No evidence of strong host resource segregation by phorid parasitoids of leaf-cutting ants

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ABSTRACT

Resource segregation by species is a cornerstone ecological concept that may result from several processes such as interspecific competition, and can help structuring communities, in particular parasitoid communities. Phorid parasitoid flies that use ants as hosts usually employ one host per individual parasitoid, and thus the pressure for segregating the host resource should be high. At a particular community, these parasitoids might segregate resources by temporal differences in activity patterns, using different host species or nests from those available. Even if parasitoid species coexist on the same nest, they can take advantage of worker polymorphism and task division, searching for ants performing different tasks at different microsites of the same nest. Here we evaluated the segregation of parasitoid species in these hypothesized axes using leaf-cutting ant phorid parasitoids as a model system. We analyzed temporal data collected at two localities with contrasting host species richness; and compared parasitoid co-occurrence at the different niche axis. For most of the hypothesized niche axes tested we found either no departures from random expectations or significantly more niche overlap than expected by chance, ruling out the existence of biologically relevant host resource segregation in this system. However, there was evidence of segregation for some species, since one parasitoid species was only found in winter and another species showed a negative correlation of its abundance over nests with other two species. Furthermore, we found that several species were flexible in host use; *Atta* phorids varied in average host sizes preferred, whereas *Acromyrmex* phorids that were generalists were able to use different host species or microsites for host location. From an applied perspective, these results are encouraging when selecting species for the control of leaf-cutting ants because parasitoids coexistence seems to be unaffected by their overlap in niche dimensions.

1. Introduction

Segregation among species sharing a resource at a given community may be the result of different mechanisms, typically to avoid inter-specific competition (Schoener, 1974), due to morphological and physiological specialization (Blum, 1981), different responses to environmental gradients (Whittaker, 1967), predation (Connell, 1970), or a mixture of mechanisms (Dunham and Tinkle, 1978). Regardless of the mechanism involved, segregation promotes species coexistence in communities (Tokeshi, 1999). In the case of parasitoids, competition is expected as it has been proved to be an important force structuring their communities (Hawkins and Goeden, 1984; Harvey et al., 2013).

Parasitoid species in the family Phoridae are dipterans that attack adult worker ants by injecting an egg into the host's body. In most species, only one larva develops per worker (Brown, 1999; Consoli et al., 2001), thus, interspecific competition might be important in

these parasitoids. Phorid parasitoids of fire ants have received the most attention as a result of their introduction in the USA to control their invasive hosts (Porter and Gilbert, 2004). Competitive displacement has been reported for these parasitoids, both in their native and introduced ranges (Lebrun et al., 2009). In addition, daily and monthly differences in the activity patterns of these phorid parasitoids seem to be important to segregate the host resource (Pesquero et al., 1996; Folgarait et al., 2003, 2007a). These parasitoids may use different nests of the same host species to segregate the resource, thus decreasing the co-occurrence of species at the same nest (Folgarait et al., 2007b). Even if phorid species coexist at the same nest, parasitoids can take advantage of the polymorphism and task partitioning that characterizes most ant species (Hölldobler and Wilson, 1990), segregating the host resource by attacking workers with different sizes or performing tasks at different microsites of the same colony (Orr et al., 1997; Morrison and Gilbert, 1998; Brown, 1999; Folgarait et al., 2006).

A group of phorid parasitoids use only leaf-cutting ants as hosts (Disney, 1994; Elizalde and Folgarait, 2011). The larvae of these parasitoids develop exclusively within leaf-cutting ant workers that are outside the nest, and in most species only one individual develops per host (Elizalde and Folgarait, 2011). Most leaf-cutting ant species are attacked by more than one phorid species (Feener and Brown, 1993; Tonhasca et al., 2001; Bragança and Medeiros, 2006; Folgarait, 2013; Elizalde et al., 2018), therefore it is highly likely that several phorid species interact over the same host. Some evidence suggest that these parasitoids might be segregating the host resource, given that there are phorids of leaf-cutting ants that attack workers at foraging trails, cutting sites and refuse piles (Tonhasca et al., 2001; Elizalde and Folgarait, 2012). However, host resource segregation by phorid parasitoids of leaf-cutting ants is poorly studied.

