{DSI}_{\mathrm{S}}$ measures relatedness but considers neither commonness nor co-occurrence. $\mathrm{DSI}_{\mathrm{A}}$ is similar to DSI but does not include co-occurrence. Values in the diagonal (in bold), are congruences between the models. Values below the diagonal are overestimations of specialization from the simpler models, while values above the diagonal are underestimations.

	Tephritidae			Tortricidae			Agromyzidae		
DSI		WRI			WRI			WRI	
	Specialist	Indiscriminate	Generalist	Specialist	Indiscriminate	Generalist	Specialist	Indiscriminate	Generalist
Specialist	**59**	3	0	**7**	0	0	**2**	0	0
Indiscriminate	9	**12**	0	9	**6**	0	7	**5**	0
Generalist	0	0	**0**	0	1	**0**	0	0	**0**
DSI		$\mathrm{DSI}_{\mathrm{S}}$			$\mathrm{DSI}_{\mathrm{S}}$			$\mathrm{DSI}_{\mathrm{S}}$	
	Specialist	Indiscriminate	Generalist	Specialist	Indiscriminate	Generalist	Specialist	Indiscriminate	Generalist
Specialist	**53**	0	0	**7**	0	0	**2**	0	0
Indiscriminate	10	**8**	0	7	**8**	0	6	**6**	0
Generalist	0	0	**0**	0	1	**0**	0	0	**0**
DSI		$\mathrm{DSI}_{\mathrm{A}}$			$\mathrm{DSI}_{\mathrm{A}}$			$\mathrm{DSI}_{\mathrm{A}}$	
	Specialist	Indiscriminate	Generalist	Specialist	Indiscriminate	Generalist	Specialist	Indiscriminate	Generalist
Specialist	**58**	4	0	**7**	0	0	**2**	0	0
Indiscriminate	0	**21**	0	2	**13**	0	0	**12**	0
Generalist	0	0	**0**	0	0	**1**	0	0	**0**

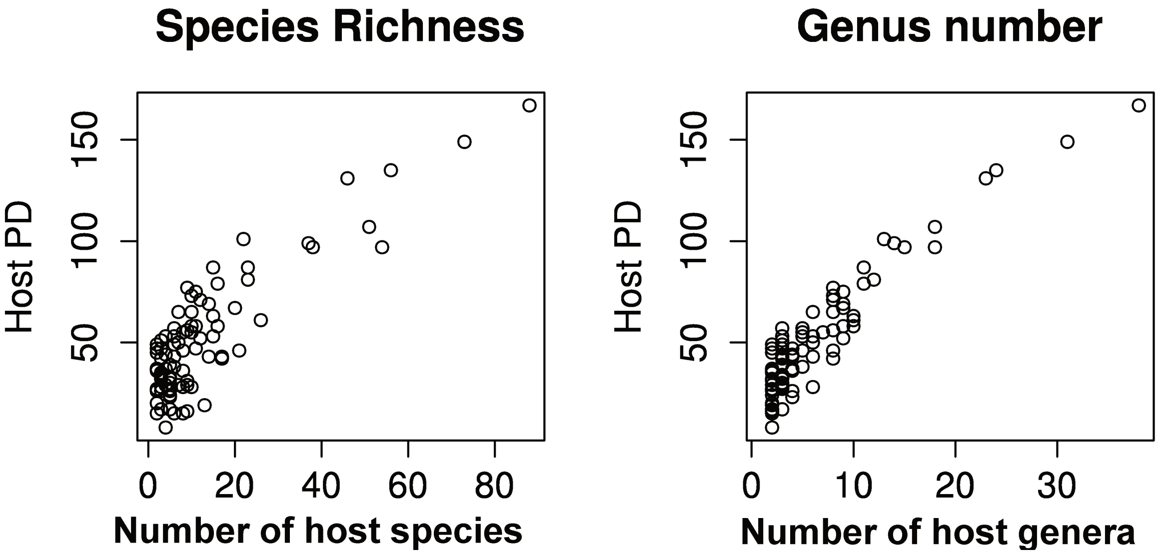

Fig S1: Relationship between Faith's Phylogenetic Diversity and number of taxonomic units for the host range of 168 species of Asteraceae flowerhead endophagous herbivores. In both cases, the correlations are highly significant: r = 0.86 for species richness and r=0.93 for genus number

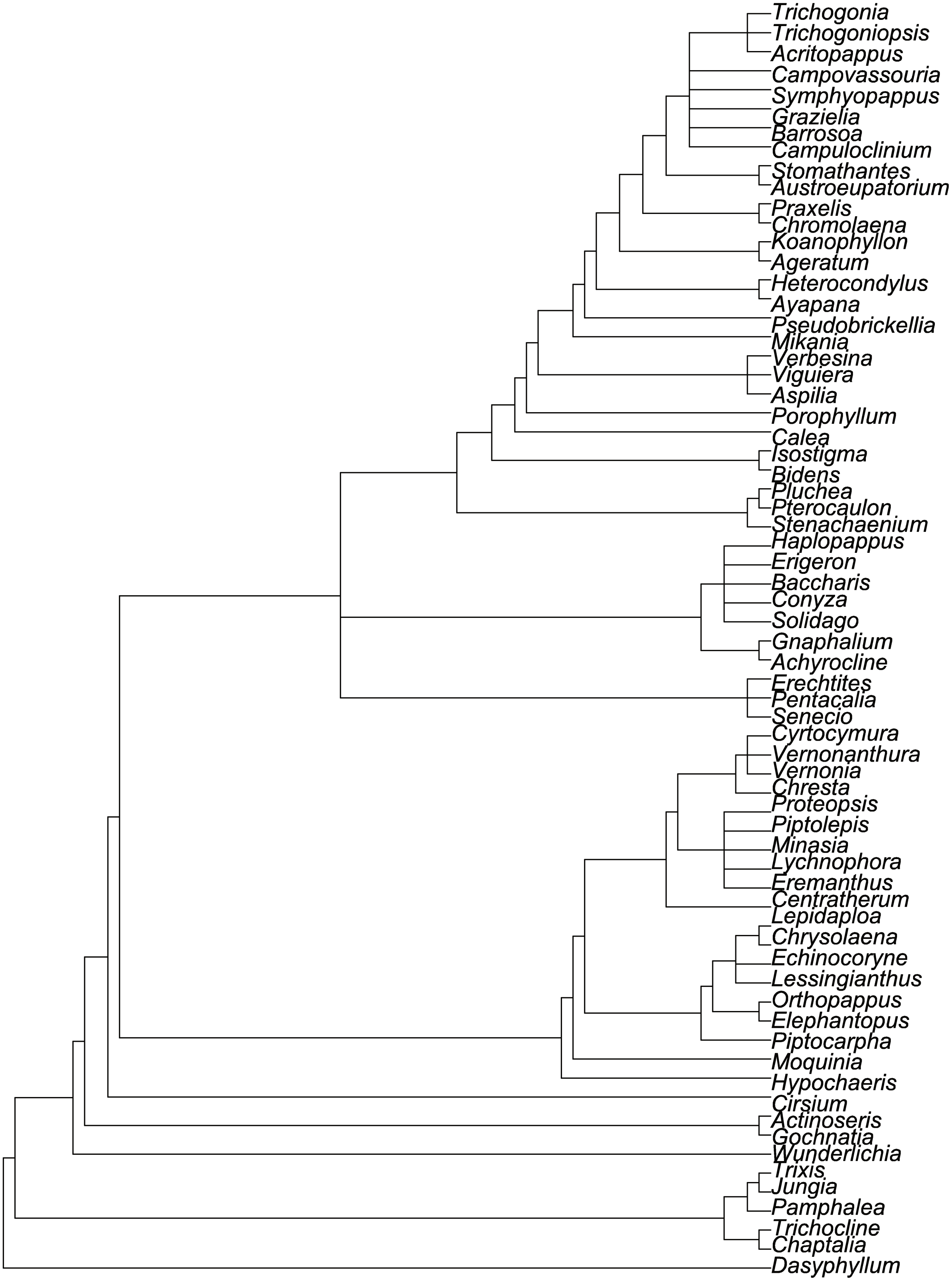

Fig. S2: Hibrid tree of all Asteraceae genera in our dataset used in the analyses.

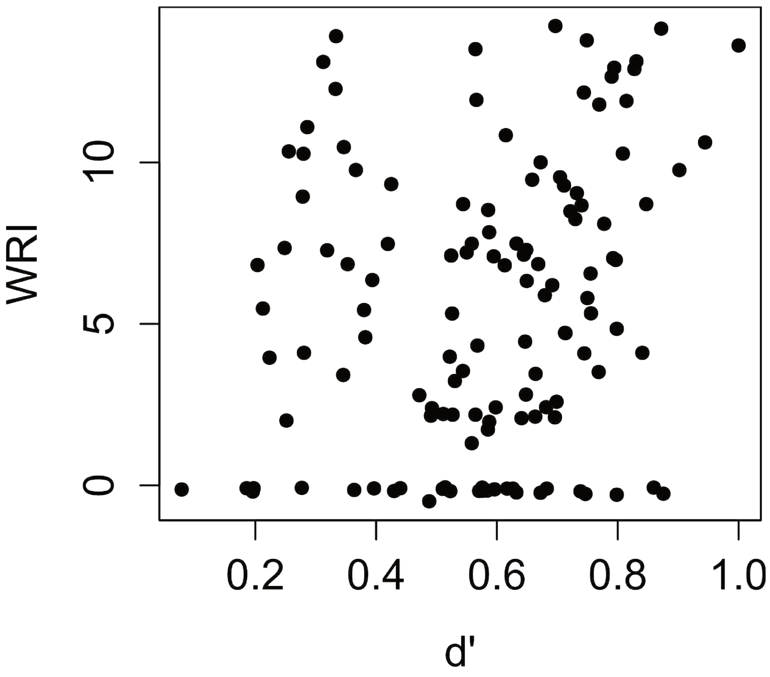

Fig. S3: Relationship between the specialization metric proposed in this study disregarding phylogenetic relatedness (WRI) and Blüthgen et al's (2006) *d′*, a metric of interaction diversity weighted for interaction frequency of the resources.

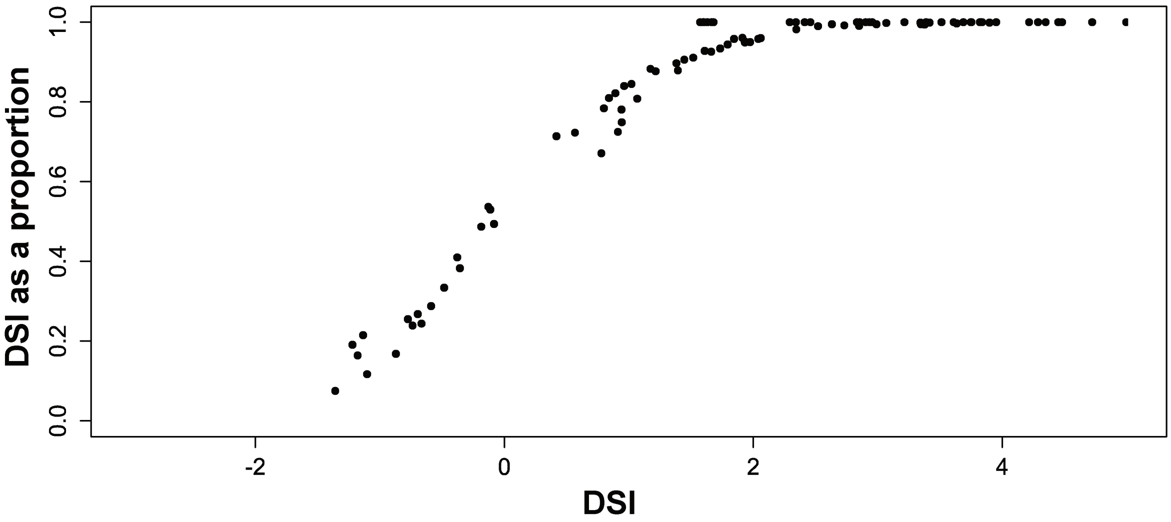

Fig. S4: Relationship between the specialization metric proposed in this study (DSI) when standardized as a Z-value and when measured as a proportion of null values below the observed. The abscissa is truncated at DSI values of 5 when measured as a Z-score (some species actually have DSI values up to 16, but when measured as a proportion it is always 1) to allow a better visualization of the linear relationship between DSI measured as a Z-score and as a proportion. All DSI values above 5 are 1 when measured as a proportion.

