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From visit to emergence: Interactions between mycophagous Drosophilidae (Insecta, Diptera) and macroscopic fungi (Basidiomycota) and their patterns in ecological networks

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ABSTRACT

Ecological interactions are diverse, variable across space and time and not always well understood. The use of interaction network analysis has become a tool that promotes a deeper understanding on ecological and evolutionary processes. The interaction between insects and fungi is an interesting research model, helping to understand colonization dynamics and species specialization in spatially aggregated and ephemeral resources. Here, we describe the interactions between Drosophilidae species and the fungal basidiocarps in a subtropical forest in Brazil. Flies were collected when were visiting basidiocarps and then the basidiocarps themselves were also collected to obtain the emerging flies whose larvae fed on the fungi. We observed 31 species of drosophilids interacting with basidiocarps of 23 fungi species. An ecological network analysis was performed for the drosophilids breeding on basidiocarps and for those visiting them as adults. We found a specialized breeding network, with stronger interactions involving *Hirtodrosophila* and *Auricularia* and *Zygothrica bilineata* and a *Marasmius* species. Our results indicate the generalist habit of most *Zygothrica* species. The visitation network was highly specialized. Despite being well represented in the sampling, most *Zygothrica* species did not emerge from any fungal species. This study advances the knowledge on patterns of Drosophilid-fungi interactions and provides insights into their drivers.

Introduction

Ecological interactions are diverse and dynamically manifest in nature, engendering the biological diversity observed in the present day (Pereira et al., 2014). These interactions, which can be either intra- or interspecific, invariably exert an influence on fitness components of the involved organisms (Chamberlain et al., 2014). A paradigmatic example is the mutualism between plants and animals, which has garnered considerable attention due to its ecological significance in sustaining biological diversity and bolstering vegetation restoration. This is primarily because a myriad of animals are instrumental in pollination and seed dispersal (Bascompte and Jordano, 2007; Forup et al., 2008; Ibanez, 2012; Losapio et al., 2015). Consequently, the employment of

interaction networks to study such interactions has emerged as an invaluable tool, furnishing insights into ecological and evolutionary processes, life histories of organisms, and ecosystem functioning (Lewinsohn et al., 2006). Networks may be depicted as graphs, where species are represented by nodes or vertices, and interactions are represented by edges or links (Lewinsohn et al., 2006). Nonetheless, organisms take part in a broad array of relationships in the environment, and the intricacies of some of these interactions, such as insect-fungi, remain elusive.

Basidiomycota mushrooms, or basidiocarps, constitute a suitable model for examining ecological interactions owing to their amenability to sampling, spatial and temporal discreteness, and the diverse array of toxic chemicals exhibited by different taxa, which renders them as coveted hosts for insects (Hanski, 1989; Courtney et al., 1990).

Typically, host fungi have been perceived as trophic resources, with mycelia being foraged upon by both adult insects and larvae. Additionally, insects may promote the dispersal of fungal propagules such as spores, hyphae, or yeast cells, which is advantageous for the fungus (Tuno, 1999; Birkemoe et al., 2018).

From the insects' perspective, various studies have underscored the importance of ecological partitioning (Takahashi et al., 2005), specialization (Yamashita et al., 2015; Valer et al., 2016; Jacobsen et al., 2018; Lunde et al., 2023), intra- and interspecific competition (Grimaldi and Jaenike, 1984; Grimaldi, 1985), aggregation of adults and larvae, spatiotemporal dynamics (Takahashi et al., 2005), and tolerance to toxic compounds (Spicer and Jaenike, 1996; Stump et al., 2011). Concerning interactions between insects and fungi, networks have been observed to exhibit high specialization. For instance, Yamashita et al. (2015) discovered that the structure of Coleoptera-fungus quantitative interaction networks in a tropical forest in Borneo was specialized and markedly influenced by the dominant fungus *Ganoderma*.

Drosophilidae, a family of insects, exhibits a pronounced ecological affinity with basidiocarps. This association appears to be evolutionarily conserved in certain lineages, including some *Drosophila* and the *Zygothrica* species (Courtney et al., 1990; Gautério et al., 2020; Zhang et al., 2021). Mycophagy likely emerged independently several times throughout the evolution of Drosophilidae, as a derivative of detritivorous habits (Throckmorton 1975). In fact, mycophagy evolved independently in at least two lineages: within the subgenus *Drosophila*, where certain lineages predominantly breed in decaying fungi; and within the *Zygothrica* genus group, which specializes in fresh fungi (Zhang et al., 2021). Consequently, the relationship with fungi transcends merely insects utilizing them as a food source, as Drosophilidae also exploit fungi for oviposition, larval breeding, and as arenas for sexual courtship (Grimaldi, 1987).