We studied whether leaf-cutting ant phorids segregate their hosts at different niche axes. At a local scale in two localities with extreme host richness, we evaluated whether these parasitoids segregate by: (1) time, (2) using different host species, (3) using different nests, (4) using different microsites for host search at the same nest, or (5) using different host sizes. Furthermore, we explored the differences between the host-parasitoid systems involving *Atta* or *Acromyrmex* as hosts, which do not share parasitoid species, and thus can be considered as different guilds (Elizalde and Folgarait, 2011; Folgarait, 2013).

2. Methods

2.1. Sampling and study system

We sampled at two localities separated by 260 km, one with eight leaf-cutting ant species (San Cristóbal, Santa Fe, Argentina, 30° 12′ S, 61° 09′ W) and the other with three leaf-cutting ant species (Noetinger, Córdoba, Argentina, 32° 19′ S, 62° 21′ W). Both localities were in the Espinal phytogeographical province, with similar landscapes of undisturbed xerophyllous subtropical forests dominated by trees such as *Prosopis* spp. (Cabrera, 1994). The parasitoid communities of both localities, together with their interactions, were described in detail in Elizalde et al. (2018). The high richness community represents the greatest local assemblage of leaf-cutting ant richness reported so far (see Elizalde et al., 2018). These species were *Atta vollenweideri*, *Acromyrmex crassispinus*, *Ac. fracticornis*, *Ac. heyeri*, *Ac. hispidus*, *Ac. lobicornis*, *Ac. lundii* and *Ac. striatus*. In Noetinger, the species were all from the genus *Acromyrmex*: *Ac. crassispinus*, *Ac. heyeri* and *Ac. lundii*. In the high richness locality there were 19 parasitoid species, with six of them using *Atta* as a host and the rest exclusively attacking *Acromyrmex*. Five of the six parasitoid species using *Atta* were quite abundant (*Apocephalus setitarsus*, *Ap. vicosae*, *Eibesfeldtphora trilobata*, *Myrmosicarius brandaoi*, *M. gonzalezae*). In contrast, most of the parasitoid species using *Acromyrmex* as host had lower abundances (with *Ap. neivai*, *M. catharinensis*, *M. cristobalensis*, *M. crudelis*, *M. longipalpis*, being the most abundant; see Elizalde et al., 2018 for more details). We found six parasitoid species in the low richness locality (with *Ap. neivai*, *M. catharinensis*, and *M. crudelis* as the most abundant ones).

At each locality, we selected six nests per leaf-cutting ant species as replicates, where we sampled for phorids along the four seasons of a year. If a nest was inactive one season, we selected a nearby nest with similar characteristics. In each nest, we sampled phorids during three time periods: morning (from the first hours of daylight to midday approximately), afternoon (from around 13 h until twilight) and night (without sunlight). Each sampling over the same nest was carried out on different days, in order to reduce the effect of parasitoid removal. The length of these sampling periods varied according to the season. In addition, during winter, most ants did not start foraging until 10:00 h, thus we continued sampling nests during midday, and that data was not included in our analysis of host segregation by attacking at different times of day (see below). We collected all the phorids during each time period with an aspirator, searching for phorids on foraging trails, over

the nests, at cutting sites, and in the external refuse piles when present. We called this "adult parasitoid collection" (APC; Elizalde and Folgarait, 2011). The order in which we started to sample nests in each season was randomized, and we left as much time as possible between sampling periods for the same nest. For nocturnal sampling, we used a low-intensity red LED light head lamp, so that ants and phorids would be visible without being disturbed.

We also performed what we called a "larvae parasitoid collection" (LPC; Elizalde and Folgarait, 2011) of phorids, which consisted in manually collecting during 30 min all the ants on a foraging trail crossing a point 2 m away from the nest entrance. This method yields more parasitoid species (Elizalde and Folgarait, 2011), and shows how species interact at a wider temporal frame (see "Temporal segregation"). We performed the LPC once per season for the three same nests after finishing the APC in all nests. We reared the parasitoids in the laboratory and identified the emerged adults to the species level (for details see Elizalde and Folgarait, 2011).