Capítulo 2 – A measure of community ecological specialization based on resource affinities.

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Keywords: feeding niche; null model; phytophagous insects; species interactions; distance-based specialization index;

ABSTRACT

Specialization in resource use is a key ecological concept, even though there is great divergence in its definition and measurement. It can be regarded as a property of communities and related to biotic and abiotic variables in macroecological or metacommunity studies. In this chapter we build on the previous one to propose a metric for community specialization that can be compared between different ecological conditions accounting for resource pool and similarity. It is measured as the mean of the distance-based specalization index (DSI) measured locally for each species. We also introduce a partitioning approach for DSI among localities and species simultaneously into three components: 1) within community interspecific differences; 2) between community interspecific differences and 3) intraspecific between community differences. We exemplify these metrics in a dataset of herbivorous insects that are endophagous on Asteraceae flower heads sampled in a metacommunity scale in the Cerrado of Brazil. We we expect that with this approach many of the macroecological questions related to interactions and specialization can be addressed in a more precise way.

INTRODUCTION

Specialization in resource use is a key ecological concept, even though there is great divergence in its definition and measurement. When applied to species, specialization is a measure of niche breadth, and if measured with reference to available resources can be interpreted as selectivity of resources. In this sense, Jorge *et al*.(2014) proposed a framework for resource specialization of consumer species including resource similarity– phylogenetic or functional similarity if resources are species or habitat similarity if resources are abiotic – and contrasting observed use with available resources. By applying this framework, the concept of specialization was disambiguated and its measure made more explicit and comparable.

Specialization can also be regarded as a property of communities and related to biotic and abiotic variables in macroecological or metacommunity studies. Especially when studying ecological networks, several other other metrics of network-wide specialization have been proposed, such as the number of links per species (L/S), Shannon diversity of the links, and H_2' (Blüthgen *et al.* 2006). These metrics have been widely applied to address diverse ecological questions, such as testing for a latitudinal gradient in specialization (Olesen & Jordano 2002; Novotny 2006; Dyer *et al.* 2007; Morris *et al.* 2014), the influence of interaction extinctions (Aizen *et al.* 2012). Here, we propose a metric of community specialization within the same framework developed for the species level – the Distance-based specialization index (DSI) (Jorge *et al.* 2014), and show that variation in DSI can be decomposed into intra- versus interspecific and local versus regional components. In the following sections we define this community metric, explain its behaviour and calculation in a worked example with a hypothetical dataset and then illustrate it to a case study of herbivorous insects feeding on Asteraceae flower heads.

A DISTANCE-BASED COMMUNITY SPECIALIZATION METRIC

We propose a metric for community specialization that can be compared between different ecological conditions either in a metacommunity framework or among equivalent local communities. It bears on the composition of individual species' resource use and accounts for resource pool and similarity to allow for comparison among communities. Our starting point is the species-level metric DSI – the Distance-based specialization index (Jorge *et al.* 2014), which uses null modelling and tools from ecophylogenetics to measure specialization as a continuous specific trait. DSI is a Z-score of the Mean pairwise distance (MPD) among resources used by a given consumer species, standardized by a null model that samples available resources, considering resource abundance and its cooccurrence with each consumer species. For more details on the measurement and properties of DSI, see Jorge et al. (2014, Chapter 1 of this thesis).

To assess specialization within a community, cooccurrence patterns can be excluded, since all resource species in the local community are available to all consumers. Therefore a simpler model is possible, accounting only for resource abundance (DSI_A in Jorge et al. 2014). In more complex sampling designs, temporal or fine-scale patterns of cooccurrence (f.i. in micro-habitats) can be incorporated in a more restricted DSI.

Given that DSI is an unbiased measure of specialization at the species level, a simple composite measure of specialization at the community level can be composed by averaging the DSI of all species coexisting in a local community. We call this measure the DSICOM index (Fig. 1a):

$$DSICOM=\frac{\sum_{i=1}^{N} DSI_i}{N}$$

When comparing DSI values of species in different communities, variation in DSICOM can be decomposed into inter- and intraspecific components, since shared species can have different levels of specialization in different local communities (i. e. local specialization, Fox & Morrow 1981). Another potential dimension for differences in DSICom is local variation among coocurring species. Thus, differences in DSI can be decomposed into three components: 1) within community interspecific differences; 2) between community interspecific differences. and 3) intraspecific between community differences. When specialization is studied at the level of the individual (e.g. Ben Halima *et al.* 1985; Bolnick *et al.* 2011), a fourth component can be included to represent within community intraspecific (interindividual) variation (Fig. 1b).

To assess these components, the pairwise distance matrices of DSI values for species (or individuals, in the case of the last component) for each pair of localities can be decomposed to provide a mean distance for each component. For component (1), the mean pairwise difference in DSI for all species in each community is calculated, and then its average between the two communities is taken. For component (2), the mean pairwise difference between communities in DSI values of all species not shared among communities is calculated. Component 3) is also a mean difference in DSI, but only for species shared among communities, for which all intraspecific differences are calculated between local communities. The sum of these components provides βDSI, representing the total difference in the specialization of species in pairs of communities (Fig. 1c).

MATERIAL AND METHODS

Dataset and analysis

Here we apply this metric to a previously published dataset of herbivorous insects that are endophagous on Asteraceae flower heads (Almeida-Neto *et al.* 2011). This study assessed the interactions of this guild of herbivores with their host plants at a metacommunity scale. In all, local communities within 20 Cerrado savannah remnants were sampled in the state of São Paulo. In each locality, 80 mL of flower heads were sampled from 20 to 30 individuals of each Asteraceae species present. In the laboratory, flower head samples were kept in plastic containers covered with mesh lids. The emergence of adult insects was checked once or twice weekly for 2 months. Adults were extracted, dry-mounted and identified by comparison with our reference collection from previous studies, and when necessary against the literature. Asteraceae abundance was estimated independently of sampling by counting the number of individuals of each species in 15 30m x 5m transects in each locality. For further details on the study sites and sampling, refer to Almeida-Neto et al. (2011).

We measured DSICOM for the 20 study sites, as detailed in the section above:

(1) The DSI metric was calculated for each herbivore species in each site separately

(2) The Mean pairwise distance of the plants from which each herbivore species was reared was calculated, using a phylogeny produced by combining the information from a composite tree of the whole Asteraceae family (Funk *et al.* 2005, 2009) for most of the genera, with the taxonomy as a surrogate for phylogenetic relationships of nodes for which no information was available. When even the taxonomy was unable to provide relationships, unresolved nodes were left as polytomies. Species were also attached as polytomies deriving from each genus (Fig. S1).

(3) The observed MPDs were then standardized as a Z-score from the distribution of a null model in which the frequency of collection of each herbivore was sampled in locally available plants weighted by their local abundance.

(4) This specific DSI value was then averaged for all herbivore species in each site, providing DSICOM.For comparison, we also calculated other widely used interaction network specialization metrics for each site: H2' (Blüthgen *et al.* 2006), Shannon diversity of links and number of links per species (Novotny 2006; Aizen *et al.* 2012; Trøjelsgaard & Olesen 2013). We also tested for a relationship of DSICOM with sampling effort and local richness of (plants, insects or both?).

Finally, we calculated βDSI and its components to compare the relative contribution of among-species variation within sites; within-species differences among local communities; and compositional differences among local communities to the observed differences in specialization among species and sites. All analyses were carried out in the R environment (R Core Team 2014). Functions to calculate DSI under different null models, DSICOM and βDSI will be made available as a R library.

RESULTS

Contrary to the strong relationship between the species metric DSI and sampling effort, observed in most herbivore groups (Fig S2), DSICOM was not influenced by local sampling effort (Fig. 2a; rho=0.37, p = 0.11). It was also not influenced by species richness of plants (Fig. 2b; rho=0.36, p = 0.12), whereas herbivore richness had a positive effect on DSICOM (Fig. 2c; rho=0.54, p = 0.02). The relationship of DSICOM in the 20 studied sites with other widely employed specialization metrics was very weak (Fig. 3a, b, c; p >0.05 for the Spearman correlation test in all cases), despite these metrics being highly correlated with each other (Fig. 3d, e, f).

When partitioning the differences in DSI between the species and pairs of locations, the three components contributed, on average, with almost equal proportions to the total variation in DSI among location pairs: (1) intracommunity interspecific mean ± SD = 34.8% ± 6.2% (2) intercommunity interspecific = 35.3% ± 4.7%; (3) intercommunity intraspecific = 29.9% ± 8.1%. The behaviour of these components showed no clear relationship with the total variation in DSI between pairs of local communities (Fig. 4a), with the differences in richness of plants and herbivores (Fig. 4b) or with species composition of plants (Fig. 4c).

DISCUSSION

The metrics we propose to measure specialization in communities and partition it into geographic and interspecific variation components build upon the DSI framework for specialization proposed by Jorge *et al*. (2014). In common with the species-level DSI, DSICOM is a statistically sound resource specialization metric, which incorporates the availability of resources and includes resource similarity into a continuous metric. Especially important for the construction of a composite metric, the measure of DSI for each consumer species in a given community is completely independent from other consumers. This occurs because, first, a different null model is run for each herbivore species in a given local community, and second, resource availabilities are not derived from interaction frequencies, but from separate abundance estimates. Thus, it is a self-evident step to average DSI among all species in a given community, in order to produce a metric of community specialization with a straightforward interpretation.

When applying this metric to a set of communities in a metacommunity context, we found that sampling effort did not affect the measurement of DSICOM (Fig. 2a), even though it does affect the measurement of DSI at the species level (Fig S2 and chapter 3 of this thesis). This sensitivity to sampling intensity is observed at the species level because for undersampled species this metric has little power to detect deviations from the null model, so that only more intensive sampling allows the detection of high values of specialization (positive DSI) or hyper-generalization (negative DSI). For DSICOM, on the other hand, communities are usually composed of both highly sampled and undersampled species. If sampling effort is comparable for different communities, there is no reason to expect a distinct bias in their comaprison. However, this is a potential problem for every measure of specialization at the comminty level, including DSICOM, and should be further ascertained in critical comparisons

The relationship of DSICOM with the richness of resources (plant species, Fig. 2b) or consumers (herbivore species, Fig. 2c) was also non-existent or very weak. Almeida-Neto *et al*. (2011) showed that in this dataset, plant richness was the main driver of herbivore richness. By classifying herbivores into three categories of specialization based on the taxonomic span of their host plants, they showed that this relationship depends on the specialization category. This result is in agreement with the relationship we observed between DSICOM and herbivore richness.

DSICOM diverged clearly from other widely used network-wide specialization metrics, Since these metrics are very similar in their rationale, correlations among them were found as expected. This is especially true for L/S and link diversity, which are equivalent to richness and diversity indexes, usually applied in communities of species, adapted to networks. H2', on the other side, is a more complex metric which is standardized by interaction frequencies and maximum and minimum possible values in each actual network. Nonetheless, since H2' includes link diversity in its calculation, a high similarity with the other established metrics was expected. DSICOM, on the other hand, incorporates relatedness and independent abundance measures to the calculus of specialization. Similarly to DSI (Jorge *et al.* 2014), these differences can be taken as strong evidence that most specialization metrics in current use have problematic shortcomings, first, by not positioning realized interactions within the pool of available resources. Second, by using interaction frequency as a proxy for resource abundance and availability, which introduces circularity between resource use and presumed resource availability and selection.

