

Spatial and temporal variation in host–parasitoid interactions: leafcutter ant hosts and their phorid parasitoids

LUCIANA ELIZALDE,^{1,*} RICHARD J. W. PATROCK,² R. HENRY L. DISNEY³ and PATRICIA J. FOLGARAIT⁴ ¹Laboratorio de Hormigas, Universidad Nacional de Quilmes, Quilmes, Argentina, ²Department of Zoology, University of Cambridge, Cambridge, U.K., ³Department of Biological and Health Sciences, Texas A&M University at Kingsville, Kingsville, Texas, U.S.A. and ⁴Laboratorio de Hormigas, Universidad Nacional de Quilmes, Quilmes, Argentina

Abstract. 1. Parasitoid–host interactions are important components of ecological communities. Although parasitoid–host interactions are strongly shaped by evolutionary history, the abundance of both the parasitoid and the host may have a role in determining the nature of the interaction once phylogenetic relationships are considered.

2. Leafcutter ants are hosts of phorid parasitoids and represent a well-defined and specialised module within a larger network of ant–symbiont interactions. A low specificity host taxa and a positive association between host abundance and parasitoid interaction frequency were expected due to the close phylogenetic relatedness of the hosts.

3. The interactions among all species of leafcutter ants and their parasitoids were quantified in two localities with different species richness. This study also characterised the spatial-temporal variability of these interactions, determined the patterns of parasitoid specificity and host selection, and tested for an association between host abundance and parasitoid interaction frequency.

4. Contrary to expectation, most parasitoid species were highly specialised and interaction frequency for parasitoid species was not related to host abundance. All host ant species were attacked by more than one phorid species. Some phorid species used more than one host species and showed preference for the same species over space and time, suggesting that there are physiological and/or behavioural restrictions on host use.

5. These results show that there is a tendency for specialisation even when hosts are highly similar in their ecology. From a biological control perspective, these parasitoids may be effective candidates, due to the high specificity of some species and little host-use variation through time.

Key words. *Acromyrmex*, *Atta*, ecological networks, electivity, host range, interaction strength.

Introduction

Parasitoid–host interactions are important components of ecological communities due to their high species richness and the key ecological function parasitoids play as regulators of

herbivore populations (Godfray, 1994; Hawkins & Sheehan, 1994). The close individual association between the parasitoid and the host, especially for endoparasitoids, suggests that parasitoid–host interactions are determined mainly by phylogenetic or physiological constraints (Ives & Godfray, 2006; Elias *et al.*, 2013; Hambäck *et al.*, 2013) rather than by neutral mechanisms. Under neutral mechanisms, stochastic factors, such as the distribution of abundance, are important in determining interaction structures (Rosindell *et al.*, 2011). However, the abundance of the parasitoid and the host may play a role in the structure

Correspondence: Luciana Elizalde, Laboratorio Ecotono, INIBIOMA, CONICET-UNComa, Pasaje Gutiérrez 1125, Bariloche 8400, Argentina. E-mail: luelizalde@gmail.com

*Current address: Laboratorio Ecotono, INIBIOMA, CONICET-UNComa, Argentina.

of the interaction once phylogenetic relationships are taken into account (Cagnolo *et al.*, 2011).

Food web tools have been increasingly applied for studying parasitoid–host interaction patterns (Memmott *et al.*, 1994; Memmott & Godfray, 1994; Rott & Godfray, 2000; Lewis *et al.*, 2002; Tylianakis *et al.*, 2007; Cagnolo *et al.*, 2011; Henri & van Veen, 2016). Classical measures of parasitoid–host interactions only consider who interacts with whom without including interaction frequencies. Quantitative parasitoid–host networks, on the other hand, incorporate information on the relative abundance of both parasitoids and hosts, as well as the frequency of interactions between them (Memmott & Godfray, 1994). The host range of a parasitoid species can be defined as the host species that the parasitoid frequently uses during development (Godfray, 1994; Shaw, 1994). Under this definition, it is critical to determine how often the parasitoid finds and oviposits into hosts of all potential host species. This allows us to differentiate between generalists (i.e. species where the frequency of interaction is proportional to host species abundance) and marginally specialised organisms or specialists (i.e. species that have a high interaction frequency with a small fraction of available hosts).

When studying parasitoid host range, host election or electivity by parasitoid species is an important ecological factor. Host election is a population measure of how prey items are utilised by a predator in relation to their availability in a given habitat or location (Lechowicz, 1982). Election is more exacting than the more commonly used terms of host selection, such as preference or discrimination, which relate to behaviours of the individual animal. Such behaviours may not be shared by all individuals in a population or, even if shared, they may not be expressed when preferred hosts are uncommon and otherwise difficult to find or capture. Given that parasitoid–host distributions are not uniform across landscapes, at a given location a host community is usually composed of species of different qualities. Thus, a parasitoid may only interact frequently with the preferred host taxa, after accounting for abundance differences. This is the reason why electivity is a preferred term when dealing with non-experimentally observed host use corrected for host abundance (Lechowicz, 1982; Singer, 2000). Meanwhile, more interactions with non-preferred hosts might be observed in communities without better-quality hosts (Fox & Morrow, 1981). In fact, studying these interactions in different communities can prove useful in discriminating the relative importance of phylogenetic versus ecological mechanisms that establish parasitoid host range (Shaw, 1994). Moreover, spatial variation in the relative abundance of species and their interactions are thought to lead to geographic mosaics of coevolution (Thompson, 2005), thus influencing the evolution of parasitoid host range. In addition, host specificity by parasitoids can have a geographic dimension, as specificity can also be manifested as the consistency in host use across a changing host landscape (Poulin *et al.*, 2011). Similar reasoning can be applied to temporal variation in interactions (Poisot *et al.*, 2015).

Interactions between phorid parasitoids that attack leafcutter ants have recently begun to be documented (for a review see Folgarait, 2013). To date these phorid species are known to utilise leafcutter ants during development and do not attack other dominant ant species in their habitat (Elizalde & Folgarait, 2011).

Thus, this system represents a well-defined module within a larger network of ant–symbiont interactions (Ivens *et al.*, 2016). In fact, all leafcutter ants belong to two sister genera from the same tribe (Attini; *Atta* and *Acromyrmex*; Wetterer *et al.*, 1998), reinforcing the function of this parasitoid–host system as a module in their communities. Upon maturity, females spend most of their lives interacting with their hosts, either searching for or attempting to oviposit in ant workers that are outside their nests. Host location is an important event in the life cycle of phorid parasitoids and is largely determined by the tasks performed by the ant hosts. The ant workers are likely to be involved in different tasks, such as foraging, performing nest maintenance or removing waste, and this influences the density and quality of the ants as hosts (Elizalde & Folgarait, 2012). Ants show highly aggressive behavioural defence mechanisms against these parasitoids, which can lead to parasitoid death (Elizalde & Folgarait, 2012). When the parasitoids manage to overcome these barriers and oviposit inside the ant, a larva hatches and feeds on the internal soft tissues of the ant, and the ant is killed only slightly ahead of parasitoid pupariation (Elizalde & Folgarait, 2011). However, the larvae develop only if they can overcome the internal defences of the hosts. Thus the capacity of the parasitoid to develop within an assumed preferred host is a key consideration in defining parasitoid–host interactions. To date most studies in this system have considered interactions between one or two focal host species at one locality rather than investigating all potential leafcutter ant hosts present (Feener & Brown, 1993; Tonhasca, 1996; Erthal & Tonhasca, 2000; Tonhasca *et al.*, 2001; Bragança *et al.*, 2002, 2003; Bragança & Medeiros, 2006; Guillade & Folgarait, 2011). Therefore studying all species interacting in a community will increase our understanding of parasitoid–host interactions involving non-hymenopteran parasitoids.

Here we quantified the interactions among all species of leafcutter ants and their phorid parasitoid species in two localities with different host species richness over the period of 1 year. Specifically, we: (i) describe the spatial-temporal variability of the interactions among hosts and parasitoids; (ii) determine parasitoid host ranges and electivities by assessing the frequency with which parasitoid species use each host in relation to host abundances; (iii) evaluate temporal variation in parasitoid specificity; and (iv) evaluate parasitoid specificity taking into account the geographic context ('geographical specificity', sensu Poulin *et al.*, 2011). Due to the phylogenetic relatedness of the hosts, which determine key traits that would influence parasitism susceptibility (such as kairomones, size, behaviour, and physiology; Feener & Brown, 1997), we expected low specificity for these parasitoids and a positive association between host abundance and parasitoid interaction frequency. To evaluate this hypothesis we used data obtained by sampling both adult parasitoids over the ants and by rearing their larvae when ants were infected.