2.2. Segregation analyses

We used null model analyses in order to evaluate deviations from random distributions of resource use by phorid parasitoids in the different niche axes. Null models use randomizations that in general are interpreted to produce a pattern that would be expected in the absence of a particular ecological mechanism (i.e., by chance). If the observed community patterns cannot be distinguished from those generated by the null model, we can conclude that the mechanism is not operating (Gotelli and Graves, 1996). We built different null models for each of the niche axes evaluated, and compared them with our data. In the case of segregation by parasitoid species in a particular axis, we expected lower overlap values in our data than in the simulated values generated by chance. This approach does not tell which species pairs may show resource partitioning, but aimed to test whether the resource utilization matrix differs or not from random expectations.

2.3. Temporal segregation

We built matrices with the abundance of each parasitoid species (in rows) at each sampling through the year (in columns), pooling the abundance of parasitoid species collected by APC for each time of the day. Matrices were built for *Acromyrmex* and *Atta* hosts separately, given that ant species of these genera do not share parasitoids (Elizalde and Folgarait, 2011; Folgarait, 2013). To assess whether these parasitoid species segregate temporally, we used the Czechanowski index that summarizes the average pairwise niche overlaps in an assemblage, by using resource utilization of each species (i.e. abundance of parasitoids over hosts) (Feinsinger et al., 1981). This index ranges from 0 to 1, indicating null and complete overlap, respectively. We compared the observed Czechanowski index with indices generated after 1000 randomizations with the ROSARIO algorithm. This randomization algorithm performs random shifts of entire distributions but preserves the temporal autocorrelation inherent in activity patterns (Castro-Arellano et al., 2010). We used the software TimeOverlap (Castro-Arellano et al., 2010). We also evaluated the temporal segregation of species with data obtained by LPC for seasonal variation. This analysis involved assessing the temporal segregation at a wider time frame because it aggregated information from several days (Elizalde and Folgarait, 2011). We expected lower values of the observed than the simulated Czechanowski index as an indication of segregation by parasitoids.

In addition, we compared the relative abundance of each parasitoid species by time of day, i.e. morning, afternoon and night (except for winter, when sampling was carried out during midday, when ants were active), in order to test if the pattern obtained differed from a random one towards significantly lower values suggesting segregation throughout the day. Data was not sufficient to build null models, so we evaluated diel segregation by directly comparing the relative

abundances.

2.4. Host species

We first measured the overlap of phorid species attacking the same host species at each season with the Czechanowski index. The matrices for these analyzes included *Acromyrmex* ant species as columns and phorid species as rows, with the summed abundance of female parasitoids collected through APC at each nest (pooling morning and afternoon, but not nocturnal periods because there were few individuals and not all species were active, see Results) for each sampling season. We only included *Acromyrmex* hosts, because only one *Atta* species was recorded in our sampling, so there was no option to segregate along this axis for the six phorid species using *Atta vollenweideri*. For each season (i.e. for each matrix), we pooled parasitoid species collected at different microsites (foraging trails, cutting sites, refuse piles). We compared the observed indices from collected data with those obtained by 1000 randomizations with the expectation of obtaining values close to cero and significantly different from the ones obtained by randomization in order to infer segregation. We used an algorithm that preserves the observed niche breadths and number of used resource categories (i.e., host species in this axis), but the use of each category is at random with respect to those of other species ("R3"; Gotelli and Ellison, 2013). We conducted these analyzes with the package EcoSimR v.1.0 for the R statistical environment (Gotelli and Ellison, 2013). We also analyzed the overlap of parasitoid species over hosts for a wider temporal frame using data from LPC, since it includes parasitized ants that have developing larvae inside as well as ants that were oviposited by phorids the same day of collection.