In this context, the present study aims to describe the patterns of interactions between drosophilids and basidiocarps of macroscopic fungi in a forest community in southern Brazil. We built two ecologically distinct interaction networks – a *visitation network*, which included all drosophilid species flying over fungi and using it for multiple purposes, and an *emergence network* which included all drosophilids emerging from fungi tissues. To elucidate patterns of resource use and specialization, we specifically used null models to test whether these networks presented specialized, modular and/or nested structure. Courtney et al. (1990) observed in their review on mycophagous Drosophilidae ecology that interactions between fungi and flies showed a low degree of specialization, with insects emerging from mushrooms of various fungal taxa. They attributed this lack of specialization to the uniform nutritional conditions and to the unpredictable nature of the utilized mushrooms. Despite most studies being conducted in temperate Northern Hemisphere areas, where mycophagous Drosophilidae and fungal diversity are lower than in the Neotropical Region, we initially expected to observe a similar pattern. We also described individual species' specialization in order to identify key resources (fungi) and fungivores.

Material and methods

Study area

The study was carried out in a Restinga forest patch, namely, Horto Botânico Irmão Teodoro Luís, which is a protected area encompassing approximately 23 hectares of forest (31°48'54"S; 52°25'48"W), situated in the municipality of Capão do Leão, Rio Grande do Sul, in southern Brazil. This forest patch is inserted in the Pampa Biome, in close proximity to the Atlantic coast, and is characterized by vegetation that is influenced by the Submontane Seasonal Semideciduous Forest (Waechter, 1985).

Climate is categorized as Mesothermal Mild Superhumid, with rainfall evenly distributed throughout the year. Climatological data from the Pelotas Agroclimatological Station (8.7 kilometers distant from the sampling site) for the interval spanning 1971 to 2000 indicate mean annual temperature of 17.8°C, with mean maximum and minimum temperatures of 28.2°C and 8.6°C, respectively. The annual precipitation sums 1,367 mm, distributed across approximately 120 days of rainfall. The annual mean relative air humidity averaged 80%, with frequent occurrences of fog. The study site is surrounded by croplands, swamps, and naturally dry or seasonally flooded grasslands.

Sampling

Surveys for basidiocarps, which were associated with soil, plant roots, leaf litter, or decaying wood, were conducted along a 200-m transect within the forest patch, extending up to 10 meters on either side. Sampling was undertaken between 9:00 a.m. and 12:00 p.m. on a monthly basis from February to May 2011 and in February, April, and June 2013. This period is the most suitable for fungi reproduction in the area which are, therefore, more locally abundant and conspicuous (personal observation). Each sampling session spanned approximately three hours, summing up 21 hours of sampling effort throughout the study period. For each basidiocarp detected, we collected insects observed flying over or landed on its surface using entomological nets or aspirators, and subsequently preserved in 70º GL ethanol. Each basidiocarp was carefully cut off using a penknife, photographed for identification purposes, and transported to the laboratory into plastic bags. In laboratory, the basidiocarps were weighed using a precision scale before placed into glass containers containing autoclaved sand which was sealed with fabric. All basidiocarps belonging to the same species and originated from the same location were designated as a single sampling unit and placed into the same container. The basidiocarps were stored in a chamber and maintained at a temperature of 25 ± 1°C for 4 to 5 weeks. During this period, the emergence of insects was monitored at intervals of 1 to 2 days. Emerging insects were aspirated and preserved in 70% ethanol. To prevent dehydration of the basidiocarps within the climatized chamber, water was periodically sprayed into the containers. All emerging insects were collected but in the present study we focused on Drosophilidae individuals.

Identification of biological material

All drosophilids collected were identified based on external morphology and analysis of male genitalia, and entailed comparison with taxonomic descriptions available in specialized literature (Burla, 1956; Grimaldi, 1987, 1990, 2018; Vilela and Bächli, 2004, 2007; Junges et al., 2019). Female drosophilids were identified solely through external morphological analysis. For cryptic species (i.e., those which females cannot be identified by external morphology), female individuals present in a sample were assigned to species following the proportion of males of each species within the sample, assuming a 1:1 sex ratio.

The preparation of male genitalia followed methods by Wheeler and Kambysellis (1966), with modifications by Kaneshiro (1969). The taxonomic identification followed classifications proposed by Frota-Pessoa (1945), Burla (1956), Wheeler and Takada (1971), Grimaldi (1987, 1990, 2018), Vilela and Bächli (2004, 2007), and Junges et al. (2016, 2019).