Materials and methods

Sampling of interactions

To record parasitoid–host interactions in order to assess temporal variation in interactions and host availability, we collected

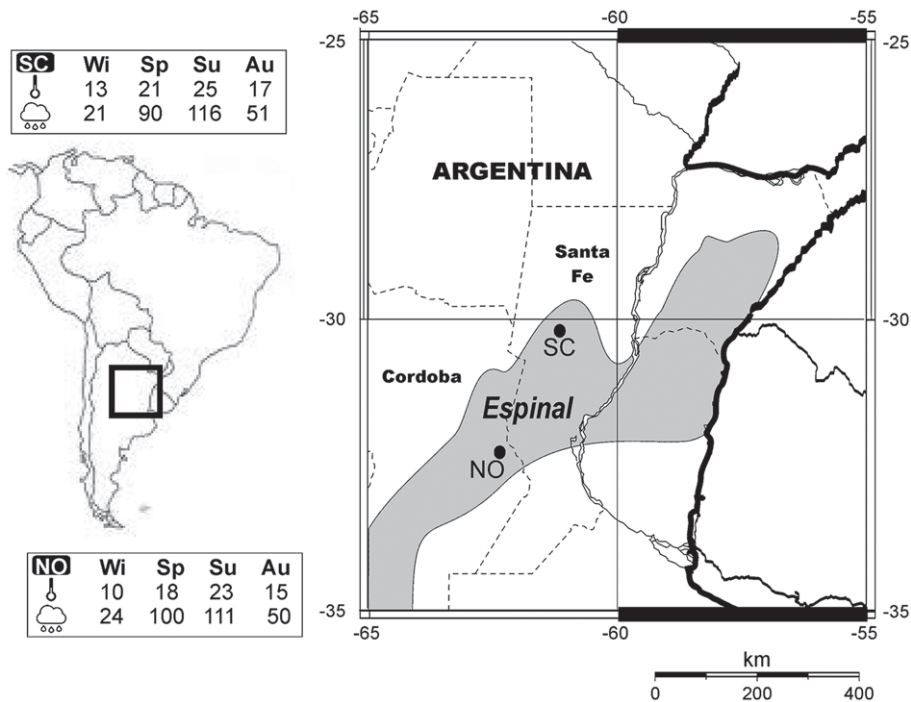


Fig. 1. Map showing the two sampling localities in Argentina, with high (SC, San Cristóbal) and low leafcutter ant species richness (NO, Noetinger), with the corresponding range of the Espinal phytogeographical region. The square on the map of South America (left) indicates the area amplified in the right panel. Mean annual temperature ($^{\circ}\text{C}$) and mean annual precipitation (mm) for each season (Wi, winter; Sp, spring; Su, summer; Au, autumn) are shown for the two localities.

data in two localities. The first study site has the highest leafcutter ant species richness reported so far throughout ant species' distribution range (seven *Acromyrmex* and one *Atta* species; San Cristóbal, Santa Fe, Argentina, $30^{\circ}12'S$, $61^{\circ}09'W$; see Elizalde & Folgarait, 2010) and the second study site, located in the Espinal phytogeographical region, 268 km from our first study site, had only three leafcutter ant species which are all present in the high species richness locality (only *Acromyrmex* species; Noetinger, Córdoba, Argentina, $32^{\circ}19'S$, $62^{\circ}21'W$; Fig. 1). Both localities had low human impact, i.e. livestock with low animal load ($< \text{one cow ha}^{-1}$). We sampled seasonally during 2 months in each locality by season (June–July 2005, October–November 2005, January–February 2006, and April–May 2006). At each study site we haphazardly selected six nests per ant species (excluding nests of the same species separated by < 50 m), and collected all observed parasitoids ovipositing on ants during the morning and afternoon on all trails of each nest. Parasitoid sampling from the same nest was conducted for ~ 3 h during the morning and another 3 h during the afternoon in each sampling season. Each survey at the same nest was separated by at least 2 days to reduce the effect of parasitoid removal. We also conducted sampling at night; however, these data were not included due to the low parasitoid abundance, and also because no additional parasitoid species were found (in fact, most species were not active at night). Further, we recorded interactions by rearing immature parasitoids from ants collected in the field, which is the classical measurement used to build parasitoid–host interaction networks

(Müller *et al.*, 1999; Salvo & Valladares, 1999; van Veen *et al.*, 2008). We collected ants within each sampling season from three nests per ant species for 15-min periods (with at least 50 ants per sample) and we transported the ants to the laboratory to rear the parasitoids (more details in Elizalde & Folgarait, 2011). This method allowed us to corroborate interactions observed in the field and to obtain parasitoid species and interactions that were not previously recorded in our adult parasitoid collection (Elizalde & Folgarait, 2011). Additionally, because host and parasitoid species can have different physiological requirements or developmental barriers, obtaining interaction data from rearing parasitoids in the laboratory should strengthen the patterns detected solely by observing the parasitoids found in the field.

All ants and parasitoids were identified using available keys (for ants Gonçalves, 1961; for parasitoids Disney *et al.*, 2006, 2008, 2009; Brown *et al.*, 2010), and voucher specimens were deposited in the Bernardino Rivadavia Museum (Buenos Aires, Argentina).

Interaction networks and specificity

To investigate parasitoid–host interactions, we built quantitative bipartite networks for each community and sampling period with the *bipartite* package in the R statistical environment (Dormann *et al.*, 2008). In the networks we included an independent measure of host abundance (see later). For parasitoids, however, we did not have a useful independent measure of their abundance beyond the record of which hosts they were found on

(i.e. their interaction frequency). In fact, Malaise traps were run for 5 days in each locality in order to obtain a parasitoid abundance measure independent of that recorded on hosts, but only five parasitoid specimens were collected after ~150 days of sampling. However, due to the high dependence of parasitoids on hosts, we expected that parasitoid abundance measured on the hosts would be highly correlated with their true abundance.

To estimate parasitoid interaction frequency (i.e. the frequency with which each parasitoid species attacked ants), host interaction frequency (i.e. the frequency with which each ant species was attacked by parasitoids), and interaction strength (i.e. frequency of parasitoid species j attacking host i ; Berlow *et al.*, 2004), we added the interaction frequency of each sampling type (i.e. adult parasitoids collected in the field and interactions recorded during parasitoid rearing). As the number of nests sampled by ant species was the same for both sampling types, frequencies of interaction were obtained by combining the number of parasitoids recorded attacking ants during adult parasitoid sampling (only females collected during the morning and afternoon, because males are rarely collected on the hosts; Elizalde & Folgarait, 2011) with the number of juvenile parasitoids reared (males and females) for each ant host species.

We obtained an independent measurement of host abundance, in addition to host interaction frequency. Nest density was obtained by counting up the number of nests recorded for each ant species sampled after carefully searching for them across 3 ha in each locality, within the area in which we sampled nests for parasitoids. Nest density did not vary substantially with time, as we recorded very few colonies that had moved between seasons. However, the number of available ant hosts foraging outside the nest was more important than nest density in accounting for parasitoid species richness (see Elizalde & Folgarait, 2010). Therefore, we first calculated a measure of host availability by multiplying ant foraging activity in each nest (measured as the number of ants passing a point on the foraging trail during 1 min) by the number of active foraging trails. Next, we calculated average values for each leafcutter ant species to obtain a measure at the host species level, and finally we multiplied that value by the nest density of each host species per locality.

We determined whether host interaction frequency could be predicted by ant abundance as has been observed in other systems (Vázquez *et al.*, 2007, but see Vizentin-Bugoni *et al.*, 2014). We used simple linear mixed regression models for each locality. In these models the interaction frequency of host species was the dependent variable, the measurement of ant availability was the independent variable (log-transformed to meet the assumptions of the test), and season was the random variable.