2.5. Nests

We measured the overlap of phorid species attacking at the same nests with the Czechanowski index calculated for each season and separately for *Atta* and *Acromyrmex* datasets. The matrices for these analyzes included ant nests as columns and phorid species as rows, with the summed abundance of female parasitoids collected through APC at each nest (pooling morning and afternoon, but not the night due to reduced richness and abundance at that sampling period) for each sampling season. For each season (i.e. for each matrix), we pooled parasitoid species collected at different microsites (foraging trails, cutting sites, refuse piles). We compared these indices with those obtained by 1000 randomizations using the R3 algorithm (Gotelli and Ellison, 2013), with the expectation of obtaining significantly lower values (and close to cero) of the observed than the simulated Czechanowski index as a suggestion of segregation by parasitoids. We conducted these analyzes with the package EcoSimR v.1.0 for the R statistical environment (Gotelli and Ellison, 2013). We also analyzed the co-occurrence of species of parasitoids using data from LPC.

To further investigate if particular parasitoid species were segregating by nests, we used Spearman correlations for the abundances of pairs of parasitoid species by nests (only for those with incidence higher than 20% over the nests mentioned previously, since the rest of the species had much lower incidences) attacking the same host, to evaluate if they were negatively correlated. Correlations were done separately for each community.

2.6. Microsites of the same nest

We compared the abundance of parasitoid species attacking at foraging trails, external refuse dumps, and cutting sites. Only a few parasitoid species attack hosts at more than one microsite of the nest (Elizalde and Folgarait, 2012). If phorids were using different microsites as a host segregation axis, we expected that these species attacked hosts at a microsite not used by other species at the same nest and time. It was not possible to test this axis with null models because we

recorded few instances of two or more species co-occurring at the same nest (see Results for Nests mainly for *Acromyrmex*). Thus, we compared the outcomes, i.e., segregating or not, when two or more species that were attacking ants at the same nest used different or the same microsites with binomial tests, separately for *Atta* and *Acromyrmex*. We also compared the outcomes for nests sampled through LPC at each season for the species *Ac. crassispinus* (at both localities), *Ac. hispidus* and *Ac. lobicornis,* from which we collected ants from both, external refuse piles and foraging trails.

2.7. Ant sizes

To test whether parasitoid species segregate by using different ant sizes from the same microsite, we compared observed indices with those obtained by 1000 randomizations using the ROSARIO algorithm for null models, that preserves the autocorrelation of this type of data involving ordered categories (Castro-Arellano et al., 2010). If phorids were segregating by using different ant sizes we expected to find significantly lower indices than those generated by the randomizations and with values close to cero. Thus, we grouped the ants into size categories. *Atta vollenweideri* foragers have head widths between 0.7 and 3.7 mm, but because foragers with heads smaller than 1.2 mm are not used by parasitoids (Elizalde and Folgarait, 2011; Guillade and Folgarait, 2011), we did not include them. Each category was divided into intervals by 0.5 mm, with the last one including ants greater than 3.0 mm. For *Acromyrmex* foragers, where parasitoids had the possibility of using different host species, we performed two null models, one pooling all parasitoid species regardless of host species, and another pooling parasitoid species that regularly use the same host species. In both cases, we pooled size data for all nests and sampling seasons to increase the sample size. Ant sizes used by parasitoid species were obtained from data collected by LPC, only for the high species richness locality (sample size was low to test for this axis at the other locality). We run these analyses for parasitoid species from which we reared more than five individuals, to be able to determine the size of the host used.

3. Results

We show the results for each segregation axis, first introducing the results of APC and afterwards for LPC, for each locality.

3.1. Temporal segregation

The parasitoid species that were attacking *At. vollenweideri* sampled through APC showed significantly more overlap throughout seasons than expected by chance (Czechanowski observed index = 0.31, mean simulated index = 0.20, P < 0.01; Fig. 1a) especially during autumn, when species richness was highest (Fig. 1a). Although observed index was higher than simulated one, it was not close to 1 (indicating full overlap). We did not find different overlap than expected by chance with species abundances obtained by LPC (Czechanowski index = 0.29, mean simulated index = 0.24, P = 0.42). Therefore, there was no suggestion of segregation of parasitoid activity throughout the day (Figs. 1a, SM1a). All the parasitoid species active during the night were also active during the day, but were always less abundant at night (*M. brandaoi*, *M. gonzalezae*, *Ap. longisetarum*, Fig. 1a).