Identification of fungi species was based on photographs of fresh basidiocarps taken in the field and consulting specialized literature (Lincoff, 1981, 2010; Putzke and Putzke, 1998; Polese, 2005; Laessoe and Lincoff, 2010). We included only fungal samples that were not in an advanced stage of decomposition, making them amenable to identification, and avoided including saprophagous Drosophilidae species.

Data analysis

To describe interaction networks, we constructed two quantitative interaction matrices. In these matrices, each row represents a species of drosophilid denoted by *i*, and each column represents a species of fungus denoted by *j*. The intersection of a cell a_{ij} in the matrix denotes the intensity of interactions between the drosophilid species *i* and the fungus species *j*. To calculate interaction intensity, we multiplied the number of drosophilids associated with each fungal species (the absolute abundance of each drosophilid species *i* in fungus *j*, n_{ij}) by the number of observations of this fly species associated with the fungus species relative to the total number of observations of the fungus species (its relative frequency, f_{ij}). To address potential overestimation of interactions due to differences in fungi availability or reproductive strategies among drosophilid species, and to minimize the influence of outliers, we standardized the interactions. This standardization involved dividing the product of n_{ij} and f_{ij} by the mass (*m*) of the fungi sampled in grams. To avoid fractional numbers, we multiply this value by 100. The final equation to calculate the interaction intensity is $(n_{ij}*f_{ij}*100)/m$. This ensured that interaction intensity accounted for the varying reproductive behaviors of drosophilid species and reduced the impact of outliers.

Biological interactions were categorized into:

[1] **Visitation -** This category comprises individuals observed flying over or landed on basidiocarps at the time of collection, which we refer hereafter as 'visits'. Drosophilid species were classified into those that [a] oviposit on fungi (as evidenced by emergence from collected basidiocarps; see below), and [b] do not oviposit, as they were not observed to emerge from the basidiocarps, implying alternative use of the fungi not related to breeding, such as foraging, courtship, or mating sites.

[2] **Emergence -** This category encompasses interactions wherein basidiocarps are recognized by female drosophilids as suitable substrates for oviposition, and where the larval stages complete their development within the basidiocarps, consuming either the fungus or associated organisms such as bacteria or yeasts.

Quantitative network analysis was executed utilizing R Program version 4.1.3 (R Core Team, 2023), with packages *bipartite* version 2.16 (Dormann et al., 2008, 2009; Dormann, 2011). We used ggplot2 package version 3.3.5 (Wickham, 2016) for graphical representation.

For each network we calculated: *connectance*, defined as the ratio of observed links to the total possible links given the set of observed species; *Complementary specialization* using the *H2'* metric, which quantifies the divergence of observed interactions from expected interactions under the assumption that resource use follows their availability, where availability is given by the matrix marginal totals. Specialization values span the range of 0 (lowest specialization possible) to 1 (highest specialization possible). *Nestedness*, which was quantified with the WNODF metric which estimate the non-overlap and decreasing fill of quantitative matrices (Almeida-Neto and Ulrich, 2011). Nestedness occurs when the less connected species (specialists) interact with subsets of the resources used by the most connected species (generalists). WNODF varies from 0 to 100 (maximum nestedness possible). *Modularity* was quantified using the metric *Q* and the optimization algorithm DIRTLPAwb+ (Beckett, 2016). A modular network occurs when subsets of species interact more among themselves than with other species in the network, forming modules. Modularity range from 0 to 1 (maximum modularity possible). We used the *r2dtable* null model to assess the statistical significance of specialization, nestedness and modularity. This null model reshuffles interactions keeping dimensions (number of species in each trophic level) and marginal total as the observed matrix. We generated 1000 random matrices, calculated each of these three metrics for each of them and then calculate the 95% confidence interval. We considered a network structure to be statistically significant when the observed values felt above the 95% confidence interval generated with the null model. We used a second (more conservative) null model, *vaznull* which also constrain network connectance. Results were qualitatively similar to the ones obtained with *r2dtable* indicating that the network structure detected is robust to null model choice.

We also calculated species-level specialization: *d'* index, derived from the *Kullback-Leibler* distance reflects the deviation of a species from a random distribution of available interaction partners. Similar to *H2'*, *d'* values also range from 0 (most generalist possible) to 1 (perfect specialist).

To estimate the sufficiency of our sampling in detecting all links (a link is a pair of consumer and resource species), we created individual-based rarefaction curves following Vizentin-Bugoni et al. (2016) using the *iNEXT* version 2.0.20 (Chao et al., 2014; Hsieh et al., 2016).