To determine parasitoid host ranges using the frequency with which parasitoid species use each host, we employed the paired difference index (PDI; Poisot *et al.*, 2012):

$$\text{PDI}_i = \frac{\sum_{i=2}^R (P_1 - P_i)}{R-1}$$

where P_1 is the strongest interaction strength, P_i is the link strength with the i th resource, and R is the number of resources in the dataset. Absolute values of PDI are straightforward to interpret, as values range from 0 to 1, with any value < 0.5 indicating

generalisation, and any value > 0.5 indicating specialisation. PDI yields more information than other available specialisation indices when assessing the degree of generalisation. This measure correctly distinguishes generalised species from those with very low degrees of specialisation (Poisot *et al.*, 2012). We obtained PDI values for the parasitoid species for each of the temporal sampling periods (i.e. winter, spring, summer, autumn) and by locality. The observed PDI values were compared with those of randomised 'ant \times parasitoid' matrices, in which combinations of ant and parasitoid species were randomised with a null model (Vázquez *et al.*, 2007; 'vaznull', 1000 permutations) using the *bipartite* package (Dormann *et al.*, 2008) of R (<http://cran.r-project.org/>). This null model constrains the connectance (i.e. number of realised interactions) to all possible interactions) by causing it to be the same as in the original network, while allowing changes in the marginal totals from the original network. A PDI lower than expected by chance highlights species that are more generalist than expected from the interaction frequencies in the system; while the opposite, i.e. a PDI higher than expected by chance, identifies specialist parasitoid species. We corrected P -values for multiple comparisons using the method of Benjamini and Hochberg (1995). To determine whether these interactions were influenced by the specificity of associations (i.e. the number of host species used) or by the specificity of impacts (i.e. interaction strength), we measured the relative importance of associations versus impacts in specificity. As PDI is necessarily lower than or equal to the number of hosts (RR; Poisot *et al.*, 2012), specificity is the sum of the specificity of associations (i.e. RR) and the specificity of impacts (I ; Bever, 2003). Thus the amount of specificity due to interaction strengths can be obtained from $I = \text{PDI} - \text{RR}$ (T. Poisot, pers. comm.). Finally, if the relative importance of associations (RR/PDI) is 1 there is a single host, and specificity is entirely explained by host number, whereas if it is 0, it is explained by interaction impacts (T. Poisot, pers. comm.).

We determined whether each parasitoid species using more than one host shows a differential electivity for some host species using Ivlev's forage ratio, $E'\alpha$ (Lechowicz, 1982):

$$E'\alpha = \frac{r_i}{p_i}$$

where p_i is the relative availability of resources in the environment (i.e. relative ant availability), and r_i is the relative utilisation of the resource (i.e. relative interaction strength). Values of this index > 1 indicate over-selection of certain host species relative to availability.

To test whether interaction frequency of parasitoids using more than one host was proportional to the abundance of their hosts, we used Fisher's exact tests. A significant result suggests that some hosts were being used more or less often than expected based on their abundance. We included all hosts that the parasitoid used at that location for each sampling season, even if in that particular sampling period the interaction was not detected (i.e. we included zeros). In contrast to the PDI, which only considers host interaction frequency, both Ivlev's forage ratio and the Fisher test allow us to evaluate the association between our measurements of host availability and interaction frequency.

We evaluated parasitoid geographic specificity (Poulin *et al.*, 2011) by comparing the ratio of the host species attacked by each parasitoid species in a community to all the host species known to be used by that parasitoid throughout its distribution that were present in that community. Data from our previous studies were used to designate the hosts that each parasitoid species is able to attack at other localities (Disney *et al.*, 2006, 2008; Brown *et al.*, 2010; Elizalde & Folgarait, 2010). As a community with higher host species richness will increase the probability of a host being part of the 'potential' host range of a parasitoid (i.e. a host that was present in a locality although not attacked by the parasitoid), we limited the comparison for the host species to those shared by the localities sampled (i.e., *Acromyrmex crassispinus*, *Acromyrmex heyeri*, and *Acromyrmex lundii*). A parasitoid species with a low ratio is more specialised at the geographical scale.

Results

We first describe the parasitoid–host communities and the temporal variation. After pooling sampling efforts by time, we found 19 parasitoid species in the community with the most hosts (13 were parasitoids of *Acromyrmex* and six only attacked *Atta vollenweideri*; Fig. 2a). In the species-poor locality, where only *A. crassispinus*, *A. heyeri*, and *A. lundii* were present, we found six parasitoid species (Fig. 2b). In the species-rich community we found eight parasitoid species parasitising these three same host species. Five parasitoid species were shared between localities (*Apocephalus neivai*, *Apocephalus noetingerorum*, *Lucianaphora folgaraitae*, *Myrmosicarius catharinensis*, *Myrmosicarius crudelis*), three were found exclusively in the species-rich community (*Apocephalus penicillatus*, *Myrmosicarius cristobalensis*, *Neodohniphora unichaeta*), and one was found in the species-poor locality only (*Myrmosicarius gracilipes*).

We found some variation in the relative abundance of the parasitoid species in both localities through time, but most parasitoids attacked ants during more than one sampling period (Fig. 2). The exception was *L. folgaraitae*, which was found to attack ants only in winter at both localities (Fig. 2). The number of hosts used by most parasitoid species was constant (i.e. parasitoid species attacked the same host species) throughout the different seasons, and some species showed a relatively constant interaction frequency with their hosts through time (e.g. *Myrmosicarius longipalpis* and *Myrmosicarius brandaoi*) (Fig. 2).

Atta vollenweideri, the unique *Atta* species present in the species-rich locality, had the highest parasitoid load (five or six species, according to season), and was attacked by almost the same parasitoid species in all sampling periods (Fig. 2a). With regard to *Acromyrmex* species, *A. crassispinus* had the highest parasitoid load at both localities when information from all sampling times was combined (Fig. 2). *Acromyrmex crassispinus* was also attacked by the same parasitoid species regardless of season. Likewise, *A. lundii* (at both localities) and *Acromyrmex lobicornis* (at the species-rich locality) were used by almost the same parasitoid species (Fig. 2). *Acromyrmex heyeri* was the only host of *M. catharinensis* in the species-rich locality,

and was used as host by two scarce parasitoid species (Fig. 2a); meanwhile, at the species-poor locality it was also regularly attacked by *A. neivai* (Fig. 2b). Most nests of *Acromyrmex striatus* and *Acromyrmex fracticornis* were inactive during winter, or had very low activity during the rest of the year. Despite such a low activity, however, they are attacked by a specific parasitoid species (*Apocephalus exstriatus*).

Host interaction frequency was predicted by ant availability in the species-rich community ($\chi_1^2 = 14.5$, $P = 0.0001$, $R^2 = 0.36$), although it explained around a third of the variation in the dataset. Moreover, this pattern was not found for the low-species-richness locality ($\chi_1^2 = 2.5$, $P = 0.11$).

The PDI values were all > 0.5 at both localities (Table 1), showing that parasitoid species in this system are highly specialised. In fact, most parasitoids had higher specificity than expected based on the distribution of interaction frequencies (Table 1), and 13 of the 20 parasitoid species were using only one host species. However, some species were marginally specialised as they differed from what was expected from the null models by reflecting a tendency to a relative generalisation (*A. neivai*, *M. cristobalensis* and *A. noetingerorum* in autumn in the species-rich community; Table 1), and other species did not differ from random in host usage according to frequency of interactions (*M. cristobalensis* in some seasons; and *A. neivai*, *A. noetingerorum*, and *M. catharinensis* in the species poor community; Table 1). However, even for these species the relative importance of associations (RR/PDI) was always > 0.5 (Table 1, except for *A. neivai* and *L. folgaraitae* in the species-poor locality), suggesting that the number of hosts was more important than the interaction strength in determining host range.

These few parasitoid species that were relative generalists when comparing observed with null PDI values showed higher electivity than expected for some hosts according to their relative abundance ($E'\alpha$ index > 1), which in general was consistent over time (Fig. 2; Table S1). For example, the parasitoid *A. neivai* showed higher electivity for *A. lundii* at all times in both communities, but it also showed higher electivity for *A. heyeri* in the community with lower species richness, and preferred *A. lobicornis* during autumn and summer in the richest community (Fig. 2; Table S1). In contrast, *M. cristobalensis* showed a high electivity for *A. fracticornis* when this species was used as a host. However, *A. fracticornis* had very low availability, and was only used as a host during the times when it was more active (Fig. 2a). *Myrmosicarius cristobalensis* also showed higher electivity for *A. lobicornis* during some sampling periods (in summer and autumn; Fig. 2a). Moreover, for these relative generalist parasitoid species, the association between the frequency of interactions and the measurement of host abundance was generally significant (Fig. 2; Fisher's exact tests, $P < 0.05$; Table S1), showing that these parasitoids did not use host species proportionately with host abundance and that they selectively choose their hosts.