Our approach to partitioning the variation in DSI proceeds in a manner equivalent to partitioning diversity into α and β components. However, specialization is partitioned simultaneously in two dimensions: space (localities) and [consumer] species. This procedure allowed us to partition differences in specialization between local communities, into intraspecific changes in specialization (local specialization – Fox & Morrow 1981), and compositional differences (interspecific turnover) with their attendant differences in specialization. Moreover, the third component, local variation among species, represents a measure of variability in DSICOM within communities.Surprisingly, in our data set the relative contribution of these three components to the differences between local communities did not produce any detectable pattern. In Fig. 4 we ordered pairs of communities according to factors capable of directly affecting the partition components, such as the total variation in DSI, differences in species richness, or compositional difference between pairs of communities. Especially regarding the compositional differences (Fig. 4c), an increase in the contribution of the interspecific component should be expected. These results indicate that the differences in specialization among local communities do not depend on species identities, which is supported by the similar magnitude of the three components in the composition of βDSI

Other aspects of DSICOM and its decomposition deserve further investigation, such as the use of significance thresholds of DSICOM to classify communities as generalized or specialized. One simple approach to apply such thresholds is to use the DSICOM values as a

Z-score and use the same criteria and classification proposed by Jorge et al. (2014) for species: communities with values above 1.96 would be classed as specialized communities. Under this criterion, only three sites are not specialized, as expected when studying a network composed of a guild of specialized habits. This result is likely to be different for other kinds of interaction or resource use, such as prey and free-living predators, generalized mutualisms (e. g. pollination, seed dispersion, cleaners) or patterns of habitat use.

By proposing a metric of specialization at the community level accounting for differences both in the resources available and the phylogenetic span of these resources, we expect that many of the macroecological questions related to interactions and specialization can be addressed in a more precise way. Further steps that can contribute to a deeper understanding of specialization include adding individual variation within species and communities into this framework, as we indicate in a general way, and also applying this partitioning into multi-scale studies instead of a simple local-regional approach, or partitioning inter-community variability into both spatial and temporal components.

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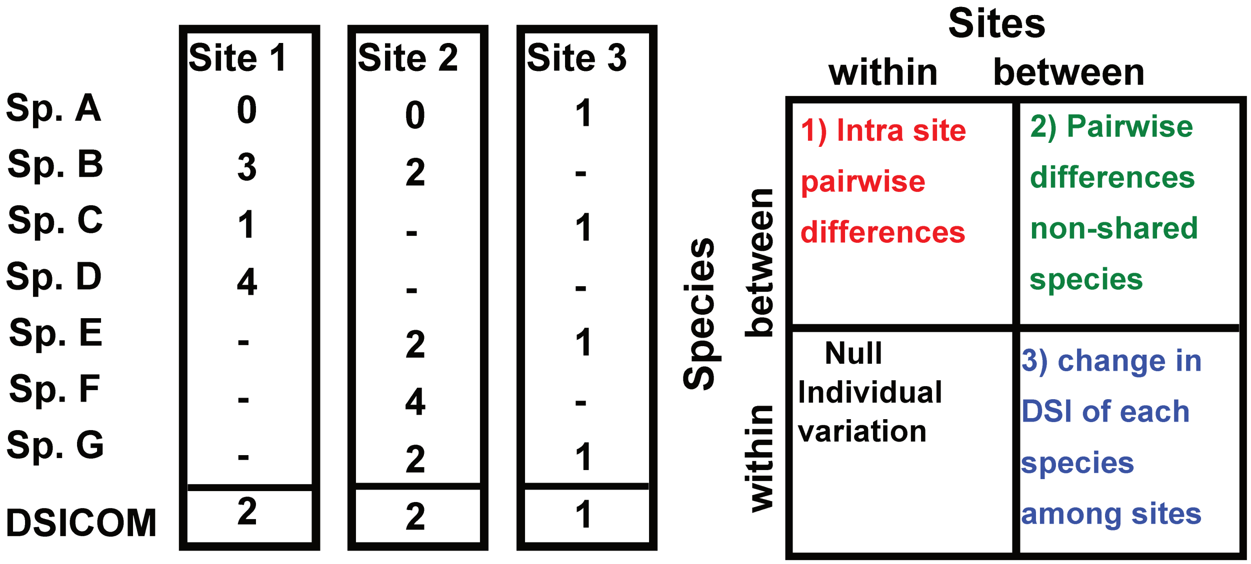

	Site 1	Site 2	Site 3
Sp. A	0	0	1
Sp. B	3	2	-
Sp. C	1	-	1
Sp. D	4	-	-
Sp. E	-	2	1
Sp. F	-	4	-
Sp. G	-	2	1
DSICOM	2	2	1

		Sites	
		within	between
Species	between	1) Intra site pairwise differences	2) Pairwise differences non-shared species
	within	Null Individual variation	3) change in DSI of each species among sites

Fig. 1: The DSICOM and βDSI metrics exemplified in a simple set of three communities. (a) DSICOM is calculated from the values of DSI for each species in each site. Sites 1 and 2 have the same DSICOM, while site 3 has smaller specialization. (b) Partition of the differences in DSI its components. (c) Details on the calculation of βDSI between the three sites. Lines of different colour represent the differences considered in each component, matching the colours in (b).

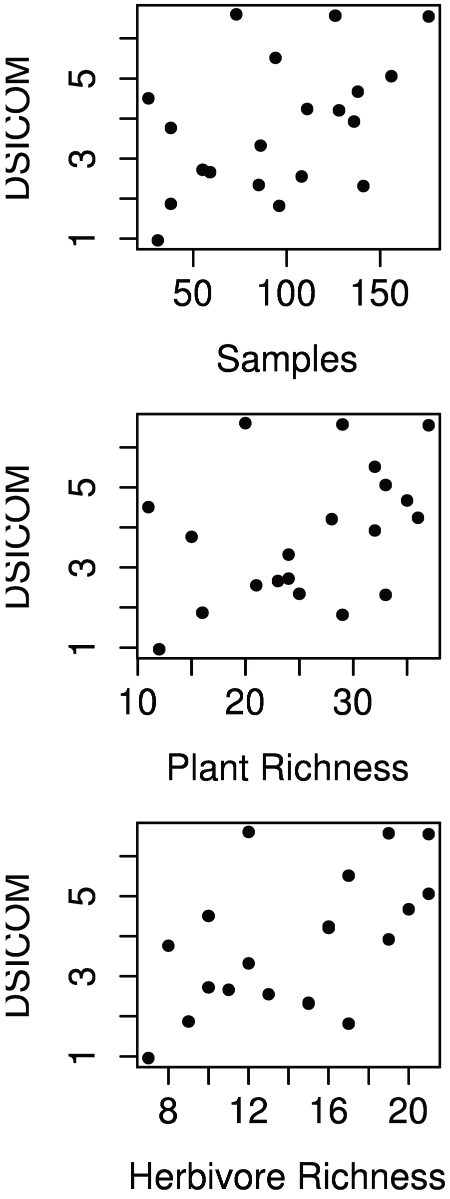

Fig. 2: Relationship between community distance-based specialization (DSICOM) of herbivorous endophages feeding on Asteraceae in 20 sites in Brazilian Cerrado and (a) sampling effort, (b) plant richness and (c) herbivore richness in each site. The only significant relationship was observed for Herbivore richness (rho=0.54, p = 0.02).

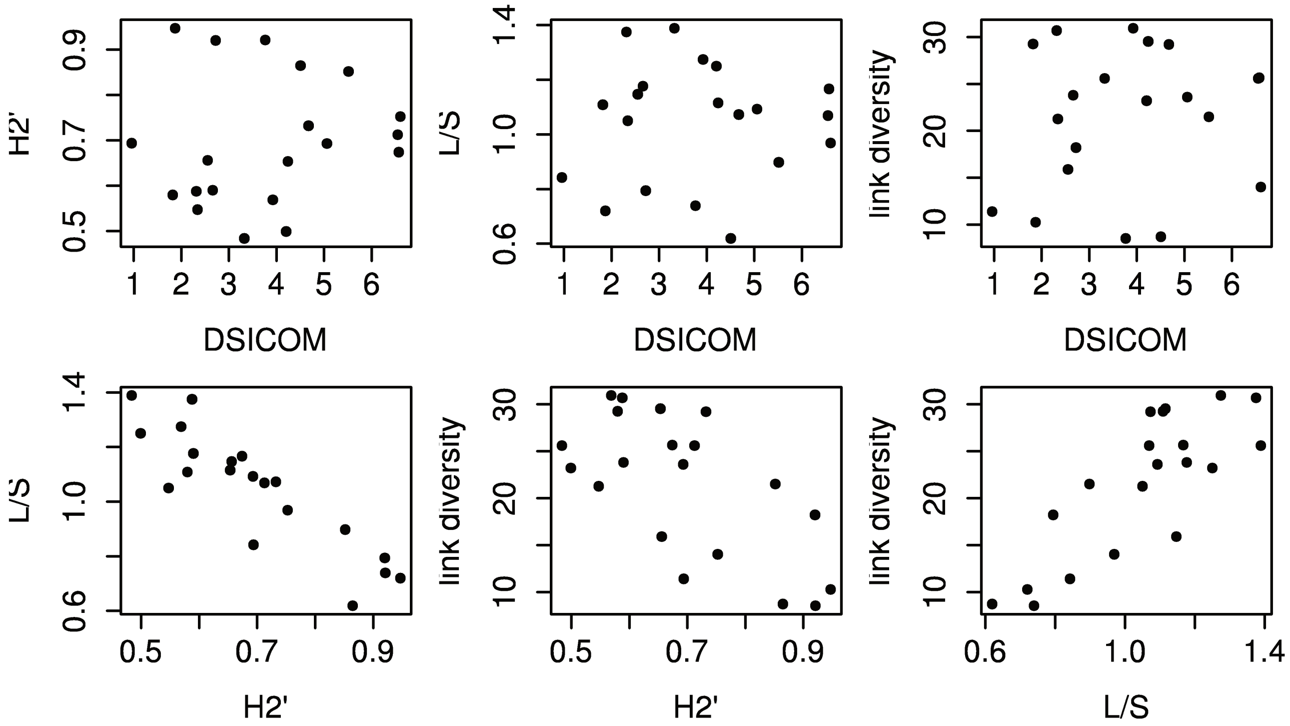

Fig. 3: Relationship between different community-wide trophic specialization metrics of herbivores endophages of Asteraceae flower heads in 20 communities in remnants of Brazilian Cerrado savannahs. In the top row, relationship of the metric proposed in this study (DSICOM) and three of the most widely used metrics: (a) H2', (b) Links per species (L/S), (c) link diversity. In the bottom row, relationships between these metrics: (d) H2' vs. L/S, (e), H2' vs. link diversity and (f), L/S vs. link diversity.