Results

We obtained a total of 149 fungal samples, out of which 118 were identifiable. Among the identifiable samples, 57 exhibited had emergence of Drosophilidae. We recognized 45 species of fungi, with 23 of them having emergence of Drosophilidae (Table 1). Additionally, our samples included 31 drosophilid species from the genera *Drosophila*, *Hirtodrosophila*, *Leucophenga*, *Mycodrosophila*, and *Zygothrica*. Out of these, 26 species comprising 3,797 individuals, emerged from 57 fungal samples, while 16 species, consisting of 277 individuals, were collected visiting 25 basidiocarp samples in the field (Fig. 1, Table S1 - Supplementary Material).
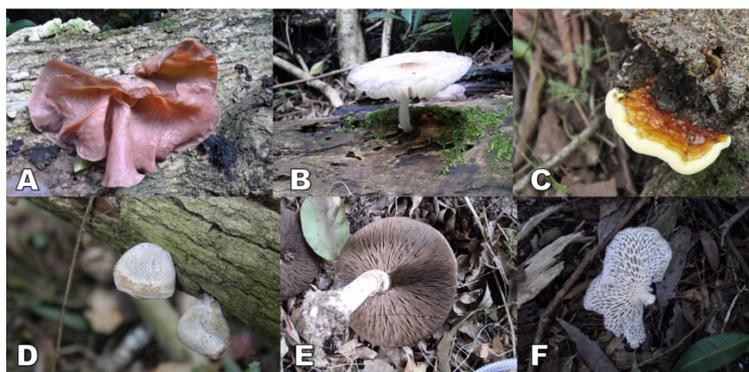

Figure 1 Images of some basidiocarps of fungi sampled. A) *Auricularia auricula-judae*. B) *Marasmius* sp. C) *Ganoderma* sp. D) *Auricularia polytricha*. E) *Agaricus* sp.4. F) *Polyporus* sp.1.

Table 1

Fungal species, number of samples collected and number of samples colonized by drosophilids in seven samplings carried out in restinga forest in southern Brazil in the years 2011 and 2013.

Species of fungi	Samples	
	Total collected	Colonized
Agaricus sp.	6	1
Agaricus sp.1	2	0
Agaricus sp.2	1	1
Agaricus sp.3	1	0
Agaricus sp.4	5	5
Agaricus sp.5	1	0
Agaricus sp.6	3	2
Agaricus sp.7	1	1
Auricula mesenterica	1	0
Auricularia auricula-judae	12	11
Auricularia polytricha	6	4
Cantharellus sp.	2	0
Clitocybe sp.	1	0
Coprinus sp.	1	0
Cortinarius sp.	1	0
Ganoderma lucidum	1	0
Ganoderma sp.2	1	1
Geastrum sp.1	1	0
Geastrum sp.2	1	0
Geastrum sp.3	1	1
Lepiota sp.	4	2
Lepiota sp.1	7	3
Lepiota sp.2	2	0
Lepiota sp.3	1	0
Marasmius sp.	3	2
Marasmius sp.2	4	2
Marasmius sp.3	2	0
Marasmius sp.4	2	1
Marasmius sp.5	2	1
Marasmius sp.6	2	0
Marasmius sp.7	7	4
Marasmius sp.8	2	1
Marasmius sp.9	3	0
Marasmius sp.10	1	0
Marasmius sp.11	1	0
Melanoleuca sp.	1	1
Panus sp.	1	0
Pleurotus sp.	1	0
Pleurotus sp.1	1	0
Pleurotus sp.2	2	2
Polyporus sp.1	8	7
Pycnoporus sp.1	2	1
Trametes sp.	3	0
Trametes sp.2	1	1
Tricholoma sp.	6	2
Number of samples	**118**	**57**

Table 2

Species-level specialization values (d') for drosophilid species and fungi emergence network in southern Brazil