Parasitoids occurring in the community with low species richness behaved more as generalists than those from the species-rich community, when interactions were considered in a geographical context. These parasitoids had higher ratios of the host species attacked there to all the host species known to

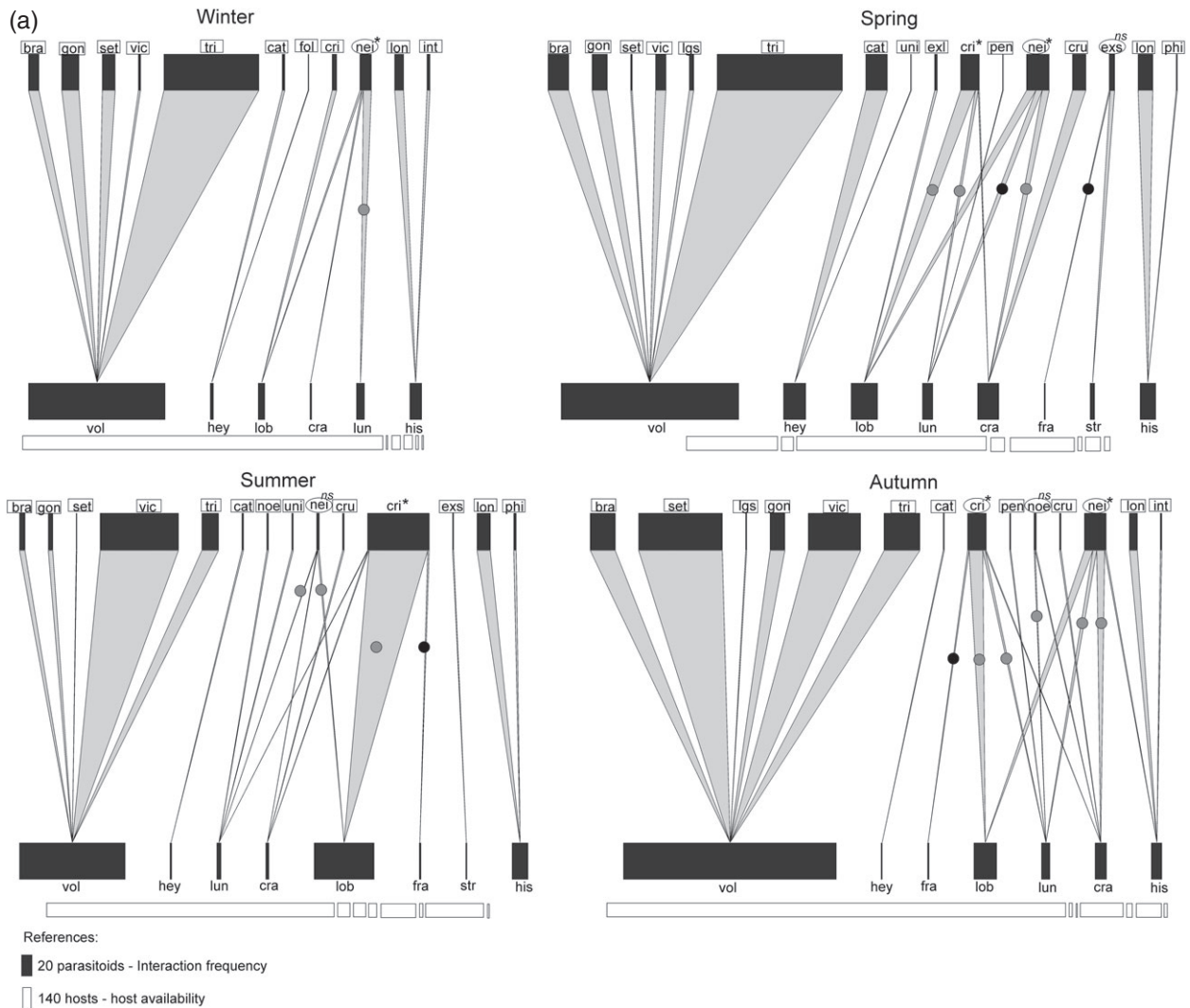


Fig. 2. Temporal bipartite networks of interactions recorded at the high (a) and low species richness localities (b). Parasitoid species are represented by the upper boxes and host ant species by the lower boxes (box width represents interaction frequencies; see References for quantitative information); white boxes represent the availability for hosts (ordered as the black boxes; see References for quantitative information). The width of the lines connecting them is proportional to the interaction strength. Specialist species [$P < 0.05$ and paired difference index (PDI) lower than the mean of null models] have their names within squares, while those that were relatively more generalised appear inside ellipses (if $P < 0.05$, with PDI higher than the mean of null models) or appear simply as text (if $P > 0.05$). Results of Fisher's exact tests to evaluate the association of generalist parasitoid abundances with host availability are indicated by 'ns' (not significant) or by an asterisk (*, significant, $P < 0.05$). Ivlev's index for generalist parasitoids is given for each sampling time as black ($E'\alpha > 5$) or grey circles ($1 < E'\alpha < 5$). Parasitoid species are abbreviated as follows: bra, *Myrmosicarius brandaoi*; cat, *Myrmosicarius catharinensis*; cri, *Myrmosicarius cristobalensis*; cru, *Myrmosicarius crudelis*; exl, *Apocephalus exlobicornis*; exs, *Apocephalus exstriatus*; fol, *Lucianaphora folgaritae*; gra, *Myrmosicarius gracilipes*; gon, *Myrmosicarius gonzalezae*; int, *Apocephalus intermedius*; lon, *M. longipalpis*; lgs, *Apocephalus longisetarum*; nei, *Apocephalus neivai*; noe, *Apocephalus noetingerorum*; phi, *Apocephalus philhispidus*; pen, *Apocephalus peniciliatus*; set, *Apocephalus setitarsus*; vic, *Apocephalus vicosa*; tri, *Eibesfeldtphora trilobata*; uni, *Neodohrniphora unichaeta*. Host species are abbreviated as follows: cra, *Acromyrmex crassispinus*; fra, *Acromyrmex fracticornis*; hey, *Acromyrmex heyeri*; his, *Acromyrmex hispidus*; lob, *Acromyrmex lobicornis*; lun, *Acromyrmex lundii*; str, *Acromyrmex striatus*; vol, *Atta vollenweideri*.

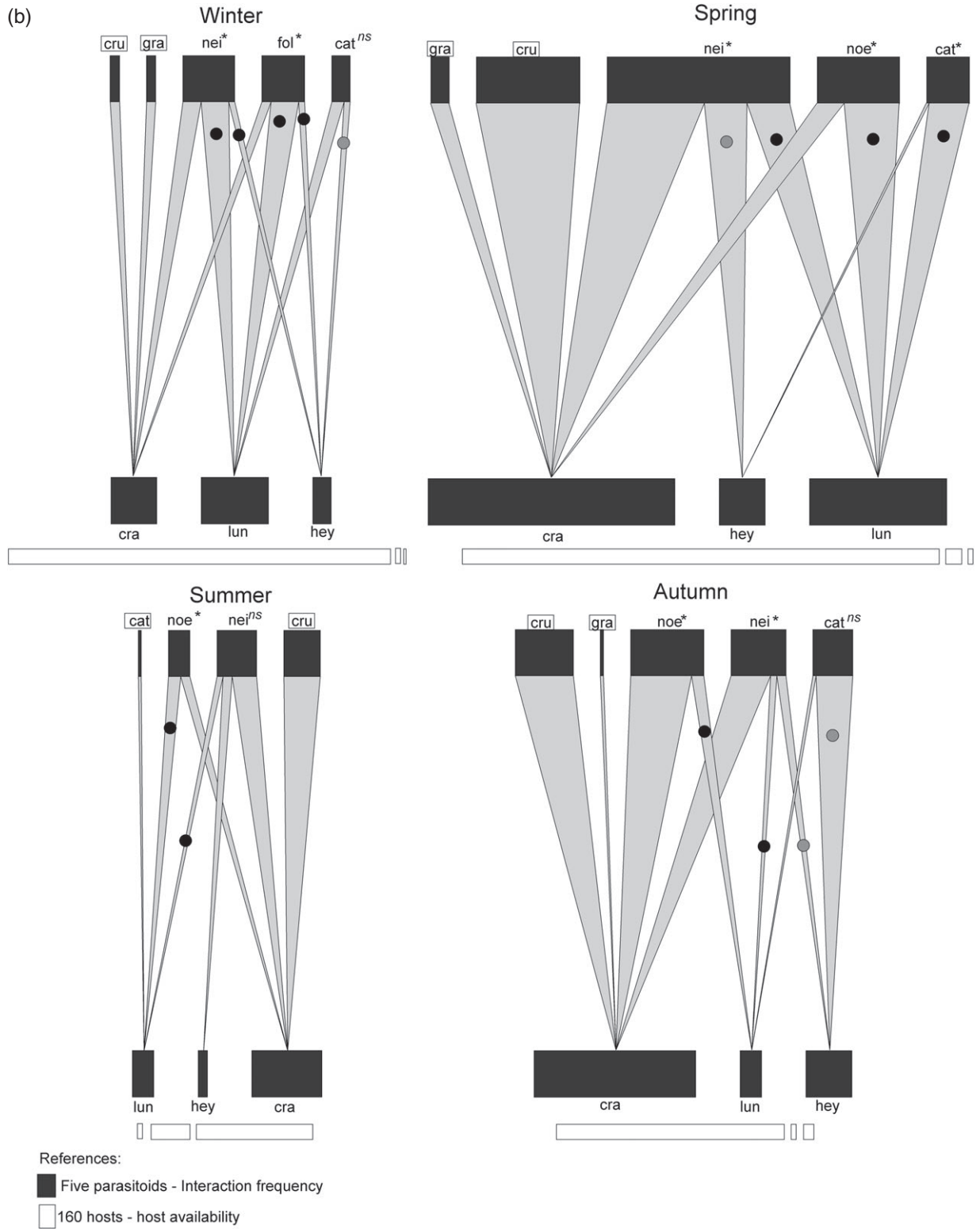


Fig. 2. Continued

Table 1. Parasitoid species that attack *Acromyrmex* or *Atta* in each season in the species-rich (a) or species-poor (b) localities, with the observed paired difference index (PDI_o), the mean of null PDI values (PDI_n), standardised effect size (SES) and the probability as the observed value to be different or the same of the simulated values. In addition, the relative importance of number of hosts accounting for specificity is shown (RR/PDI).