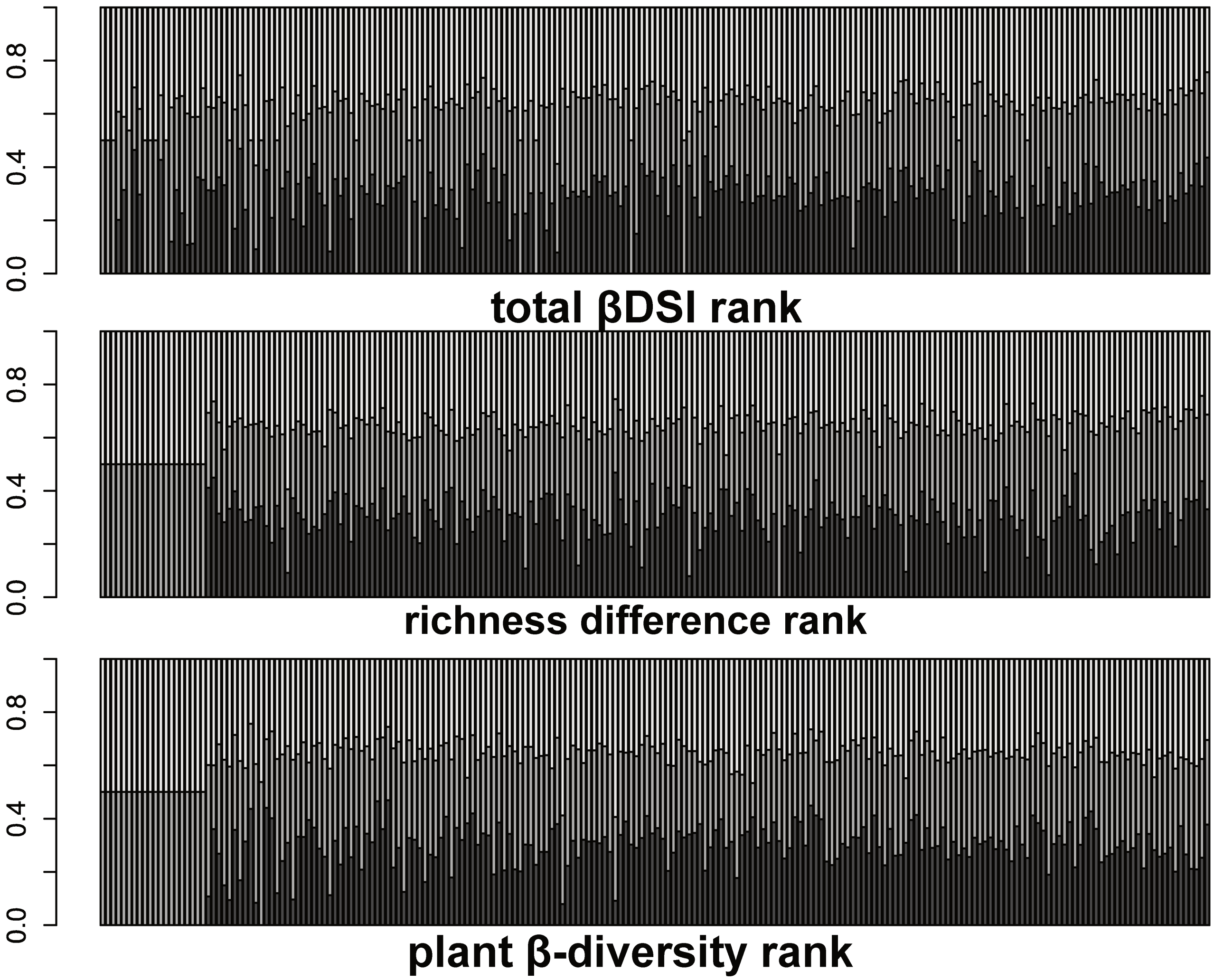

Fig. 4: Proportional contribution of different components of the variation in DSI between all pairs of localities ordered by different criteria. Each bar is a pair of communities, with colours coding the three components: dark grey: intra-specific differences between sites, medium grey: inter-specific variation within a site and light grey: inter-specific variation between sites. (a) pairs of species are ranked according to the total βDSI between the pair, (b) pairs are ranked according to richness differences, (c) pairs are ranked according to compositional β-diversity (Jaccard index) among pairs of sites.

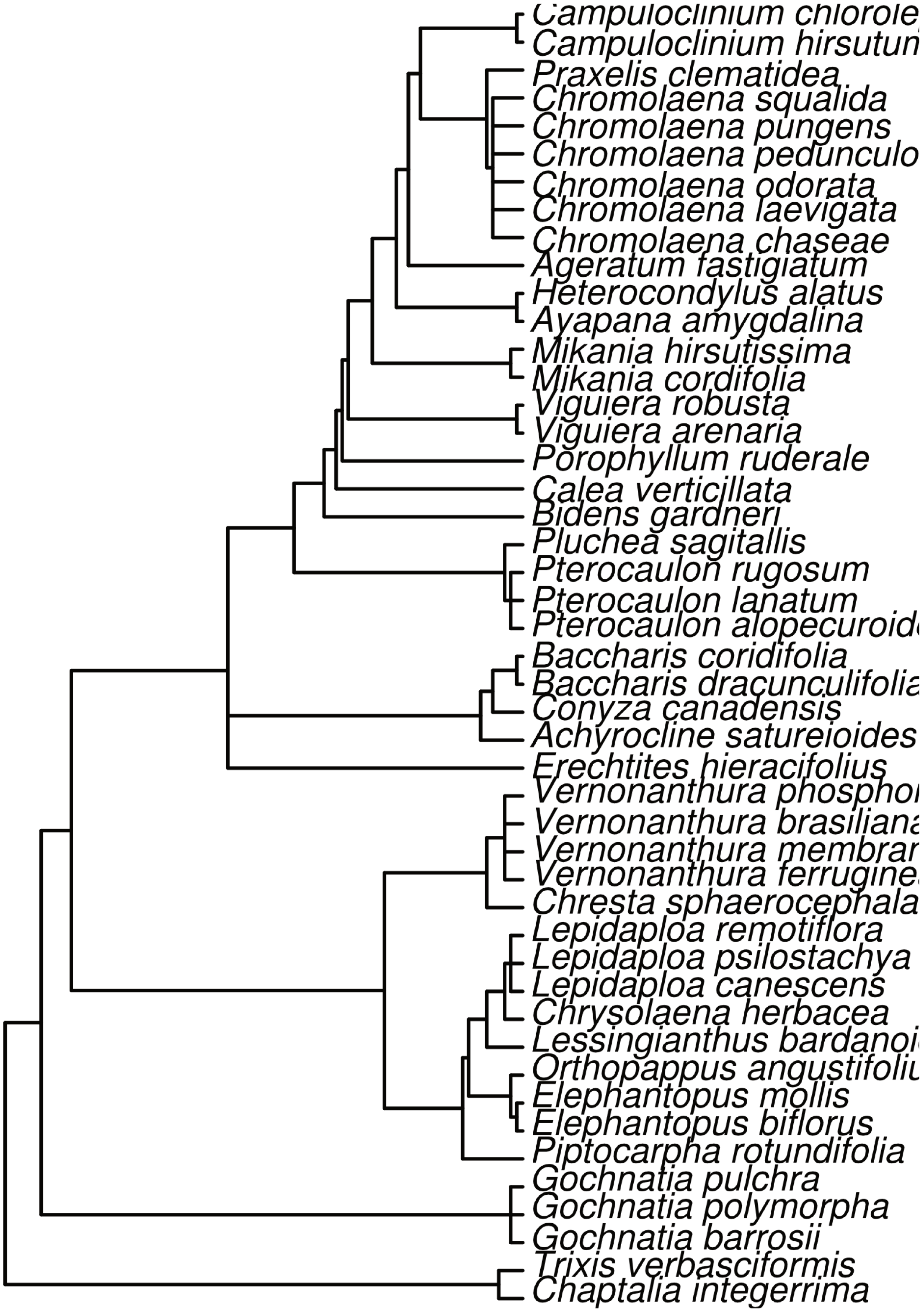

Fig. S1: Hybrid phylogeny used in this study

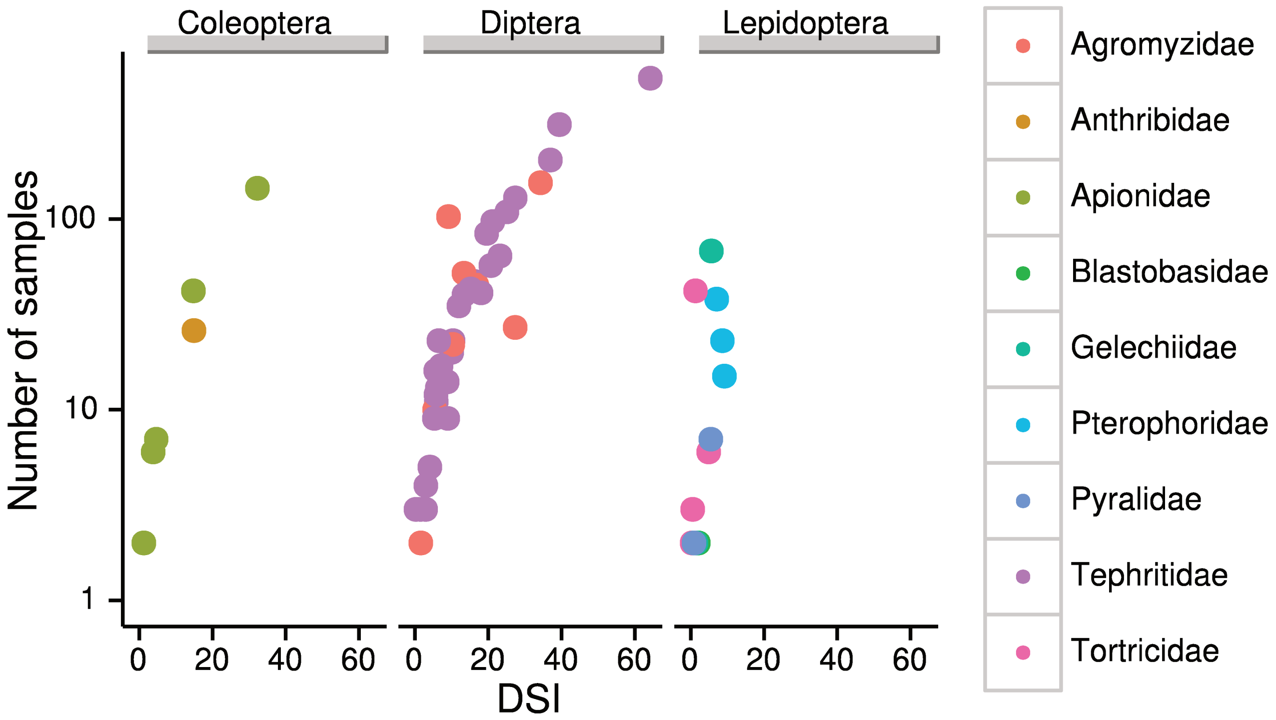

Fig. S2: DSI measured at the regional scale for the species included in this study.

Capítulo 3 – The relationship of geographic range and diet breadth is contingent on choice of specialization metric

Leonardo R. Jorge & Thomas M. Lewinsohn

ABSTRACT

Niche breadth is among the preferred explanations of geographic range size of species. Even though there is strong evidence for a relationship between environmental and habitat niche breadth with range size, the pattern for diet breadth is less clear. Given the great diversity of feeding specialization metrics in the current literature, that take different aspects into account, it is difficult to pinpoint a cause for these equivocal results. In this chapter we employ a distance-based specialization index (DSI) to assess the role of resource availability and phylogenetic relatedness on the relationship between geographic range size and diet breadth of 168 species of herbivorous insects that feed inside Asteraceae flowerheads sampled in 26 localities in four regions in Brazil. For each species we measured richness, mean pairwise phylogenetic distance (MPD) and DSI of host plants and related with the range size of each species. The results were very different for each specialization metric, with a strong relationship with range size for host plant richness, a weaker relationship for MPD and a relationship strongly dependent on herbivore family for DSI. This shows that the relationship of diet breadth with range size may be a result of differences of resource availability and differences in sampling abundance for widespread and rare species.

INTRODUCTION

One of the main issues addressed by macroecology is investigating which ecological factors determine the size of the distribution ranges of species (Brown 1995; Gaston & Blackburn 2000). Among these factors, one of the preferred explanations is niche breadth (Brown 1984). This is a simple prediction, stating that species with broader niche requirements (whether environmental, habitat or trophic) should encounter more extensive areas with suitable conditions and so should be more widespread. However, several issues have long precluded a clear demonstration of this hypothesis. Among the problems is the multidimensionality of the niche, along with the absence of correlation in the specialization of different niche dimensions (Brown 1984; Gaston & Blackburn 2000). Also, all environmental conditions, resources or habitats are not equally frequent or available; therefore niche position within each dimension may be more important than its size. Thus, even very specialized species can have a large distribution if the conditions they specialize in are common and widespread, such as an abundant and ubiquous host species (Gaston & Blackburn 2000). On the other hand, a widespread species may be restricted in the resources it can use but even though apparently use a large number of resources simply because it has more resources available. There is also a pervasive methodological issue, which is the relationship between the distribution range and sampling effort of species. Widely distributed species tend to be sampled more frequently and/or intensively, so that niche breadth measurements can be biased and produce a spurious positive relationship of distribution and niche breadth due in fact to differences in sampling extent.

Regardless of these issues, a recent meta-analysis (Slatyer *et al.* 2013) showed that the published literature supports the positive relationship between range size and niche breadth. Nevertheless, different niche axes show varying degrees of fit. Habitat and environmental breadth variables tend to show a highly significant relationship with geographical range, whereas the evidence for a relationship with diet breadth is equivocal. Diet breadth is much more contingent on availability throughout the range and on the taxonomic extent and resolution on which it is evalauted, compared to other abiotic components of the niche. Especially for herbivorous insects and other kinds of intimate interactions, diet is highly phylogenetically restricted (Ehrlich & Raven 1964; Rasmann & Agrawal 2011). Therefore widespread herbivore species are expected to feed on related plants throughout their ranges; they may, however change the particular species they feed upon depending on local availability of their hosts, whose ranges may be more restricted due to their own niche axes(Strong *et al.* 1984; Hughes 2000). Thus, diet breadth measures suitable to test this relationship need to be more exact by, first, incorporating local availability and second, using a phylogenetic scale, which is less susceptible to sampling effort than the count of species or of higher taxonomic entities, which are often used to gauge trophic speciazlization (see chapter 1).