| Drosophilidae		Basidiomycota	
Z. bilineata	0.90	*A. auricula-judae*	0.90
H. crioula	0.81	*Marasmius* sp.4	0.88
D. griseolineata	0.81	*A.* polytricha	0.82
D. ornatifrons	0.81	*Agaricus* sp.7	0.77
Leucophenga sp.2	0.75	*Agaricus* sp.2	0.69
H. levigata	0.69	*Tramates* sp.2	0.70
Leucophenga sp.4	0.68	*Lepiota* sp.	0.65
D. willistoni	0.67	*Ganoderma* sp.2	0.64
M. projectans	0.66	*Pycnoporus* sp.1	0.63
D. paraguayensis	0.66	*Agararicus* sp	0.61
H. subflavohalterata aff.2	0.64	*Agaricus* sp.4	0.61
H. mendeli	0.64	*Marasmius* sp.7	0.60
H. subflavohalterata aff.1	0.61	*Marasmius* sp.2	0.60
Drosophila sp Z2	0.54	*Melanoleuca* sp.	0.59
Leucophenga sp.3	0.53	*Geastrum* sp.3	0.57
L. maculosa cf.	0.52	*Marasmius* sp.	0.51
Z. ptilialis	0.51	*Agaricus* sp.6	0.48
Leucophenga sp.5	0.51	*Marasmius* sp.5	0.48
H. morgani	0.49	*Lepiota* sp.1	0.36
H. subflavohalterata	0.47	*Polyporus* sp.1	0.30
D. nappae	0.41	*Tricholoma* sp.	0.29
Leucophenga sp.1	0.40	*Marasmius* sp.8	0.22
H. pleuroestrigata	0.36	*Pleurotus* sp.2	0.21
D. melanogaster	0.26		
Hirtodrosophila sp.2	0.06		
Hirtodrosophila sp.1	0.06		
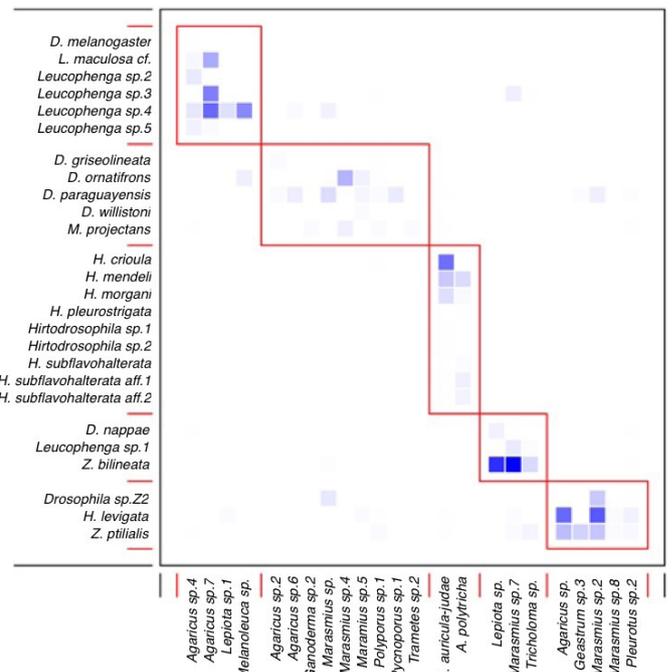
Figure 2 Interaction network between fungal and drosophilid species generated from emergence data in southern Brazil, showing each module (red rectangles). Shades of blue indicate interaction intensity.

In the emergence network reflecting fungi used as breeding sites (Fig. 2), strong interactions were observed between *Hirtodrosophila crioula* Junges, Robe and Gottschalk, 2019 and *Auricularia auricula-judae* (Bull.) J. Schröt., as well as between *Zygothrica bilineata* (Williston, 1896) and *Marasmius* sp.7 and *Lepiota* sp. When considering species-level specialization, *Z. bilineata* (d' = 0.90) and *A. auricula-judae* (d' = 0.90) were the most specialized species of Drosophilidae and Basidiomycota, respectively (Table 2). Furthermore, the emergence network had low connectance (0.14), and a high complementary specialization ($H2'$=0.72).

Table 3
Metrics calculated to describe the structure of the visitation and emergence drosophilid-fungi interaction networks in a *restinga* forest community in Brazil. In parenthesis are shown 95% confidence intervals of the metrics generated by both null models used. Bold indicates metrics whose observed value falls above the null model expectation and, therefore, are considered statistically significant.

Network type	Connectance	Specialization (H_2')	Nestedness (WNODF)	Modularity (Q)	Number of modules	Null model
Visitation	0.19	**0.65**	18.99	**0.58**	5	r2dtable
		(0.12 - 0.22)	(23.81 - 42.50)[ns]	**(0.18 - 0.27)**		
Emergence	0.14	**0.74**	10.92	**0.72**	5	r2dtable
		(0.02 - 0.03)	(66.75 - 75.02)[ns]	**(0.05 - 0.07)**		
Visitation	0.19	**0.65**	18.99	**0.58**	5	vaznull
		(0.30 - 0.63)	(11.80 - 27.52)[ns]	**(0.25 - 0.51)**		
Emergence	0.14	**0.74**	10.92	**0.72**	5	vaznull
		(0.20 - 0.33)	(27.27 - 37.36)[ns]	**(0.16 - 0.27)**		

Table 4
Species-level specialization values (*d'*) for drosophilid species and fungi visitation network in southern Brazil.