Parasitoids	Winter						Spring						Summer						Autumn												
	PDI _o	PDI _n	SES	P	RR/PDI	PDI _o	PDI _n	SES	P	RR/PDI	PDI _o	PDI _n	SES	P	RR/PDI	PDI _o	PDI _n	SES	P	RR/PDI	PDI _o	PDI _n	SES	P	RR/PDI						
	(a) Species-rich locality																														
<i>Acromyrmex</i> hosts																															
cat	<i>I</i>	0.998	0.129	<0.01	1	<i>I</i>	0.988	0.387	<0.01	1	<i>I</i>	0.998	0.124	<0.01	1	<i>I</i>	0.999	0.116	<0.01	1	<i>I</i>	0.999	0.120	<0.01	1	<i>I</i>	0.999	0.106	<0.01	1	
cri	<i>I</i>	0.997	0.149	<0.01	1	0.96	0.990	-1.114	0.06	0.69	0.99	0.966	0.597	0.34	0.69	0.94	0.990	-1.942	0.03	0.53	<i>I</i>	0.998	0.147	<0.01	1	<i>I</i>	0.998	0.147	<0.01	1	
cru	<i>I</i>					<i>I</i>	0.992	0.312	<0.01	1	<i>I</i>	0.999	0.103	<0.01	1	<i>I</i>	0.999	0.103	<0.01	1	<i>I</i>	0.999	0.097	<0.01	1	<i>I</i>	0.999	0.097	<0.01	1	
exl	<i>I</i>					<i>I</i>	0.999	0.098	<0.01	1	0.97	0.999	0.098	<0.01	1	<i>I</i>	0.999	0.097	<0.01	1	<i>I</i>	0.999	0.097	<0.01	1	<i>I</i>	0.999	0.097	<0.01	1	
exs	<i>I</i>					0.97	0.998	-2.440	<0.01	0.83	<i>I</i>	0.999	0.097	<0.01	1	<i>I</i>	0.999	0.097	<0.01	1	<i>I</i>	0.999	0.097	<0.01	1	<i>I</i>	0.999	0.097	<0.01	1	
fol	<i>I</i>	0.999	0.048	<0.01	1	<i>I</i>	0.999	0.048	<0.01	1	<i>I</i>	0.999	0.048	<0.01	1	<i>I</i>	0.999	0.048	<0.01	1	<i>I</i>	0.999	0.048	<0.01	1	<i>I</i>	0.999	0.048	<0.01	1	
int	<i>I</i>	0.997	0.141	<0.01	1	<i>I</i>	0.997	0.141	<0.01	1	<i>I</i>	0.997	0.141	<0.01	1	<i>I</i>	0.997	0.141	<0.01	1	<i>I</i>	0.997	0.141	<0.01	1	<i>I</i>	0.997	0.141	<0.01	1	
lon	0.91	0.994	0.264	<0.01	1	<i>I</i>	0.994	0.264	<0.01	1	<i>I</i>	0.994	0.264	<0.01	1	<i>I</i>	0.994	0.264	<0.01	1	<i>I</i>	0.994	0.264	<0.01	1	<i>I</i>	0.994	0.264	<0.01	1	
nei	0.91	0.993	-3.995	0.02	0.73	0.8	0.987	-6.079	0.04	0.83	<i>I</i>	0.999	0.107	<0.01	1	<i>I</i>	0.999	0.107	<0.01	1	<i>I</i>	0.999	0.107	<0.01	1	<i>I</i>	0.999	0.107	<0.01	1	
noe	<i>I</i>					<i>I</i>	0.999	0.042	<0.01	1	<i>I</i>	0.999	0.042	<0.01	1	<i>I</i>	0.999	0.042	<0.01	1	<i>I</i>	0.999	0.042	<0.01	1	<i>I</i>	0.999	0.042	<0.01	1	
pen	<i>I</i>					<i>I</i>	0.999	0.084	<0.01	1	<i>I</i>	0.999	0.084	<0.01	1	<i>I</i>	0.999	0.084	<0.01	1	<i>I</i>	0.999	0.084	<0.01	1	<i>I</i>	0.999	0.084	<0.01	1	
phi	<i>I</i>					<i>I</i>	0.999	0.031	<0.01	1	<i>I</i>	0.999	0.031	<0.01	1	<i>I</i>	0.999	0.031	<0.01	1	<i>I</i>	0.999	0.031	<0.01	1	<i>I</i>	0.999	0.031	<0.01	1	
uni	<i>I</i>					<i>I</i>	0.999	0.031	<0.01	1	<i>I</i>	0.999	0.031	<0.01	1	<i>I</i>	0.999	0.031	<0.01	1	<i>I</i>	0.999	0.031	<0.01	1	<i>I</i>	0.999	0.031	<0.01	1	
<i>Atta</i> hosts																															
bra	<i>I</i>	0.993	0.273	<0.01	1	<i>I</i>	0.988	0.395	<0.01	1	<i>I</i>	0.988	0.395	<0.01	1	<i>I</i>	0.988	0.395	<0.01	1	<i>I</i>	0.988	0.395	<0.01	1	<i>I</i>	0.988	0.395	<0.01	1	
gon	<i>I</i>	0.989	0.322	<0.01	1	<i>I</i>	0.993	0.302	<0.01	1	<i>I</i>	0.996	0.221	<0.01	1	<i>I</i>	0.996	0.221	<0.01	1	<i>I</i>	0.996	0.221	<0.01	1	<i>I</i>	0.996	0.221	<0.01	1	
lgs	<i>I</i>					<i>I</i>	0.999	0.088	<0.01	1	<i>I</i>	0.999	0.088	<0.01	1	<i>I</i>	0.999	0.088	<0.01	1	<i>I</i>	0.999	0.088	<0.01	1	<i>I</i>	0.999	0.088	<0.01	1	
set	<i>I</i>	0.992	0.311	<0.01	1	<i>I</i>	0.995	0.256	<0.01	1	<i>I</i>	0.995	0.256	<0.01	1	<i>I</i>	0.995	0.256	<0.01	1	<i>I</i>	0.995	0.256	<0.01	1	<i>I</i>	0.995	0.256	<0.01	1	
vic	<i>I</i>	0.999	0.080	<0.01	1	<i>I</i>	0.997	0.172	<0.01	1	<i>I</i>	0.997	0.172	<0.01	1	<i>I</i>	0.997	0.172	<0.01	1	<i>I</i>	0.997	0.172	<0.01	1	<i>I</i>	0.997	0.172	<0.01	1	
tri	<i>I</i>	0.952	0.720	<0.01	1	<i>I</i>	0.928	0.916	<0.01	1	<i>I</i>	0.990	0.439	<0.01	1	<i>I</i>	0.990	0.439	<0.01	1	<i>I</i>	0.990	0.439	<0.01	1	<i>I</i>	0.990	0.439	<0.01	1	
(b) Species-poor locality																															
cat	0.75	0.755	-0.028	0.48	0.67	0.96	0.883	0.483	0.45	0.52	<i>I</i>	0.965	0.294	<0.01	1	0.96	0.876	0.623	0.37	0.52	<i>I</i>	0.96	0.876	0.623	0.37	0.52	<i>I</i>	0.96	0.876	0.623	0.37
cru	<i>I</i>	0.865	0.745	<0.01	1	<i>I</i>	0.763	1.422	<0.01	1	<i>I</i>	0.800	1.242	<0.01	1	<i>I</i>	0.800	1.242	<0.01	1	<i>I</i>	0.800	1.242	<0.01	1	<i>I</i>	0.800	1.242	<0.01	1	
gra	<i>I</i>	0.838	0.785	<0.01	1	<i>I</i>	0.942	0.459	<0.01	1	<i>I</i>	0.942	0.459	<0.01	1	<i>I</i>	0.942	0.459	<0.01	1	<i>I</i>	0.942	0.459	<0.01	1	<i>I</i>	0.942	0.459	<0.01	1	
fol	0.72	0.608	0.577	0.47	0	0.56	0.698	-0.994	0.25	0	0.69	0.813	-0.846	0.19	0	0.62	0.863	-1.543	0.15	0.81	0.81	0.857	-0.364	0.37	0	0.81	0.857	-0.364	0.37		
nei	0.56	0.578	-0.105	0.48	0	0.75	0.802	-0.308	0.45	0.67	0.75	0.802	-0.308	0.45	0.67	0.75	0.802	-0.308	0.45	0.67	0.75	0.802	-0.308	0.45	0.67	0.75	0.802	-0.308	0.45	0.67	
noe																															