In the present chapter we assess the effect of resource similarity and availability on the relationship between niche breadth and geographic range of 168 species of herbivorous insects that feed inside Asteraceae flowerheads. We use a recently developed resource specialization metric that takes resource similarity and availability into account (Jorge *et al.* 2014) and compare it with two other diet breadth metrics in common use: number of host plants, or the mean pairwise phylogenetic distance between used hosts. We predict that, when measuring diet breadth as number of used host-plant species, large differences in total availability of resources between widespread and restricted herbivore species will create a strong relationship between their range and trophic niche breadth. Conversely, when measuring diet breadth as the relatedness among host plants, the effect of host availability should be smaller. Finally, when using a metric which incorporates both resource availability and resource phylogenetic similarity, most confounding factors are adjusted, so that any detected relationship of geographic range and trophic breadth can be deemed a net biological effect which cannot be ascribed to host availability or sampling effort.

MATERIAL AND METHODS

Sampling methods and database

We used a large dataset on endophagous herbivore insects that breed in Asteraceae flowerheads, reared out from plants collected over eight years in four regions in Brazil – montane grasslands in the Espinhaço and Mantiqueira ranges, *Cerrado* vegetation in São Paulo state and coastal lower to montane grasslands in southern Brazil. In these regions, spanning 15º latitude, 26 localities were sampled (Table 1; Fig. 1), most of them more than once and in different seasons. We followed the plant sampling and insect rearing procedures described in Prado *et al*. (2002): flowerheads in different developmental stages of all flowering Asteraceae species in each locality were collected and kept in plastic vials covered with a mesh cap to await adult emergence.

The sampling unit in the dataset is a population of a given Asteraceae species in a site, and each entry is a recorded interaction, for which we have both plant and herbivore identity, locality (with exact geographic coordinates in most cases), number of reared individuals and total weight of flowerheads. The dataset comprises 3309 interactions among insects from two orders: Diptera (especially Tephritidae and Agromyzidae) and Lepidoptera (Tortricidae, Pterophoridae, Pyralidae, Gelechiidae, and three other occasional families); and Asteraceae belonging to 372 species and 83 genera; other reared herbivorous groups were more difficult to identify or separate into morphospecies. In the present study, we restricted analyses to the four insect families that are taxonomically better resolved at the species level: Tephritidae (TE, 106 species), Tortricidae (TO, 32), Agromyzidae (AG, 20) and Pterophoridae (PT, 10). This subset of the database comprises 2690 interactions and 337 plant species from 66 genera.

Analysis

We used three different metrics of resource range: the number of host plant species used by each herbivore, the mean pairwise phylogenetic distance between the set of host-plants used by a given herbivore species (MPD), and the distance-based specialization index (DSI), proposed by Jorge *et al*. (2014; chapter 1). This index is a Z-score of the observed MPD of the resources used by a given species, standardized by a null model that samples the expected MPD from the set of host plants the herbivore can feed on, according to the entire data base.

To measure MPD and DSI, we built a hybrid tree for all plant genera in our dataset (Fig. S2). This was produced by combining the information from a composite tree of the whole Asteraceae family for most of the genera (Funk *et al.* 2005, 2009), with the taxonomy as a surrogate for phylogenetic relationships of nodes for which no information was available. When even the taxonomy was unable to provide relationships, unresolved nodes were left as polytomies. MPD was calculated using the *picante* package in R (Kembel *et al.* 2010) and DSI was calculated using scripts available as a supplement of Jorge et al. (2014).

We used the dispersion of the geographic coordinates of the localities where each species was collected to measure the geographic range of the herbivores. For each herbivore species, the dispersion is obtained by calculating, the square root of the squared distances of each locality where it was recorded, to the centroid of the set of localities where it occurred. Thus, geographic range here is entirely based on the sampled data set; no records from other studies, museum specimens or the literature were added. Therefore we avoid diverging identifications and sampling procedure is consistent for all herbivores and their hosts.

To assess the relationship between the different measures of resource range and the area of distribution, accounting for differences among herbivore families and the variation of sampled abundances of species, we applied a model selection approach. For each specialization metric we built a set of six models including different groups of the following variables and their interactions: range of distribution, herbivore family and sample abundance(Table 2). We then used the corrected Akaike information criterion (AICc - Burnham & Anderson 2004) to rank models according to their fit.

RESULTS

We observed a large variation both in geographic range as in the two measures of host range, with some species occurring from only one locality up to all the 26 studied localities, and also using from one to 88 host species. For all specialization metrics, the complete model including the range of distribution, insect family and the number of insect records was the one which best explained differences in specialization among species (Table 2). However, the proportion of variation explained by the best model, and the importance of each variable for the model varied among specialization metrics (Figs. 2-4).

To predict the number of host species (Fig. 2), the relationship between area and richness was very strong, with a small difference among herbivore families in this relationship. The relationship of area of occurrence with MPD was much more variable among families (Fig. 3), and even the best model predicted poorly the variation in MPD, with a high variability in MPD unrelated to the range size and herbivore family. For DSI, a negative relationship with the distribution range is expected, because it is a specialization metric instead of a resource range one as in the case of host richness or MPD. Even though, the relationship was highly dependent on the insect family (Fig. 4), with a positive relationship for Tephritidae and Pterophoridae, no relationship for Agromyzidae, and a slightly negative one for Tortricidae.

DISCUSSION

Since Brown's (1984) proposition of a relationship between niche breadth and range size as an explanation for the general positive relationship of local abundance to geographical range, there has been recurrent discussion of the possibility that different availability of resources and sampling effort might create a spurious relationship (Gaston & Blackburn 2000). By using different metrics of resource range, here we were able to disentangle the role of these factors and show that both availability and sampling effort are important in creating the relationship between range size and diet breadth. Nonetheless, when incorporating them, this relationship was weak and contingent on the herbivore family.

Our results show a clear positive relationship between number of host plants and range size within all herbivore families. This result is commonly observed for insect herbivores when such simple specialization measures are used (Hughes 2000; Slove & Janz 2011). An alternative explanation for this pattern is that species have different geographic ranges independently of diet breadth, and use all the plants they are able to feed on (potential host range) within their area of distribution; since the number of potential hosts is subject to a simple species-area effect, widespread herbivores will intersect more potential hosts. Our results suggest that this explanation is supported by a very stronger observed relationship of herbivore geographic range with the simple number of host species, compared to MPD; and this relationship is contingent on herbivore family for DSI. The number of host species is much more sensitive to beta diversity in the plant species, which is observed in our data set (Lewinsohn et al., unpublished). DSI controls for resource availability and thus it is more robust to differences in host and herbivore abundance. Also, by incorporating plant relatedness DSI can be directly connected to the process most widely considered to influence the choice of host plants by phytophagous insects (Ehrlich & Raven 1964; Rasmann & Agrawal 2011). The pattern we observe, with a large variability of phylogenetic host range independent of herbivore geographic range, suggests that herbivores feed on a set of related plants, regardless of both their geographic range and the richness of the group they specialize on (cf. Prado *et al.* 2002).

A recent meta-analysis (Slatyer *et al.* 2013) showed that contrary to environmental tolerance and habitat range, diet breadth tends to have a weak relationship with geographic range size in several groups. Given this general relationship for other niche axes, the absence of a relationship for diet breadth could be due to two factors. Either the diet is a less relevant niche axis, with little influence on range size, or measures of diet breadth currently employed do not capture this niche axis properly. Given the large diversity of diet breadth metrics currently employed, derived from different concepts of specialization and that take different factors into account (as discussed in Chapter 1), a larger variability in the relationship with range size is expected and precludes a robust test of the importance of diet breadth for range size. In our dataset, that controlled for the common problems of measures of diet breadth, the former explanation is more likely, at least in the geographical scope of our study. A different pattern is possible in larger (i.e. continental) scales, given that some of the Asteraceae groups used by these herbivores have a limited distribution outside the region encompassed in our field study.

This study shows with a very straightforward example the high potential of application of the recently developed ecophylogenetic approaches for the measurement and interpretation of patterns of interaction and resource use. We expect that, as these more robust tests become widespread, the understanding of the broad patterns proposed by macroecological theory are improved and its mechanisms are further unveiled.

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Table 1: List of localities used in this study, with the geographic region they belong to and the average latitude and longitude of each one of them.

State	Locality	Region	Latitude	Longitude
SP	Agudos	Cerrado	22°27,93'	48°54,97'
RS	Aparados	Sul	29°4,29'	50°4,95'
SP	Águas Sta. Bárbara	Cerrado	22°48,82'	49°13,76'
SP	Assis	Cerrado	22°45,58'	49°54'
SP	Bauru	Cerrado	22°20,82'	49°0,49'
SC	Bom Jardim da Serra	Sul	28°17,62'	49°39,42'
MG	Cabral	Espinhaço	17°42,43'	44°13,33'
MG	Serra do Cipó	Espinhaço	19°14,97'	43°33,25'
SP	Campos do Jordão	Mantiqueira	22°38,94'	45°34,2'
MG	Diamantina	Espinhaço	18°12,42'	43°44,03'
MG	Grão Mogol	Espinhaço	16°34,22'	42°55,46'
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SC	Mafra	Sul	26°8,51'	49°48,09'
SP	Marinópolis	Cerrado	22°15,14'	51°6,56'
SC	Matos Costa	Sul	26°32,76'	51°1,78'
SP	Mogi-Guaçu	Cerrado	22°15,78'	47°16,21'
MG	Ouro Branco	Espinhaço	20°30,27'	43°39,2'
SP	Pedregulho	Cerrado	20°12,05'	47°22,8'
SP	Passa Quatro	Mantiqueira	22°24,9'	45°3,13'
SC	Sul Litoral	Sul	29°24,55'	50°0,29'
SP	Santa Rita do Passa Quatro	Cerrado	20°30,39'	46°18,68'
MG	Visconde de Mauá	Mantiqueira	22°17,04'	44°31,83'

Table 2: Results of model selection for the relationship of three different specialization metrics with herbivore range size, sampling effort and family of 168 species of endophagous insects.

		Richness		MPD		DSI	
Model	df	ΔAICc	weight	ΔAICc	weight	ΔAICc	weight
Area*Family+log(Samp)+log(Samp):Area	11	0.0	1	0.0	1	0.0	1
Area+Family+log(Samp)	7	211.6	<0.001	22.8	<0.001	45.6	<0.001
Area+log(Samp)	4	218.9	<0.001	54.5	<0.001	94.5	<0.001
log(Samp)	3	234.9	<0.001	55.1	<0.001	95.2	<0.001
Area	3	262.2	<0.001	60.2	<0.001	143.0	<0.001
Null	2	378.5	<0.001	87.1	<0.001	159.4	<0.001

Fig. 1: Map of the 26 localities sampled in this study.