Drosophilidae		Basidiomycete	
D. paraguayensis	1.00	*Marasmius* sp.	1.00
Drosophila sp. Z2	0.73	*Marasmius* sp.2	0.88
Z. bilineata	0.63	*A. auricula-judae*	0.50
Z. ptilialis	0.46	*Marasmius* sp. 7	0.49
Z. Z002	0.44	*Marasmius* sp.5	0.48
D. nappae	0.42	*Tricholoma* sp.	0.40
D. willistoni	0.42	*Polyporus* sp.1	0.36
H. levigata	0.39	*Agaricus* sp.	0.23
H. crioula	0.33	*Pleurotus* sp.2	0.00
H. mendeli	0.32		
Z. dispar	0.28		
Z. prodispar	0.28		
H. morgani	0.26		
Z. orbitalis	0.04		
H. subflavohalterata aff.1	0.04		
Z. vittimaculosa	0.04		

Figure 3 Interactions network between fungal and drosophilid species generated from visitation data in southern Brazil, showing each module (red rectangles). Shades of blue indicate interaction intensity.

Network structure was not nested (WNODF= 10.92) but was modular (Q=0.72), presenting five modules (Table 3; Fig. 2). The third module (Fig. 1) includes a strong interaction between *A. auricula-judae* and *A. polytricha* (Mont.) Sacc. and *Hirtodrosophila* species, with the interaction between *H. crioula* and *A. auricula-judae* being the strongest within this module.

The first module (Fig. 1) featured a somewhat strong interaction between the fungus *Agaricus* sp.7 and two undescribed species of the *Leucophenga* genus. The other members of module, which included fungi from the *Lepiota* and *Melanoleuca* genera as well as flies from the *Drosophila* genus, engaged in weaker interactions.

In the visitation network, eleven Drosophilidae species interacted exclusively with a single fungus species; however, species specialization values (*d'*) were generally low, with the notable exception of *D. paraguayensis* Duda, 1927 and *Marasmius* sp. (Table 4). Notably, nine species were collected in association with *A. auricula-judae*, five of which exclusively interacted with this fungus.

Additionally, *Zygothrica dispar* Wiedemann, 1830 and *Z. prodispar* Duda, 1925 were observed exclusively visiting *Marasmius* sp.7. On the other hand, the genus *Drosophila* exhibited only exclusive interactions: *D. nappae* Vilela, Valente and Basso-da-Silva, 2004 and *D. willistoni* Sturtevant, 1916 interacted with *Polyporus* sp.1, *Drosophila* sp.Z2 exclusively with *Marasmius* sp.2, and *D. paraguayensis* with *Marasmius* sp. This latter interaction was the most specialized within this network.

Among the species observed visiting the basidiocarps, *Z. dispar*, *Z. prodispar*, *Z. orbitalis* Sturtevant, 1916, *Z. vittimaculosa* Burla, 1956,
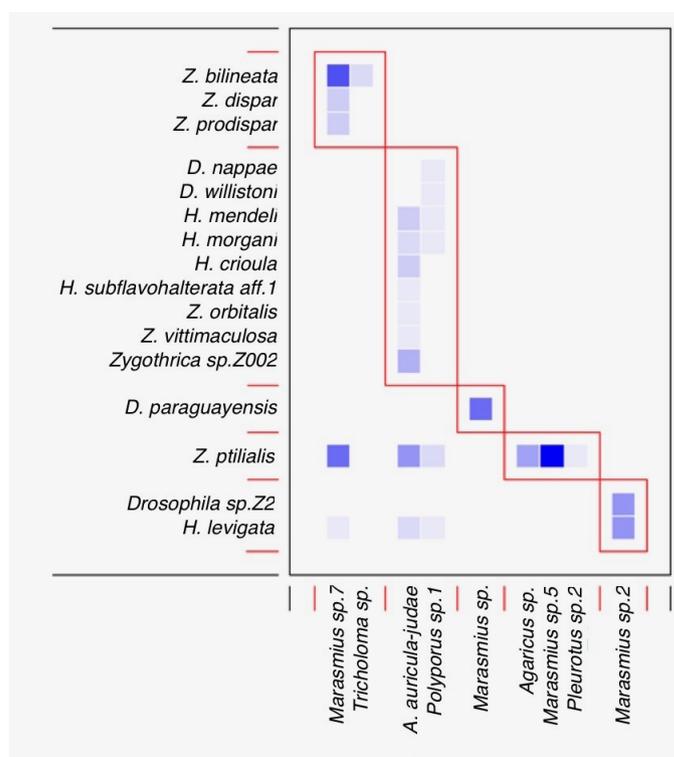
Z. ptilialis, and *Zygothrica* Z002 were not recorded emerging from any fungal species. Notably, all interactions of these species were exclusively with a single fungus species.