Parasitoids of *Acromyrmex* collected by APC also showed significantly more overlap through seasons than expected by chance at both localities (Czechanowski index = 0.31, mean simulated index = 0.19, P = 0.01 for parasitoids at the high richness locality, Fig. 1b; Czechanowski index = 0.43, mean simulated index = 0.31, P = 0.03 for parasitoids at the low richness locality, Fig. 1c). The observed indices were higher than simulated ones, although they were not close to 1. The most abundant phorids that attack *Acromyrmex* at the high richness locality, *Ap. neivai* and *M. cristobalensis,* had similar abundances through time (Fig. 1b). Therefore, it seems that these two
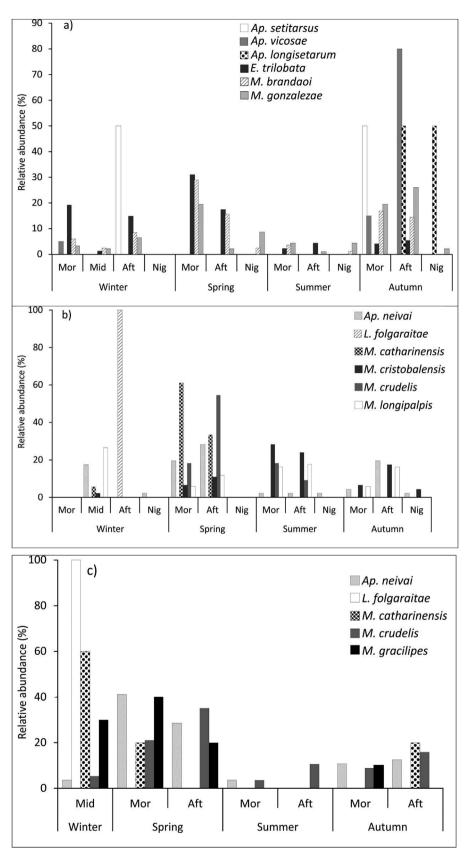
Fig. 1. Seasonal and daily variation of the percentage of relative abundance of parasitoid species attacking a) *Atta* or b) *Acromyrmex* at the high richness locality, and c) *Acromyrmex* at the low richness locality. Mid = midday, Mor = morning, Aft = afternoon, Nig = night. For parasitoid genera, *Ap.* = *Apocephalus*, *E.* = *Eibesfeldtphora, L.* = *Lucianaphora, M.* = *Myrmosicarius*.

Table 1
Overlap of phorid species on host species discriminated by seasons, measured with the Czechanowski index (Co: observed index; Cs: simulated index, ranging from 1 to 0, with 1 indicating full overlap) for a) *Acromyrmex* at the high richness locality, and b) *Acromyrmex* at the low richness locality. Probability values for less (P ≤) or more overlap (P ≥) than that expected by chance.

		Parasitoids collected as adults (APC)				Parasitoids collected as larvae (LPC)			
		Co	Cs	P ≤	P ≥	Co	Cs	P ≤	P ≥
a)	Winter	0.06	0.20	0.09	0.95	0.16	0.19	0.39	0.61
	Spring	0.06	0.20	0.08	0.96	0.04	0.21	0.06	1.00
	Summer	0.14	0.22	0.32	0.77	0.11	0.17	0.08	0.93
	Autumn	0.28	0.23	0.52	0.51	0.11	0.17	0.20	0.82
b)	Winter	0.41	0.36	0.75	0.26	0.60	0.33	1.00	0.33
	Spring	0.38	0.34	0.85	0.20	0.67	0.54	0.85	0.14
	Summer	0.00	0.33	0.33	1.00	0.66	0.49	0.85	0.18
	Autumn	0.45	0.33	0.89	0.22	0.43	0.36	0.74	0.33

parasitoid species were not segregating their activity by using different times of the day, and at night we only found *Ap. neivai* attacking ants at the high richness community (Fig. 1b), whereas no parasitoids were active at night at the low richness locality (Fig. 1c). Although two individuals of *M. cristobalensis* were also collected at night, no attacks to ants were observed.

Through LPC we found that parasitoid species that used *Acromyrmex* as host showed more coincidental activity than expected by chance at the high richness locality (Czechanowski index = 0.38, mean simulated index = 0.22, P = 0.02, Fig. SM1b), whereas no significant differences at the low richness locality were found (Czechanowski index = 0.43, mean simulated index = 0.38, P = 0.36, Fig. SM1c).