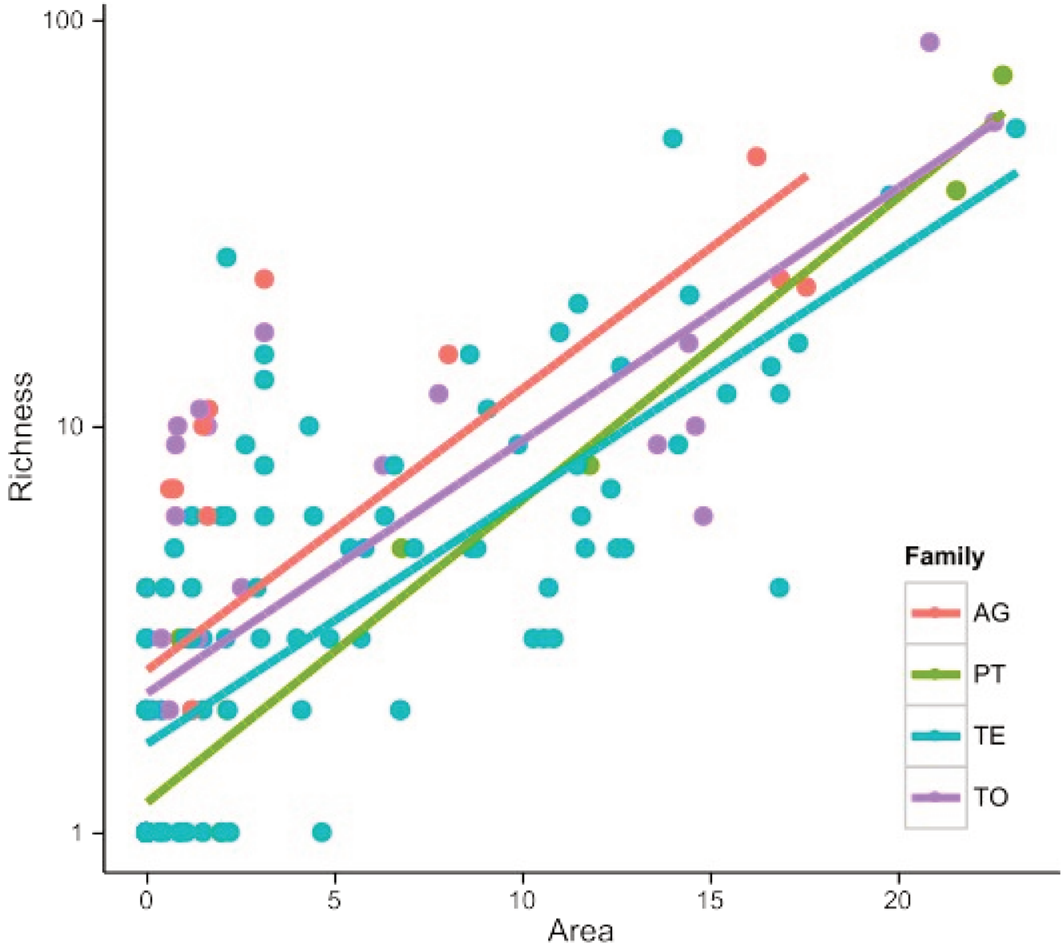

Fig. 2: Relationship between geographic range, measured as the dispersion of the localities where each insect species was collected, and the number of species of host-plant of 168 species of Asteraceae flowerhead endophagous insects belonging to four families. Diptera - AG: Agromyzidae, TE: Tephritidae. Lepidoptera: PT: Pterophoridae, TO: Tortricidae.Multiple R^2=0.91

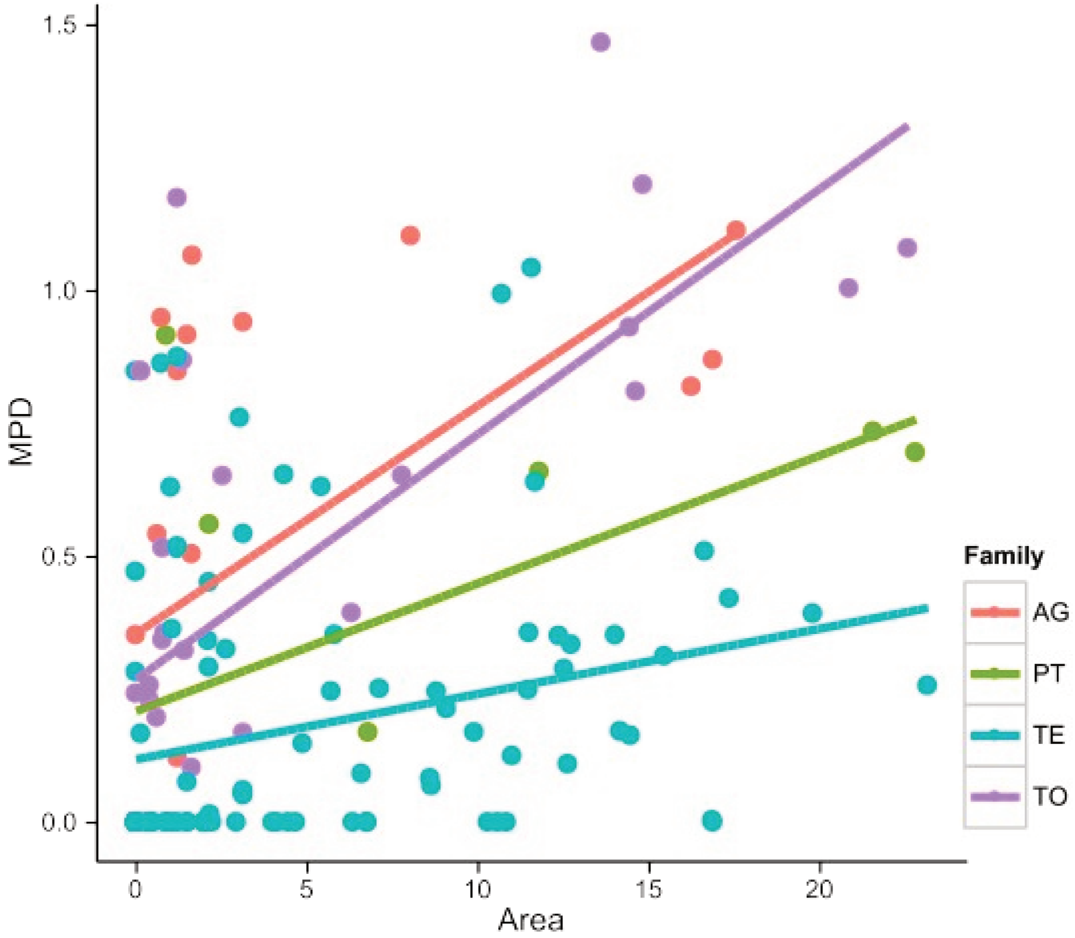

Fig. 3: Relationship between geographic range, measured as the dispersion of the localities where each insect species was collected, and the mean pairwise phylogenetic distance (MPD) of the plants eaten by 168 species of Asteraceae flowerhead endophagous insects belonging to four families. Diptera - AG: Agromyzidae, TE: Tephritidae. Lepidoptera: PT: Pterophoridae, TO: Tortricidae. Multiple R^2=0.47.

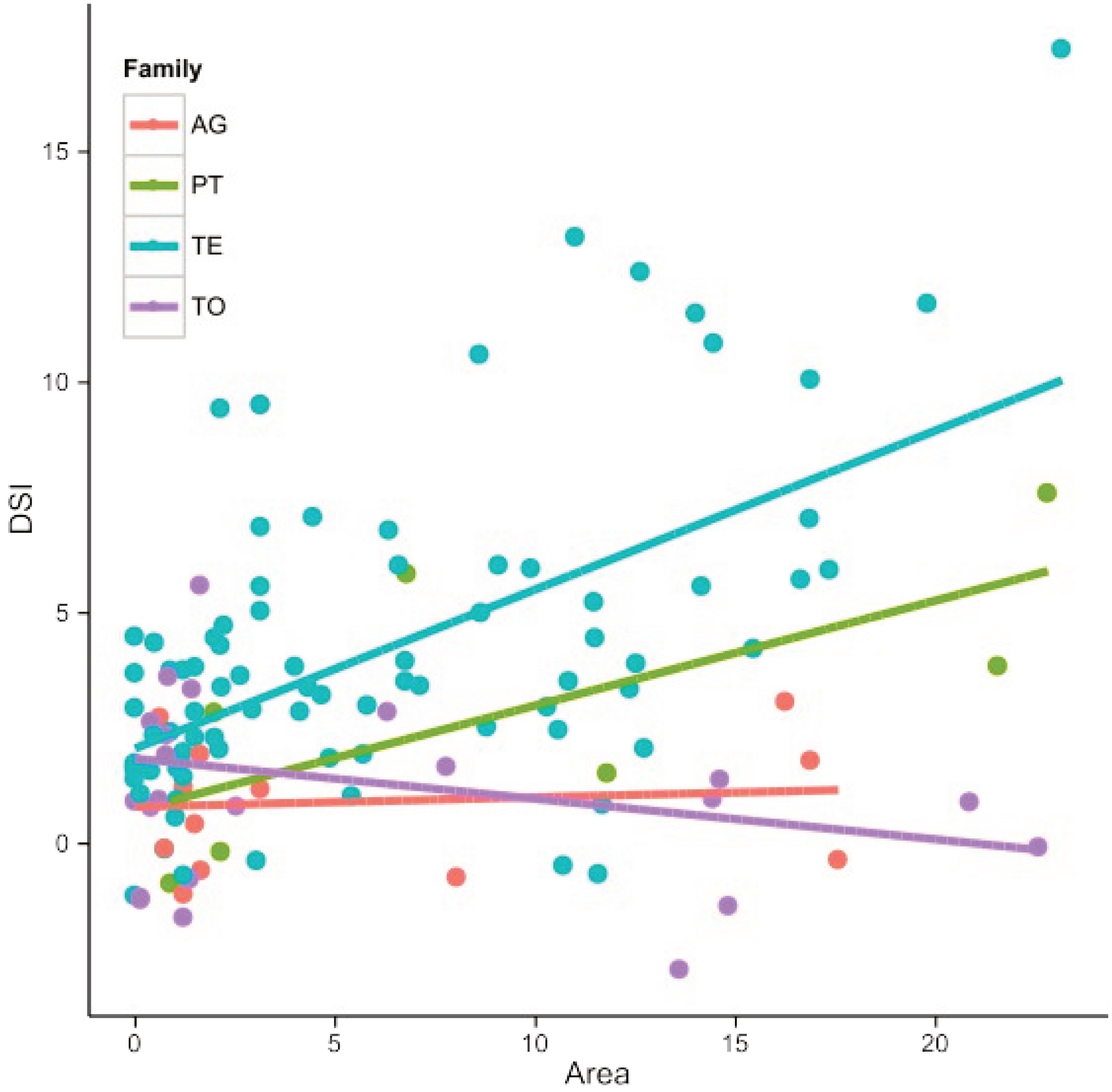

Fig. 4: Relationship between geographic range, measured as the dispersion of the localities where each insect species was collected, and the the distance-based specialization index (DSI) of 168 species of Asteraceae flowerhead endophagous insects belonging to four families. Note that contrary to the previous figures, a higher value of DSI indicates higher specialization or a smaller resource range. Diptera - AG: Agromyzidae, TE: Tephritidae. Lepidoptera: PT: Pterophoridae, TO: Tortricidae. Multiple R^2=0.75.

Capítulo 4 – Phylogenetic diversity does not improve the resource-consumer richness relationship

Leonardo R. Jorge & Thomas M. Lewinsohn

ABSTRACT

Herbivore insects are the richest macroscopic organisms on earth, and explanations for this richness usually bear on their tight relationship with plants. Thus, a linear relationship between herbivore and plant richness in communities is a widespread pattern. Given the strong phylogenetic structure in herbivore diet selection, the relationship with herbivore richness is expected to be even stronger when measuring plant phylogenetic diversity instead of species richness. To test this prediction we used a dataset on four families of endophagous herbivore insects that breed in Asteraceae flowerheads reared from 337 plant species in 26 locations from four regions in Brazil. Both plant richness and phylogenetic diversity were strong predictors of herbivore richness. However, plant richness was the best predictor for the total, Tephritidae and Agromyzidae richness. Tortricidae richness showed a negative relationship with mean pairwise phylogenetic distance of plants. Contrary to our expectations, phylogenetic diversity does not increase the prediction of local herbivore richness.

INTRODUCTION

The relationship between the diversity of plants and herbivore species in communities is a very strong pattern, with consequences for the estimation of local and global species richness (Erwin 1982; Ødegaard *et al.* 2000). Plant richness can be considered a null expectation for herbivore richness when investigating other potential drivers (Lewinsohn & Roslin 2008), such as the ones related to interactions (feeding specialization, beta-diversity in interactions and spatiotemporal cooccurrence) or environmental variables directly affecting the herbivores. For herbivorous insects, the plant-herbivore richness relationship was shown to be significant in most studies that test it, regardless of region or insect taxa studied (Lewinsohn & Roslin 2008).

This fundamental relationship is expected because herbivores tend to be restricted to a small subset of the hosts available, with a high phylogenetic conservatism in use of hosts (Ehrlich & Raven 1964; Rasmann & Agrawal 2011; Jorge *et al.* 2014). Given the phylogenetic constraint of host usage, one can expect the diversity of plant lineages in a given community to be a better predictor of the diversity of herbivores than plant richness, since the overlap of associated herbivore faunas among unrelated plants is expected to be small while closely related plants are should share most of their herbivore species.