The visitation network showed low connectance (0.19) and high specialization (*H2'* = 0.65). It was also not nested (*NODF* = 18.99) but it was modular (Q=0.58), having five modules (Fig. 3; Table 3). The strongest interactions occur in the fourth module between *Z. ptilialis* and *Marasmius* sp.5 and in first module between *Z. bilineata* and the fungus *Marasmius* sp.7, repeating the configuration seen in emergence modularity. Differently of the emergence network, *Z. bilineata* interacts weakly with *Tricholoma* sp., represented in the first module. The composition of modules in the visitation network was totally different from that obtained in the emergence network, with the exception of the interaction between *Z. bilineata* and *Marasmius* sp.7, which stands out in both. Analysis of sampling sufficiency indicates that most links (pairwise interactions) in the community were recorded for both interaction types as indicated by the asymptotic trend of the rarefaction curves (Fig. 4).
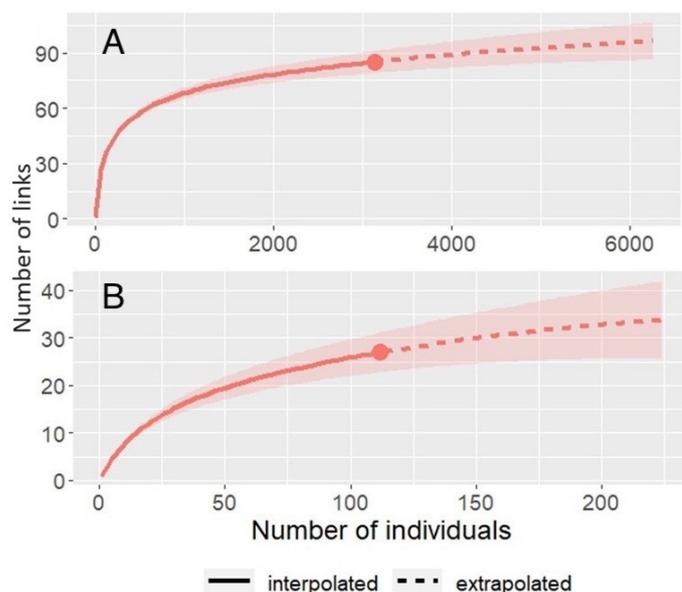
Figure 4 Individual-based rarefaction curves estimating sampling sufficiency of interactions (i.e., links between fungal and drosophilid species). A) Data on the emergence of flies (breeding sites). B) Visitation data of basidiocarps by flies.

Discussion

Drosophilidae family evolved and specialized in utilizing a variety of resources, including fruits (Atkinson and Shorrocks, 1977), flowers (Schmitz and Valente, 2019; Cordeiro et al., 2020), cacti (Manfrin and Sene, 2006), and even bat guano (Tosi et al., 1990). Among these, fungi are noteworthy resources due to their often high concentration of toxic chemicals (Courtney et al., 1990). Moreover, Hanski (1989) and Birkemoe et al. (2018) highlight that, with a few well-known exceptions of insects specializing on hard fungi (such as certain species of beetles specializing in polypore fungi), the level of specialization between fungi and insects is generally lower compared to that between plants and insects. However, our results indicate a high degree of specialization in interactions between drosophilids and fungi, consistent with the findings of Põldmaa et al. (2016) for fungus and gnat flies. Additionally, the modular and specialized network demonstrated for beetles associated with wood-decomposing fungi (Jacobsen et al., 2018) further supports this notion. It seems that not only the hardness of fungi (associated with greater persistence and predictability) may be linked to insect species specialization, but also other, yet unknown, factors.

Our study identified a large diversity of basidiomycete fungi, totaling 45 species, 23 of which showed some type of interaction with drosophilids at different developmental stages. We observed 26 fly species emerging from basidiocarps, indicating a close relationship between them and fungi, as all phases of the flies' development interact directly with fungi. Despite the wide array of available resources for colonization, we observed an almost exclusive interaction between *H. crioula* and the gelatinous fungus *A. auricula-judae*. However, the specialization value for the species (d') does not indicate maximal specialization ($d' = 0.30$), suggesting that network specialization was not determined by *H. crioula*'s strong interaction for *A. auricula-judae*. Similarly, other observed *Hirtodrosophila* species demonstrated higher occurrences with fungi of the *Auricularia* genus (*A. auricula-judae* and *A. polytricha*), as previously described in other studies (Valer et al., 2016; Grimaldi, 2018; Junges et al., 2019). The exception was *H. levigata* (Burla, 1956), which was the only species in the genus that showed no preference.