3.2. Host species

We found that phorid species did not differ from random in the use of *Acromyrmex* host species at either locality, both for APC and LPC, given that the observed Czechanowski index was in all cases similar to the mean of the simulated one (Table 1). However, there was a tendency towards less overlap of parasitoid species in the use of host species during winter and spring at the high richness locality for APC, and during spring and summer for LPC (Table 1). At the low richness locality, the indices were higher -but not significant-than the simulated ones and also than those of the high richness locality (Table 1).

3.3. Nests

All *Atta vollenweideri* nests had phorids collected by APC (except for one nest in winter) and 90% of the nests had two or more phorid species attacking simultaneously (Fig. 2a). The Czechanowski indices for parasitoid species over nests were not significant, and in general, greater than those generated by the randomizations (Table 2). The co-occurrence was even greater with data from LPC, but it was only marginally significant for winter and autumn (Table 2). In fact, we found a positive correlation between the abundances of *Eibesfeldtphora trilobata* and *M. brandaoi* at the same nest (Spearman correlation rho = 0.45, P = 0.03, N = 23) and a marginally positive correlation between the abundances of *M. brandaoi* with *M. gonzalezae* (rho = 0.38, P = 0.06, N = 23). The abundances calculated by LPC also showed a positive correlation for *E. trilobata* and *Ap. setitarsus* (rho = 0.73, P < 0.01, N = 23), but the abundances of *E. trilobata* and *Ap. vicosae* were negatively correlated (rho = −0.55, P = 0.01, N = 23) and marginally significant in the case of *Ap. vicosae* and *Ap. setitarsus* (rho = −0.43, P = 0.07, N = 23). The abundances of the remaining parasitoid species were not correlated (P > 0.10).

In contrast, a large proportion of *Acromyrmex* nests had no parasitoids at both localities according to both sampling types (range 54–75% of nests sampled with APC and 21–47% of nests sampled with LPC at the high richness locality, Fig. 2b; 22–80% of nests sampled by

APC and 0–63% of nests sampled by LPC at the low richness locality, Fig. 2c). The presence of two or three species at the same nest was very uncommon (Fig. 2). In fact, there were no differences of the observed Czekanowski's indices of overlap in comparison to those generated by the randomizations for parasitoids species attacking *Acromyrmex* at nests collected both with APC and LPC (Table 2; just the *Acromyrmex* nests in winter of the low richness locality show higher overlap that expected by chance, although the value of the index was close to zero).

If we consider the *Acromyrmex* host species attacked by more phorid species, *Ac. crassispinus*, the co-occurrence of parasitoid species on nests was higher than when all *Acromyrmex* species were considered (45 and 63% of nests with more than one phorid species collected at the same time by APC at the high and low richness localities, respectively; for the LPC, 67 and 40% of the ant nests had more than one phorid species developing simultaneously, at the high and low richness localities, respectively). Two phorid species, *Ap. neivai* and *M. crudelis*, had incidences higher than 20% over *Ac. crassispinus* nests; however their abundances at nests were not correlated at neither locality or with data from APC or LPC (all P > 0.22). The only significant correlation between the abundances of parasitoids attacking *Ac. crassispinus* at the same nest was positive, and it occurred between *Ap. noetingerorum* and *M. crudelis* with LPC at the low richness locality (rho = 0.63, P = 0.04, N = 11).

3.4. Microsites of the same nest

While most species attacked ants at foraging trails, some species oviposited at refuse piles or cutting sites. For parasitoids using *At. vollenweideri* as host, *Apocephalus setitarsus* was the only species recorded attacking ants while they were cutting leaves. In the few instances that we observed this in the field, phorids from other species were attacking ants in the same nest at the foraging trails (only 3 parasitoids in 2 nests).