To test this expectation, different metrics of phylogenetic diversity can be employed. Metrics that at present are widely used are based either on total branch lengths in a given community (Phylogenetic diversity – PD; Faith 1992) or on average relatedness between coexisting species (Mean pairwise phylogenetic distance - MPD; (Webb *et al.* 2002). If total lineage diversity is the foremost parameter, PD should be the best predictor of herbivore richness, outperforming species richness and MPD. Note that in this chapter the metric of specialization developed in the previous chapters is not useful, as only the overall phylogenetic diversity available in each locality is considered, not phylogenetic patterns of host plant use. In the present study we test this prediction by comparing the effect of plant richness, phylogenetic diversity and mean pairwise phylogenetic distance on the richness of herbivores in a guild of endophagous insects on flowerheads of Asteraceae in Brazil.

MATERIAL AND METHODS

Sampling methods and database

We used a large dataset on endophagous herbivore insects that breed in Asteraceae flowerheads, reared out from plants collected over eight years in four regions in Brazil – montane grasslands in the Espinhaço and Mantiqueira ranges, *Cerrado* vegetation in São Paulo state and coastal lower to montane grasslands in southern Brazil. In these regions, spanning 15º latitude, 26 localities were sampled (Table 1; Fig. 1), most of them more than once and in different seasons. We followed the plant sampling and insect rearing procedures described in Prado *et al*. (2002): flowerheads in different developmental stages of all flowering Asteraceae species in each locality were collected and kept in plastic vials covered with a mesh cap to await adult emergence.

The sampling unit in the dataset is a population of a given Asteraceae species in a site, and each entry is a recorded interaction, for which we have both plant and herbivore identity, location (with exact geographic coordinates in most cases), number of reared individuals and total weight of flowerheads. The dataset comprises 3309 interactions among insects from two orders: Diptera (especially Tephritidae and Agromyzidae) and Lepidoptera (Tortricidae, Pterophoridae, Pyralidae, Gelechiidae, and three other occasional families); and Asteraceae belonging to 372 species and 83 genera; other reared herbivorous groups were more difficult to identify or separate into morphospecies. We selected the four insect families that are taxonomically better resolved at the species level: Tephritidae (TE, 106 species), Tortricidae (TO, 32), Agromyzidae (AG, 20) and Pterophoridae (PT, 10). This subset of the database comprises 2690 interactions and 337 plant species from 66 genera.

Analysis

We used three different metrics of plant diversity: richness, phylogenetic diversity (PD) and mean pairwise phylogenetic distance (MPD). To measure PD and MPD, we built a hybrid tree for all plant genera in our dataset (Fig. S2). This was produced by combining the information from a composite tree of the whole Asteraceae family for most of the genera (Funk *et al.* 2005, 2009), with the taxonomy as a surrogate for phylogenetic relationships of nodes for which no information was available. When even the taxonomy was unable to specify relationships, unresolved nodes were left as polytomies. Both phylogenetic metrics were calculated using the *picante* package in R (Kembel *et al.* 2010).

For each metric, we evaluated the power to predict the species richness of all herbivores and also separately for three of the families: Tephritidae, Agromyzidae and Tortricidae. As there are only 10 species of Pterophoridae in our dataset, with a maximum of 4 locally cooccurring species, there was not enough power to test for associations and the famiiy was not analised separately. We used separate Ordinary Least Squares (OLS) models with the plant diversity measures – Richness, PD and MPD – as explanatory variables of herbivore richness. We compared the performance of each measure of plant diversity by means of the corrected Akaike information criterion (AICc - Burnham & Anderson 2004). All tests were processed in the R programming environment (R Core Team 2014).

RESULTS

The overall richness of herbivores in a local assemblage was strongly related to the richness of plants (Fig. 2, $F_{1,24}=237.7$, $p<0.001$). With regard to the richness of each herbivore family, a significant relationship with plant richness was observed only for Tephritidae ($F_{1,24}=117.3$, $p<0.001$) and Agromyzidae ($F_{1,24}=100.7$, $p<0.001$), and only marginally for Tortricidae ($F_{1,24}=3.89$, $p=0.06$). Noticeably, while plant richness was the best predictor of herbivore richness overall and for Tephritidae and Agromyzidae, it ranked badly for Tortricidae (Table 2).

For the relationship of PD with herbivore richness, there was an overall positive relationship (Fig. 3, $F_{1,24}=93.4$, $p<0.001$). Nonetheless, this model was not as well fitted as plant richness (Table 2). When considering herbivore families separately, the results were very similar to those observed for plant richness for all three families (Tephritidae – $F_{1,24}=83.9$, $p<0.001$; Agromyzidae – $F_{1,24}=88.1$, $p<0.001$ and Tortricidae – $F_{1,24}=0.52$, $p<0.48$), although the model with PD as sole predictor was always a worse performer than plant richness (Table 2).

There was no significant relationship of MPD with total herbivore richness (Fig. 4, $F_{1,24}=0.23$, $p=0.63$), nor with Tephritidae ($F_{1,24}=0.89$, $p=0.35$) or Agromyzidae ($F_{1,24}=1.84$, $p=0.19$) separately. For Tortricidae, on the other hand, there was a significant negative relationship between MPD and richness ($F_{1,24}=14.01$, $p=0.001$). Model selection showed MPD as the worst model for the overall relationship and for Tephritidae and Agromyzidae, whereas it was the best model for Tortricidae (Table 2).

DISCUSSION

Contrary to our expectations, plant richness was the best predictor of local herbivore richness, rather than phylogenetic diversity. This was a strong effect, valid both for the whole set of species and for most herbivore families separately. Given that in this system the herbivore species show a very strong phylogenetic affinity with their hosts (Prado *et al.* 2002; Jorge *et al.* 2014), this is a surprising result. Furthermore, for Tortricidae, the family with the highest proportion of indiscriminate (i.e. non-selective) feeders and generalist species (Jorge *et al.* 2014), an opposing pattern was observed, with no relationship with plant richness and a negative relationship with MPD.

Plant richness is widely accepted as the main driver of herbivore richness in communities. However, plant phylogenetic diversity is supposed to have a major role in this relationship, as host plant usage by herbivores is under a strong phylogenetic constraint. Dinnage et al. (2012) showed a significant role of phylogenetic diversity on herbivore richness in an experimental plant biodiversity gradient, with a no-linear effect of plant richness and phylogenetic diversity increasing herbivore richness. The metric of phylogenetic diversity employed in their study is similar to an abundance-sensitive measure of PD standardized by host richness. High diversity is then achieved when plant individuals are equally spread along the phylogeny, so that closely related taxa are less abundant and more distinct ones are more abundant (Cadotte *et al.* 2010). Even though it is not directly comparable to any of the phylogenetic measures we employed, we expect the fundamental differences in our results are not an artefact of metric formulations, but have a true biological meaning. Both our predictions and this study hypothesized that whenever herbivores have a strong phylogenetic structure in their diets, plant phylogenetic diversity should be the main driver of herbivore richness.

Among potential explanations for this result, the first possibility is that our phylogeny is only resolved to the genus level and that many specialized herbivore species in our study system (e.g. in the Tephritidae and Agromyzidae) feed on congeneric or cotribal plants, whereas few are truly monophages (Prado *et al.* 2002, T. M. Lewinsohn et al., unpublished)

However, even within genera or tribes, specialized diets may be restricted to certain rather than all species. Plant richness would be a better predictor of herbivore richness because the phylogeny would not be resolved in sufficient scale to capture the phylogenetic patterns of plant use within genera; moreover, some remaining polytomies encompass large and frequent genera and species-groups.

Almeida-Neto et al. (2011) showed that the relationship between plant and herbivore richness is dependent on the degree of specialization of herbivores, by analyzing relationships separately for monophages, oligophages and polyphages. Another approach that may prove effective is to include the overall specialization in each community as a further predictor of herbivore richness with an analytical framework already developed by us (Chapter 2 of this thesis). In a similar manner to the decomposition of herbivore richness between specialization and plant richness components at the species level proposed by Lewinsohn & Roslin (2008), this decomposition at the phylogenetic level should also be feasilbe. This would allow to test effectively whether differences in phylogenetic specialization reduce the power of plant phylogenetic diversity to determine herbivore richness in local communities.

ACKNOWLEDGEMENTS

We thank many colleagues, especially Paulo I. Prado, Mario Almeida-Neto, Adriana Almeida, Umberto Kubota and Carlos Fonseca, and students for help with field work, rearing and sorting insects, and many specialists for help with plant and insect identification. Field work was supported by FAPESP grants to TML (94/02837-2 and Biota/Fapesp 98/05085–2).

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SP	Mogi-Guaçu	Cerrado	22°15.78'	47°16.21'
MG	Ouro Branco	Espinhaço	20°30.27'	43°39.2'
SP	Pedregulho	Cerrado	20°12.05'	47°22.8'
SP	Passa Quatro	Mantiqueira	22°24.9'	45°3.13'
SC	Sul Litoral	Sul	29°24.55'	50°0.29'
SP	Santa Rita do Passa Quatro	Cerrado	20°30.39'	46°18.68'
MG	Visconde de Mauá	Mantiqueira	22°17.04'	44°31.83'

Table 2: Model selection results of the performance of Asteraceae richness, phylogenetic diversity (PD) and mean pair-wise phylogenetic distance (MPD) in explaining the richness of insects endophagous on Asteraceae floweheads in 26 localities in Brazil. The results are shown for models for the richness of all herbivores and for three families separately: Tephritidae, Agromyzidae and Tortricidae.

		All families			Tephritidae			Agromyzidae			Tortricidae		
Model	df	ΔAICc	weight	R^2	ΔAICc	weight	R^2	ΔAICc	weight	R^2	ΔAICc	weight	R^2
Plant Richness	3	0.0	1	0.91	0.0	0.97	0.83	0.0	0.8	0.81	8.1	0.02	0.14
Plant PD	3	20.9	<0.001	0.80	7.0	0.03	0.78	2.7	0.2	0.79	11.4	0.003	0.02
Plant MPD	3	61.9	<0.001	0.01	45.1	<0.001	0.04	40.9	<0.001	0.07	0.0	0.98	0.37

Fig. 1: Map of the 26 localities sampled in this study.

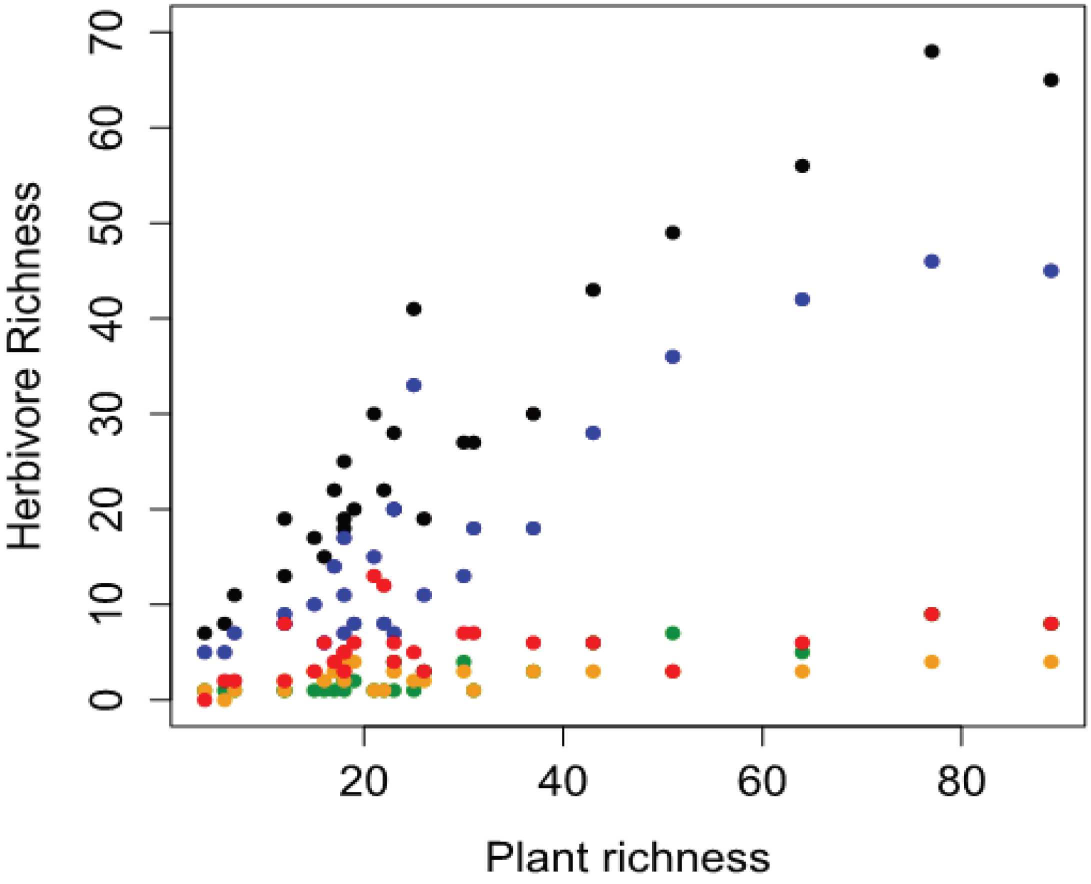

Fig. 2: Relationship between the richness of endophagous insects of Asteraceae flowerheads and the richness of Asteraceae species in 26 localities in Brazil. Black dots represent richness of all insect species, blue dots is richness of Tephritidae species, green for Agromyzidae, red for Tortricidae and orange for Pterophoridae.