The strong interaction of *Hirtodrosophila* for *Auricularia*, coupled with *Z. bilineata*'s interaction with *Marasmius* sp.7, determined the specialization of the network, as the most frequent relationships involved these particular species. This is further emphasized by the network modularity, where the strongest interactions are observed between these species. However, the nature of ecological interactions changes significantly when considering only the visitation of fungi by drosophilids, as there are many singular interactions involving these insects. Since basidiocarps serve to various functions for drosophilids (such as feeding, oviposition, and courtship sites) (Courtney et al., 1990), our study found that not all species visiting fungi subsequently emerged from them; this is particularly evident in the *Zygothrica* genus. Of these, only *Z. ptilialis* and *Z. bilineata* emerged from the basidiocarps of the collected fungi. Being the more generalist species in the genus, they interact with six and two fungal species respectively, suggesting a preference for different substrates. However, they appear more selective regarding oviposition sites, indicating the need for specific characteristics for this process. Conversely, the other *Zygothrica* species that did not emerge demonstrated specialist relationships in our study, being observed with only one fungal species. This observation could be attributed to the challenges in standardizing adult collections. We only collected organisms that were visiting the fungi at the precise moment of sampling. Therefore, we might have missed those that had visited earlier or would visit later. Consequently, there is a temporal gap between the actual interactions and the moment of sampling, which may suggest that at the time of our collection, the species were using the basidiocarps for courtship, as documented by Grimaldi (1987), and would only lay eggs at a later stage, a detail that was not captured in our data.

Among the various resources available, *Hirtodrosophila*'s preference for *Auricularia* is notable. The *Auricularia* genus is characterized by ear-shaped gelatinous basidiocarps, which are rich in polysaccharides (Miao et al., 2020). Generally, the consistency of a fungus is linked to its persistence in the environment. Less consistent basidiocarps tend to be more ephemeral and, consequently, available for colonization for a shorter period (Jonsell and Nordlander, 2004; Graf et al., 2018). Moreover, the colonization of less consistent fungi may be associated with the larval development time. For instance, dipterans, which have a short development period, are predominant in such fungi (Hanski, 1989). The colonization of *Auricularia* by drosophilids has also been documented in several recent studies (Gottschalk et al., 2009; Valer et al., 2016; Junges et al., 2019; Santa-Brígida et al., 2019).

Our study observed that *D. paraguayensis* exhibited high specialization in the visiting network as it was only associated with *Marasmius* sp.. Interestingly, when examining the breeding sites, *D. paraguayensis* is deemed generalist, as it emerged from ten different species of fungi. Although it is associated with fungi (being part of the *immigrans-tripunctata* radiation), it can also be commonly found in trap samples with banana baits in the same locality (Mendes et al., 2017). This contrasts with species of the genus *Hirtodrosophila*, which are exclusively mycophagous and rely on fungi for both nourishment and reproduction (Courtney et al., 1990). *Hirtodrosophila*'s specialization was evident through the substantial sampling of *A. auricula-judae* in our study. In this regard, mycophily implies a high degree of specialization for organisms that have evolved and adapted to utilize this resource, considering that basidiocarps are transient structures reliant on specific environmental conditions, such as light, temperature, and nutrients (Sakamoto, 2018). Due to these specific traits, collecting adults visiting basidiocarps is less effective and challenging in representing the entire community, as suggested by our rarefaction curves.

Nonetheless, our findings contribute to the existing knowledge regarding mycophily in Drosophilidae by describing the interaction network structures, which had not been previously explored for this group.

Our study also observed the specialization of the network of drosophilids emerging from basidiocarps. However, expanding this information to visitation interactions remains challenging due to a methodological bias in adult collection, with gelatinous fungi being preferred by species of the genus *Hirtodrosophila*.

Conflicts of interest

The authors declare no conflicts of interest.

Author contribution statement

LBD, FBV and MSG Conceptualization; Data Curation. FBV and MSG Methodology; Insect identification. JVB Formal Analysis. EB Fungus identification. LBD, MSG and VLV Writing – Original Draft Preparation. VLV and MSG Funding acquisition. LBD, FBV, MSG, JVB, EB and VLV Writing – Review & Editing.

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Supplementary material

The following online material is available for this article:

Table S1 - SM1: Drosophilidae abundance table by fungus species.

Table S2 - SM2: Abbreviations of fungal species present in the abundance table (SM1).

This document has an erratum: https://doi.org/10.1590/1806-9665-RBENT-2023-0097er