PDI_o and *P*-values ≤ 0.05 indicating relative generalist species are in bold; for specialists, PDI and *P*-values ≤ 0.05 are in italics. bra, *Myrmosciarius brandaui*; cat, *Myrmosciarius catharinensis*; cri, *Myrmosciarius cristobalensis*; cru, *Myrmosciarius crudelis*; exl, *Apocephalus exultans*; exs, *Apocephalus exultans*; fol, *Lucitanaphora folgaraitae*; gon, *Myrmosciarius goncalvesae*; gra, *Myrmosciarius gracilipes*; int, *Apocephalus intermedius*; lgs, *Apocephalus longispalpis*; nei, *Myrmosciarius longispalpis*; nei, *Apocephalus neivai*; noe, *Apocephalus noetingeronum*; pen, *Apocephalus penicillatus*; phi, *Apocephalus philispidis*; set, *Apocephalus setitarsus*; tri, *Eibesfeldtphora trilobata*; uni, *Neodohrniphora unichaeta*; vic, *Apocephalus vicosa*.

Table 2. Ratios of host species attacked by each parasitoid species in a community to all the host species known to be used by that parasitoid, throughout its geographic distribution range, that are present in that community. Ratios are shown for the species-poor and species-rich communities, and for the reduced species-rich community, i.e. considering only the hosts species shared by both communities. Means and coefficients of variation (CV) are given.

Parasitoid spp.	Species-poor community	Reduced-species rich community	Species-rich community
cat	0.75	0.33	0.20
cri		0.67	0.57
cru	1.00	1.00	1.00
exl			1.00
exs			1.00
fol	1.00	0.33	0.33
gra	1.00		
int			1.00
lon			1.00
nei	1.00	0.67	0.60
noe	1.00	1.00	1.00
pen		1.00	0.50
phi			1.00
uni			0.50
Mean	0.96	0.71	0.75
CV	0.11	0.42	0.40

cat, *Myrmosicarius catharinensis*; cri, *Myrmosicarius cristobalensis*; cru, *Myrmosicarius crudelis*; exl, *Apocephalus exlobicornis*; exs, *Apocephalus exstriatus*; fol, *Lucianaphora folgaritae*; gra, *Myrmosicarius gracilipes*; int, *Apocephalus intermedius*; lon, *Myrmosicarius longipalpis*; nei, *Apocephalus neivai*; noe, *Apocephalus noetingerorum*; pen, *Apocephalus peniciliatus*; phi, *Apocephalus philhispidus*; uni, *Neodohrniphora unichaeta*.

be used by that parasitoid throughout their known distribution range (Table 2).

Discussion

Here we report on the web of interactions among leafcutter ants and their specific phorid parasitoids in two communities with contrasting host richness. Further, we evaluate three aspects of host specificity: (i) the role of host relative abundance; (ii) variation in host specificity through time; and (iii) consistency in host specificity in a spatial context with variable host species composition. We show that parasitoid species are highly specialised in this system, even when all hosts are closely related. We also found that interaction frequency between these parasitoids and their leafcutter ant hosts is not accounted for by host availability. We provide several lines of evidence that ant availability has little influence on parasitoid–host interactions in this system, when comparing within host genera. This is shown by the higher influence of the specificity of associations (i.e. the number of host species used) than the specificity of impacts (i.e. interaction strength) when accounting for specialisation, by the many significant Fisher tests and the electivity of most generalist parasitoids to certain host species shown by Ivlev's index. These unexpected results suggest that other factors apart from abundance play a key role in the structure of these parasitoid–host

interactions. Factors probably involved in shaping these interactions include those related to the host's immune responses, the host's behavioural defences and/or the host's location cues or parasitoid interspecific interactions.

In contrast to a previous investigation of a parasitoid–host interaction network for leaf miner hosts (Lewis *et al.*, 2002), and to what is known for other phorid ant parasitoids (*Pseudacteon* phorids–*Solenopsis* ants; Patrock *et al.*, 2009) and hymenopteran ant parasitoids (Lachaud & Perez Lachaud, 2012), we found that these phorid parasitoids of leafcutter ants are extremely specialised, as most use only one ant species as host. Furthermore, they showed low host species turnover throughout time and space. Even when some parasitoid species could use more than one hosts species, they tended to specialise on a small array of hosts (one or two species). This was surprising given that hosts of these parasitoids belong to the same genus, and probably have similar physiological and behavioural defences. This specialisation may be associated with the constancy of the host community, because most ant nests were at the same site throughout the year and ants were active all year round for most species. There were two species that did not show continuous annual activity – *A. striatus* and *A. fracticornis* – and both had a very low parasitoid load.

In this system, interactions are split along a phylogenetic axis, as *Atta* and *Acromyrmex* hosts differed in parasitoid species composition (Elizalde & Folgarait, 2011), species richness (Elizalde & Folgarait, 2010), and the frequency of interactions (this work; although only one species was included in this study, unpublished data for *Atta saltensis* show a similar pattern, [L. Elizalde] as do other *Atta* species; Tonhasca, 1996; Erthal & Tonhasca, 2000; Tonhasca *et al.*, 2001; Bragança & Medeiros, 2006). In addition to the effect of phylogeny, the high interaction levels may also be associated with much higher resource availability in *Atta* species, i.e. they have more populous colonies with more foragers and thus represent more resources for parasitoids (Elizalde & Folgarait, 2010, 2012). When differences in host genera were taken into account by restricting the analyses to parasitoid species that only use one leafcutter ant genus, parasitoid–host interaction frequencies did not increase with ant availability.

Unlike other systems (Hawkins & Sheehan, 1994; Arneberg *et al.*, 1998; Krasnov *et al.*, 2002; but see Stanko *et al.*, 2002), *Acromyrmex* hosts for which parasitoid species showed higher interaction frequency or electivity were not the most abundant species. This was especially clear in *A. lundii*, a host with relatively low availability for which most parasitoids using it as a host showed high electivity. In addition, in the few instances when *A. fracticornis* was used by parasitoids, they showed high electivity, even when the ant was scarce. Parasitoid species using more than one host consistently showed high electivity for the same host across different sampling periods and in both localities in general (*A. neivai* and *A. noetingerorum* for *A. lundii*; *M. cristobalensis* for *A. lobicornis*; and *M. catharinensis* for *A. heyeri*). This electivity could be related to host location behaviours (e.g. the use of ant pheromones as kairomones; Feener & Brown, 1997), higher host resource quality, or local adaptation of parasitoids to particular host species. This seems to be the case for the exclusive use of *A. heyeri* by *M.*

catharinensis in the species-rich locality, even though the other three host species present there were used at other localities by this parasitoid [*A. lundii* at the species-poor locality (Fig. 2b); and *A. crassispinus* and *A. lobicornis* at different locations (Disney *et al.*, 2006)]. In addition, it is likely that the local species richness of potential hosts influences parasitoid host range because this parasitoid species, for instance, also showed differences in host range (both for PDI and for the ratio of realised to possible hosts used) in the two localities studied. For example, the fact that there were higher ratios of realised to possible hosts used in the species-poor community may be related to a less efficient host search in communities with many hosts due to a higher probability of host errors (Straub *et al.*, 2011). Because the information available for this system is still limited, we were not able to determine which of these mechanisms were acting.

Interactions were quite stable through time. Abundant specialist parasitoids attacked their hosts at all sampling times and generalist parasitoids interacted with their preferred host in most sampling times. Thus, even if few parasitoid species were active during one sampling period, interactions in these localities were quite constant and the level of specificity did not differ through time for most of them. For parasitoid species where variation in interaction frequencies was most evident, there appeared to be an association with climatic tolerances. For example, *M. cristobalensis* was present in northern and hotter localities, and it was located at microsites with higher temperatures than other species (Elizalde & Folgarait, 2010). In fact, the interaction webs sampled throughout the year showed that *M. cristobalensis* was most abundant in summer and interacted more in this season with *A. lobicornis*, a host species that prefers to nest in open areas (L. Elizalde, pers. obs.), than with *A. crassispinus* or *A. lundii* (species that nest in quite closed forest in the study sites; L. Elizalde, pers. obs.). The opposite was the case with *M. catharinensis*, which was less abundant in summer and was present in the southern localities. Moreover, *M. catharinensis* has the most southern distribution of these phorids where it is the only species attacking *A. lobicornis* ants (a preferred host) along the Patagonian steppe (40°56'S 71°03'W; L. Elizalde, pers. obs.). Similarly, *M. brandaoi* and *Eibesfeldtphora trilobata* both use *A. vollenweideri* as host, although the former was also collected attacking another *Atta* species, *A. saltensis* (Disney *et al.*, 2006). In this case, *M. brandaoi* is more abundant in the northern and hotter communities, while *E. trilobata* is more abundant in the most southern community where *Atta* was recorded (Elizalde & Folgarait, 2010), and was more abundant during winter.