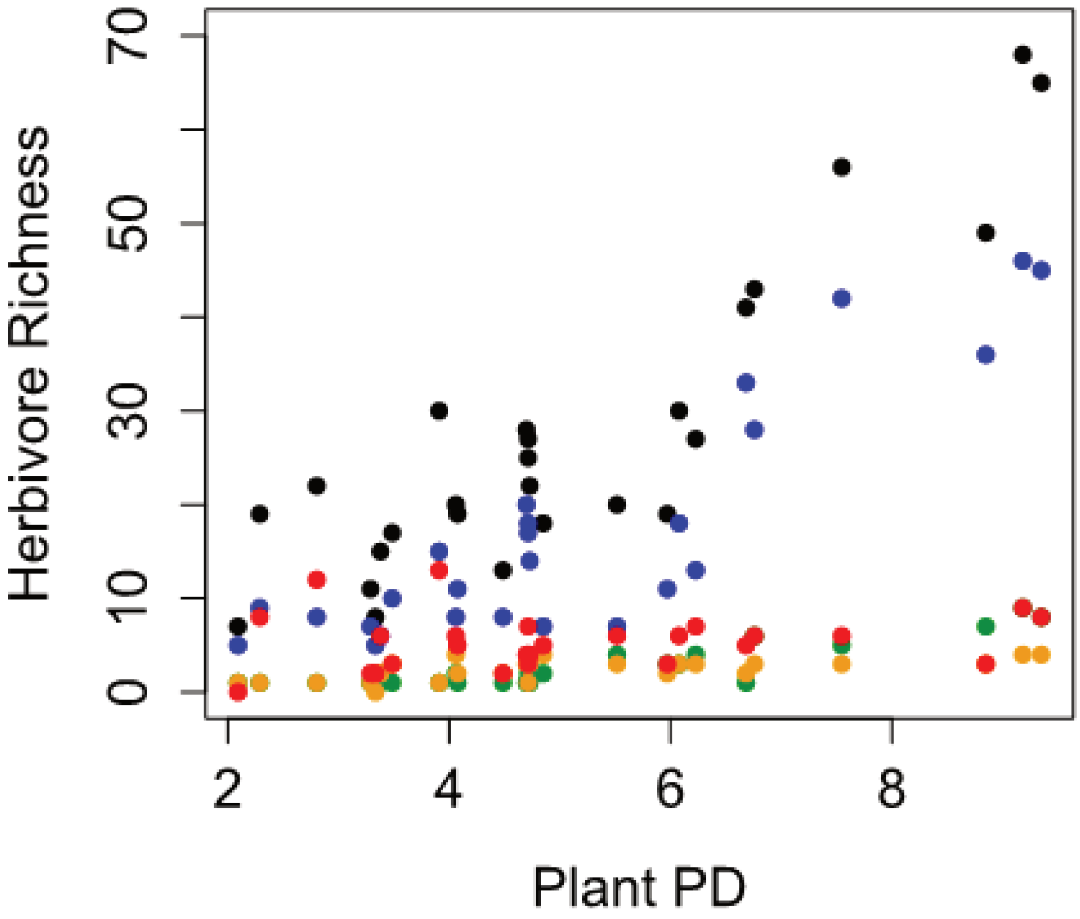

Fig. 3: Relationship between the richness of endophagous insects of Asteraceae flowerheads and the phylogenetic diversity (PD) of Asteraceae species in 26 localities in Brazil. Color indicates species richness respectively of all insect species (black), Tephritidae (blue), Agromyzidae (green), Tortricidae (red), and Pterophoridae (orange).

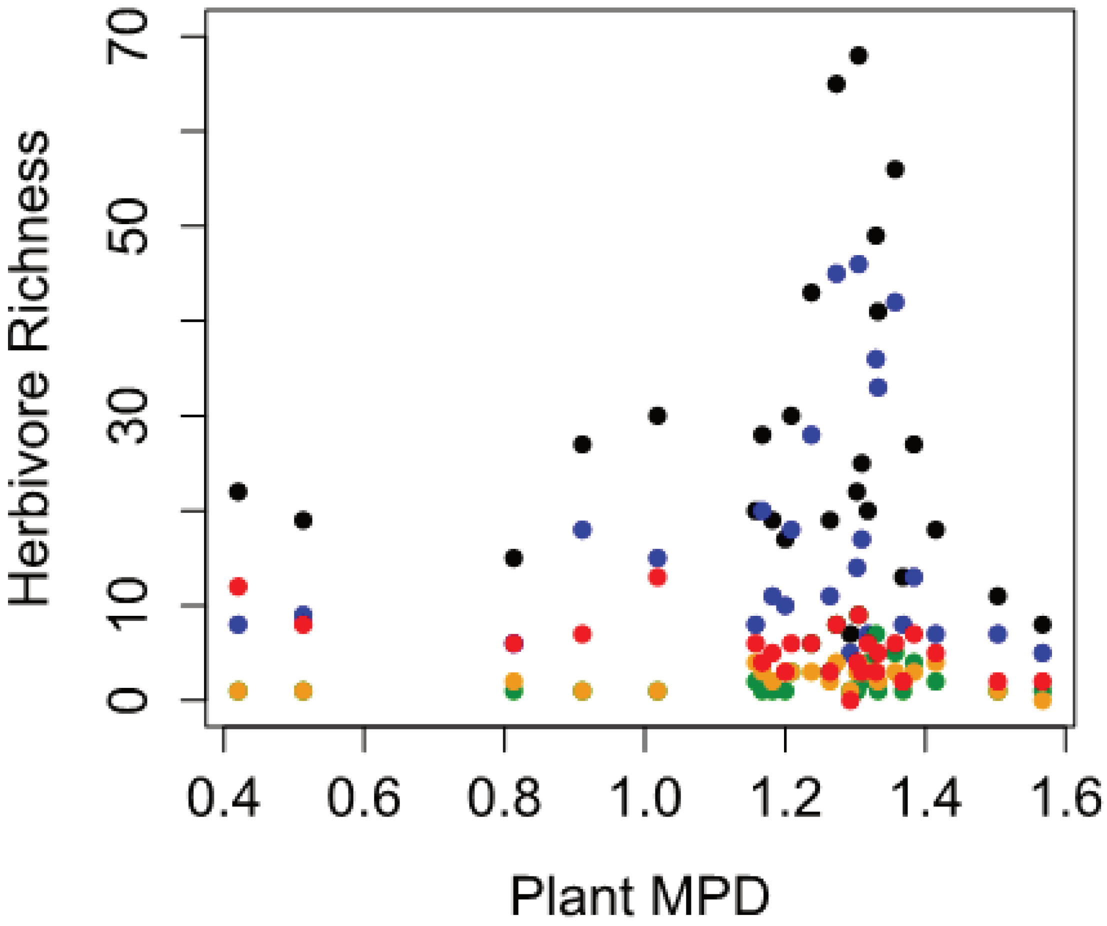

Fig. 4: Relationship between the richness of endophagous insects of Asteraceae flowerheads and the mean pairwise phylogenetic distance (MPD) of Asteraceae species in 26 localities in Brazil. Color indicates species richness respectively of all insect species (black), Tephritidae (blue), Agromyzidae (green), Tortricidae (red), and Pterophoridae (orange).

Conclusões

Nesta tese, buscamos reconciliar diferentes formas de conceituar a especialização ecológica em um único panorama teórico. Essa reformulação consiste em incorporar a não-independência de recursos e sua disponibilidade em um conceito integrado de especialização. A não-independência é incorporada na forma de parentesco filogenético ou similaridade fenotípica, quando os recursos são espécies, e similaridade ambiental, quando os recursos são habitat. Um determinado conjunto de recursos com alta similaridade demanda o mesmo tipo de adaptações para permitir seu uso, enquanto recursos muito dissimilares demandam diferentes adaptações e alta generalidade no uso e seleção de recursos. É importante ressaltar que essa similaridade é, ao menos em parte, independente do número de recursos utilizados. Esse é o principal avanço em se considerar os recursos de forma hierárquica, pois assim os mecanismos de seleção e adaptações necessárias ao uso de recursos podem ser investigados diretamente.

A disponibilidade é incorporada nessa conceituação a partir de modelos nulos, que abrangem a abundância de recursos disponíveis e seu padrão de co-ocorrência com os consumidores. Assim, é possível detectar se o padrão de similaridade de recursos observado para cada espécie se desvia do esperado pelo acaso. Consequentemente, pode-se diferenciar se restrições na disparidade de recursos utilizados são causadas por um mecanismo intrínseco ao consumidor, como seleção ativa de recursos, ou se resultam de contingências, como baixa abundância de determinados itens ou a ocorrência disjunta do consumidor e desses recursos .

Entre as principais limitações para uma ampla aplicação desse conceito para estudos de especialização está a dificuldade em obter toda a informação necessária. Para medir a especialização de uma espécie com relação a dieta ou uso de habitat, tanto o seu padrão de uso de recursos deve ser investigado em diferentes populações em toda a sua área de distribuição, como a abundância dos recursos em cada uma dessas populações deve ser mensurada; além disto, há necessidade de uma medida de similaridade dos recursos, com base em uma filogenia ou uma caracterização fenotípica/ambiental. Poucos estudos possuem toda essa informação de forma robusta e para um número razoável de espécies de consumidor. Ao mesmo tempo em que isso representa uma limitação para a aplicação desse conceito, podemos concluir que, sendo tais fatores importantes para a definição do que se entende por especialização, há muito trabalho de obtenção de dados primários por fazer, e uma grande possibilidade de que nossa compreensão da especialização de sistemas tróficos mude com a incorporação desses fatores.

Ao expandir esse conceito de especialização para comunidades, a principal contribuição deste trabalho é fornecer uma forma de repartir a especialização entre espécies e comunidades, de forma bidimensional, levando em consideração tanto diferenças em especialização de uma mesma espécie em diferentes comunidades (especialização/generalização local) como diferenças entre espécies (efeitos da composição). Um próximo passo possível nessa abordagem é relacionar a partição dos padrões de especialização com a partição da diversidade de organismos de diferentes níveis tróficos que interagem em diferentes comunidades. Tal abordagem poderá contribuir para estudos que investigam mecanismos causais dos padrões de riqueza e diversidade em comunidades, já que especialização é um dos facilitadores potenciais da coexistência de espécies que utilizam determinados conjuntos de recursos. Portanto uma abordagem que integre padrões de diversidade, especialização e diferenças de composição poderá avançar no esclarecimento do papel desses fatores para a estruturação de comunidades.

Finalmente, a abordagem proposta nesta tese permite desvendar parte dos processos que determinam a amplitude de nicho de espécies e comunidades, separando a seleção ativa de recursos do uso indiscriminado, além de separar restrição (uso de recursos similares) de dispersão (uso de recursos dissimilares) nessa seleção de recursos. No entanto, um passo adicional consistirá na investigação dos mecanismos geradores dessa seleção. Nesse sentido, é possível antever como avanço natural, a exploração de modelos mecanísticos de construção de comunidades. Esses modelos deverão incluir espécies de consumidor com diferentes tipos de seleção de recurso combinados com comunidades de recursos com diferentes padrões de similaridade, abundância e distribuição geográfica. Isso permitirá que os diferentes padrões de partição de riqueza e especialização de consumidores sejam relacionados de forma robusta a diferentes mecanismos de montagem de comunidades.