The results obtained in this study add key information to our understanding of parasitoid–host interactions. To our knowledge, this is the first time an interaction web involving phorid parasitoids has been quantitatively evaluated. The results of this study, particularly the high specificity of these parasitoids and their tendency to specialise in a subset of possible hosts – both the opposite of what we expected – are relevant from both ecological/evolutionary and applied perspectives. From an ecological or evolutionary perspectives, our work shows that hosts are attacked by specialist parasitoids, but most hosts were attacked by more than one parasitoid species. This asymmetry probably

makes it more difficult for hosts to mount specific, presumably more efficient, defences (Rigaud *et al.*, 2010; Elizalde & Folgarait, 2012), leaving the hosts at a relative disadvantage (Lapchin & Guillemaud, 2005). From an applied point of view, the results reported here show that these parasitoids offer an intriguing option due to their high host specificity and little variation with time. As these parasitoids are candidates for use in the biological control of leafcutter ants, an important pest group in the Neotropics (Della Lucia, 2003), this information may be helpful in characterising the potential non-target effects of parasitoids released for biological control initiatives on pest hosts. Finally, our results contribute to the ecology of non-hymenopteran parasitoid communities, which are poorly known (Stireman, 2016). Specifically, this work characterises the interactions among a highly specialised parasitoid guild and all their hosts in a locality with the highest known host diversity.

Acknowledgments

We are grateful to Luciano Cagnolo, the Insect Seminar Group of Laboratorio Ecotono, Julia Jones, and two reviewers for their comments to improve manuscript quality, as well as to Constanza González Campero, Sebastián Lorenzatti and Gabriel Maceiras for help with field or laboratory work. We thank the following people or organisations for logistical help and/or permits to perform field work: familia Capovilla in San Cristóbal, Administración de Parques Nacionales de Argentina, and INTA. We also thank Timothée Poisot for sharing his idea on how to decompose specificity. Field and laboratory work was financially supported by National Geographic Society (grant 7539-03 to PJF) and Universidad Nacional de Quilmes (grant 0340/03 to PJF). LE and PJF thank the Consejo Nacional de Ciencia y Tecnología, Argentina (CONICET), and LE thanks Fundación Bunge y Born. RJWP thanks Lawrence Gilbert and the University of Texas Fire Ant Laboratory for his introduction to the South American ant–phorid community. RHLD's work on Phoridae is currently funded by the Balfour-Browne Trust (University of Cambridge). Contribution of authors: LE designed the study and collected the data. LE and RJWP analysed the data. LE and RHKD identified parasitoid specimens. LE, RJWP and PJF wrote the article. All authors read and approved the article.

Supporting Information

Additional Supporting Information may be found in the online version of this article under the DOI reference: 10.1111/een.12477

Table S1. Ivlev's index ($E'\alpha$) for generalist parasitoids at each sampling time in the high and low species richness localities. Numbers in bold indicate a host species that was selected more than was expected according to its abundance ($E'\alpha > 1$). Host species are abbreviated as in Fig. 1. The last column for each sampling time shows probability values (P) for exact Fisher's tests for the interaction strength of generalist species and host abundance (availability of ants).

References

- Arneberg, P., Skorping, A., Grenfell, B. & Read, A.F. (1998) Host densities as determinants of abundance in parasite communities. *Proceedings of the Royal Society of London Series B: Biological Sciences*, **265**, 1283–1289.
- Benjamini, Y. & Hochberg, Y. (1995) Controlling the false discovery rate: a practical and powerful approach to multiple testing. *Journal of the Royal Statistical Society. Series B*, **57**, 289–300.
- Berlow, E.L., Neutel, A.-M., Cohen, J.E., De Ruiter, P.C., Ebenman, B.O., Emmerson, M. et al. (2004) Interaction strengths in food webs: issues and opportunities. *Journal of Animal Ecology*, **73**, 585–598.
- Bever, J.D. (2003) Soil community feedback and the coexistence of competitors: conceptual frameworks and empirical tests. *New Phytologist*, **157**, 465–473.
- Bragança, M.A.L., Della Lucia, T.M.C. & Tonhasca, A. Jr. (2003) First record of phorid parasitoids (Diptera: Phoridae) of the leaf-cutting ant *Atta bisphaerica* Forel (Hymenoptera: Formicidae). *Neotropical Entomology*, **32**, 169–171.
- Bragança, M.A.L. & Medeiros, Z.C.S. (2006) Ocorrência e características biológicas de forídeos parasitóides (Diptera: Phoridae) da saúva *Atta laevigata* (Smith) (Hymenoptera: Formicidae) em Porto Nacional, TO. *Neotropical Entomology*, **35**, 408–411.
- Bragança, M.A.L., Tonhasca, A. Jr. & Moreira, D.D.O. (2002) Parasitism characteristics of two phorid fly species in relation to their host, the leaf-cutting ant *Atta laevigata* (Smith) (Hymenoptera: Formicidae). *Neotropical Entomology*, **31**, 241–244.
- Brown, B.V., Disney, R.H.L., Elizalde, L. & Folgarait, P.J. (2010) New species and new records of *Apocephalus* Coquillett (Diptera: Phoridae) that parasitize ants (Hymenoptera: Formicidae) in America. *Sociobiology*, **55**, 165–190.
- Cagnolo, L., Salvo, A. & Valladares, G. (2011) Network topology: patterns and mechanisms in plant-herbivore and host-parasitoid food webs. *The Journal of Animal Ecology*, **80**, 342–351.
- Della Lucia, T.M.C. (2003) Hormigas de importancia económica en la región Neotropical. *Hormigas de la Región Neotropical* (ed. by F. Fernández), pp. 337–349. Instituto de Investigación de Recursos Biológicos Alexander von Humboldt, Bogotá, Colombia.
- Disney, R.H.L., Elizalde, L. & Folgarait, P.J. (2006) New species and revision of *Myrmosicarius* (Diptera: Phoridae) that parasitize leaf-cutter ants (Hymenoptera: Formicidae). *Sociobiology*, **47**, 771–809.
- Disney, R.H.L., Elizalde, L. & Folgarait, P.J. (2008) New species and records of scuttle flies (Diptera: Phoridae) associated with leaf-cutter ants and army ants (Hymenoptera: Formicidae) in Argentina. *Sociobiology*, **51**, 95–117.
- Disney, R.H.L., Elizalde, L. & Folgarait, P.J. (2009) New species and new records of scuttle flies (Diptera: Phoridae) that parasitize leaf-cutter and army ants (Hymenoptera: Formicidae). *Sociobiology*, **54**, 601–631.
- Dormann, C.F., Gruber, B. & Fründ, J. (2008) Introducing the bipartite package: analysing ecological networks. *R News*, **8**, 8–11.
- Elias, M., Fontaine, C. & van Veen, F.J.F. (2013) Evolutionary history and ecological processes shape a local multilevel antagonistic network. *Current Biology*, **23**, 1355–1359.
- Elizalde, L. & Folgarait, P.J. (2010) Host diversity and environmental variables as determinants of the species richness of the parasitoids of leaf-cutting ants. *Journal of Biogeography*, **37**, 2305–2316.
- Elizalde, L. & Folgarait, P.J. (2011) Biological attributes of Argentinian phorid parasitoids (Insecta: Diptera: Phoridae) of leaf-cutting ants, *Acromyrmex* and *Atta*. *Journal of Natural History*, **45**, 2701–2723.
- Elizalde, L. & Folgarait, P.J. (2012) Behavioral strategies of phorid parasitoids and responses of their hosts, the leaf-cutting ants. *Journal of Insect Science*, **12**, 135.
- Erthal, M. Jr. & Tonhasca, A. Jr. (2000) Biology and oviposition behavior of the phorid *Apocephalus atrophilus* and the response of its host, the leaf-cutting ant *Atta laevigata*. *Entomologia Experimentalis et Applicata*, **95**, 71–75.
- Feener, D.H. Jr. & Brown, B.V. (1993) Oviposition behavior of an ant-parasitizing fly, *Neodohrniphora curvinervis* (Diptera: Phoridae), and defense behavior by its leaf-cutting ant host *Atta cephalotes* (Hymenoptera: Formicidae). *Journal of Insect Behavior*, **6**, 675–688.
- Feener, D.H. Jr. & Brown, B.V. (1997) Diptera as parasitoids. *Annual Review of Entomology*, **42**, 73–97.
- Folgarait, P.J. (2013) Leaf-cutter ant parasitoids: current knowledge. *Psyche: A Journal of Entomology*, **2013**, 1–10.
- Fox, L.R. & Morrow, P.A. (1981) Specialization: species property or local phenomenon? *Science*, **211**, 887–893.
- Godfray, H.C.J. (1994) *Parasitoids: Behavioral and Evolutionary Ecology*. Princeton University Press, Princeton, NJ.
- Gonçalves, C.R. (1961) O gênero *Acromyrmex* no Brasil (Hym. Formicidae). *Studia Entomologica*, **4**, 113–180.
- Guillade, A.C. & Folgarait, P.J. (2011) Life-history traits and parasitism rates of four phorid species (Diptera: Phoridae), parasitoids of *Atta vollenweideri* (Hymenoptera: Formicidae) in Argentina. *Journal of Economic Entomology*, **104**, 32–40.
- Hambäck, P.A., Weingartner, E., Ericson, L., Fors, L., Cassel-Lundhagen, A., Stenberg, J.A. et al. (2013) Bayesian species delimitation reveals generalist and specialist parasitic wasps on *Galerucella* beetles (Chrysomelidae): sorting by herbivore or plant host. *BMC Evolutionary Biology*, **13**, 92.
- Hawkins, B.A. & Sheehan, W. (1994) *Parasitoid Community Ecology*. Oxford University Press, Oxford, U.K.
- Henri, D.C. & van Veen, F.J.F. (2016) Link flexibility: evidence for environment-dependent adaptive foraging in a food web time-series. *Ecology*, **97**, 1381–1387.
- Ivens, A.B.F., Beeren, C.v., Blüthgen, N. & Kronauer, D.J.C. (2016) Studying the complex communities of ants and their symbionts using ecological network analysis. *Annual Review of Entomology*, **61**, 353–371.
- Ives, A.R. & Godfray, H.C.J. (2006) Phylogenetic analysis of trophic associations. *The American Naturalist*, **168**, E1–E14.
- Krasnov, B., Khokhlova, I. & Shenbrot, G. (2002) The effect of host density on ectoparasite distribution: an example of a rodent parasitized by fleas. *Ecology*, **83**, 164–175.
- Lachaud, J.-P. & Perez Lachaud, G. (2012) Diversity of species and behavior of hymenopteran parasitoids of ants, a review. *Psyche*, **2012**, 1–24.
- Lapchin, L. & Guillemaud, T. (2005) Asymmetry in host and parasitoid diffuse coevolution: when the red queen has to keep a finger in more than one pie. *Frontiers in Zoology*, **2**, 4.
- Lechowicz, M.J. (1982) The sampling characteristics of electivity indices. *Oecologia*, **52**, 22–30.
- Lewis, O.T., Memmott, J., LaSalle, J., Lyal, C.H.C., Whitefoord, C. & Godfray, H.C.J. (2002) Structure of a diverse tropical forest insect–parasitoid community. *Journal of Animal Ecology*, **71**, 855–873.
- Memmott, J. & Godfray, H.C.J. (1994) The use and construction of parasitoid webs. *Parasitoid Community Ecology* (ed. by B. A. Hawkins and W. Sheehan), pp. 300–316. Oxford University Press, New York, New York.
- Memmott, J., Godfray, H.C.J. & Gauld, I.D. (1994) The structure of a tropical host-parasitoid community. *Journal of Animal Ecology*, **63**, 521–540.
- Müller, C.B., Adriaanse, I.C.T., Belshaw, R. & Godfray, H.C.J. (1999) The structure of an aphid-parasitoid community. *Journal of Animal Ecology*, **68**, 346–370.

- Patrock, R.J.W., Porter, S.D., Gilbert, L.E. & Folgarait, P.J. (2009) Distributional patterns of *Pseudacteon* associated with the *Solenopsis saevissima* complex in South America. *Journal of Insect Science*, **9**, 60.
- Poisot, T., Canard, E., Mouquet, N. & Hochberg, M.E. (2012) A comparative study of ecological specialization estimators. *Methods in Ecology and Evolution*, **3**, 537–544.
- Poisot, T., Stouffer, D.B. & Gravel, D. (2015) Beyond species: why ecological interactions vary through space and time. *Oikos*, **124**, 243–251.
- Poulin, R., Krasnov, B.R. & Mouillot, D. (2011) Host specificity in phylogenetic and geographic space. *Trends in Parasitology*, **27**, 355–361.
- Rigaud, T., Perrot-Minnot, M.-J. & Brown, M.J.F. (2010) Parasite and host assemblages: embracing the reality will improve our knowledge of parasite transmission and virulence. *Proceedings of the Royal Society of London B: Biological Sciences*, **277**, 3693–3702.
- Rosindell, J., Hubbell, S.P. & Etienne, R.S. (2011) The unified neutral theory of biodiversity and biogeography at age ten. *Trends in Ecology & Evolution*, **26**, 340–348.
- Rott, A.S. & Godfray, H.C.J. (2000) The structure of a leafminer-parasitoid community. *Journal of Animal Ecology*, **69**, 274–289.
- Salvo, A. & Valladares, G. (1999) Parasitoid assemblage size and host ranges in a parasitoid (Hymenoptera)–agromyzid (Diptera) system from central Argentina. *Bulletin of Entomological Research*, **89**, 193–197.
- Shaw, M.R. (1994) Parasitoid host ranges. *Parasitoid Community Ecology* (ed. by B. A. Hawkins and W. Sheehan), pp. 111–144. Oxford University Press, New York, New York.
- Singer, M.C. (2000) Reducing ambiguity in describing plant-insect interactions: “preference”, “acceptability” and “electivity”. *Ecology Letters*, **3**, 159–162.
- Stanko, M., Miklisová, D., Gotý de Bellocq, J. & Morand, S. (2002) Mammal density and patterns of ectoparasite species richness and abundance. *Oecologia*, **131**, 289–295.
- Stireman, J. (2016) Community ecology of the ‘other’ parasitoids. *Current Opinion in Insect Science*, **14**, 87–93.
- Straub, C.S., Ives, A.R. & Gratton, C. (2011) Evidence for a trade-off between host-range breadth and host-use efficiency in aphid parasitoids. *The American Naturalist*, **177**, 389–395.
- Thompson, J.N. (2005) *The Geographic Mosaic of Coevolution*. The University of Chicago Press, Chicago, IL.
- Tonhasca, A. Jr. (1996) Interactions between a parasitic fly, *Neodohrniphora declinata* (Diptera: Phoridae), and its host, the leaf-cutting ant *Atta sexdens rubropilosa* (Hymenoptera: Formicidae). *Ecotropica*, **2**, 157–164.
- Tonhasca, A. Jr., Bragança, M.A.L. & Erthal, M. Jr. (2001) Parasitism and biology of *Myrmoscarius grandicornis* (Diptera, Phoridae) in relationship to its host, the leaf-cutting ant *Atta sexdens* (Hymenoptera, Formicidae). *Insectes Sociaux*, **48**, 154–158.
- Tylianakis, J.M., Tscharntke, T. & Lewis, O.T. (2007) Habitat modification alters the structure of tropical host–parasitoid food webs. *Nature*, **445**, 202–205.
- Vázquez, D.P., Melián, C.J., Williams, N.M., Blüthgen, N., Krasnov, B.R. & Poulin, R. (2007) Species abundance and asymmetric interaction strength in ecological networks. *Oikos*, **116**, 1120–1127.
- van Veen, F.J.F., Müller, C.B., Pell, J.K. & Godfray, H.C.J. (2008) Food web structure of three guilds of natural enemies: predators, parasitoids and pathogens of aphids. *Journal of Animal Ecology*, **77**, 191–200.
- Vizentin-Bugoni, J., Maruyama, P.K. & Sazima, M. (2014) Processes entangling interactions in communities: forbidden links are more important than abundance in a hummingbird-plant network. *Proceedings of the Royal Society of London Series B: Biological Sciences*, **281**, 20132397.
- Wetterer, J.K., Schultz, T.R. & Meier, R. (1998) Phylogeny of fungus-growing ants (tribe Attini) based on mtDNA sequence and morphology. *Molecular Phylogenetics and Evolution*, **9**, 42–47.

Accepted 8 September 2017

First published online 5 October 2017

Associate Editor: Chris Hassall