Myrmosicarius crudelis, *M. gracilipes*, and *M. longipalpis* were ovipositing *Acromyrmex* ants while were depositing waste outside the nest. The latter species always attacked at refuse piles, while the first two also oviposited at foraging trails. Using different microsites was not an important host segregation axes for *M. crudelis* or *M. gracilipes* (binomial tests P > 0.17). In 25% of the times that *M. crudelis* parasitoids were sampled, they were attacking at trails of *Acromyrmex* whereas in 75% of the cases they were on refuse piles; in 72% of the times they were attacking at foraging trails where there were other parasitoid species also attacking there. A similar pattern was detected for *M. gracilipes*, where 43% of the parasitoids collected were attacking at foraging trails (always when other parasitoid species were also attacking at this microsite), and 57% of the times they were attacking on refuse piles. *Myrmosicarius longipalpis* exclusively attacked at refuse piles of *Ac. hispidus*, but almost no parasitoids were attacking this host at foraging trails (no parasitoid was recorded with APC at foraging trails of *Ac.*
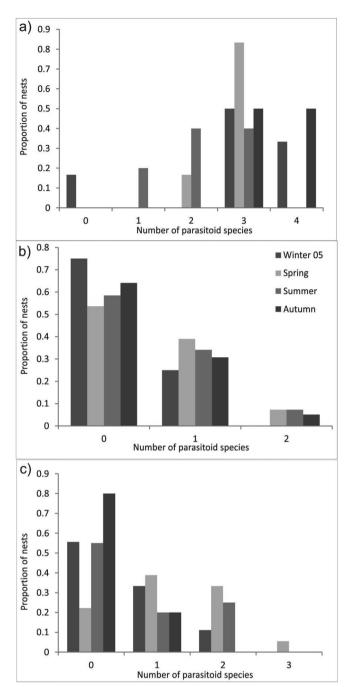
Fig. 2. Co-occurrence for parasitoid species collected attacking ants over the same nests, discriminated by season, for a) *Atta* b) *Acromyrmex* at the high richness locality, and c) *Acromyrmex* at the low richness locality.

Table 2
Overlap of phorid species on nests collected attacking ants and by rearing parasitoids from ants by seasons, measured by the Czechanowski index (Co: observed index; Cs: simulated index, ranging from 1 to 0, with 1 indicating full overlap) for a) *Atta* b) *Acromyrmex* at the high richness locality, and c) *Acromyrmex* at the low richness locality. Probability values for less (P ≤) or more overlap (P ≥) than that expected by chance.

		Adult parasitoids				Larval parasitoids			
		Co	Cs	P ≤	P ≥	Co	Cs	P ≤	P ≥
a)	Winter	0.32	0.26	0.84	0.15	0.35	0.27	0.94	0.06
	Spring	0.50	0.45	0.78	0.22	0.39	0.47	0.19	0.85
	Summer	0.55	0.42	0.92	0.07	0.74	0.24	1.00	0.23
	Autumn	0.32	0.33	0.47	0.52	0.58	0.43	0.95	0.06
b)	Winter	0.00	0.03	0.43	1.00	0.63	0.56	0.88	0.13
	Spring	0.03	0.05	0.13	0.87	0.08	0.12	0.62	0.77
	Summer	0.08	0.07	0.62	0.41	0.04	0.05	0.50	0.50
	Autumn	0.18	0.07	0.93	0.07	0.07	0.03	0.81	0.31
c)	Winter	0.18	0.08	0.96	0.05	0.05	0.05	0.65	0.35
	Spring	0.14	0.08	0.89	0.11	0.06	0.004	0.23	1.00
	Summer	0.00	0.07	0.80	1.00	0.50	0.06	1.00	0.12
	Autumn	0.10	0.08	0.80	0.22	0.09	0.18	0.25	0.91

3.5. Ant sizes

We found significantly greater overlap than expected by chance for the sizes used by all parasitoid species attacking *Atta* (Czechanowski observed index = 0.63, mean simulated index = 0.40, P = 0.02; Fig. 3), and by the most abundant parasitoid species attacking this ant (*M. brandaoi*, *M. gonzalezae* and *E. trilobata*; Czechanowski observed index = 0.60, mean simulated index = 0.41, P = 0.08). However, the overlap in the ant sizes used was asymmetrical, as *E. trilobata* overlapped 4% with *M. brandaoi*, but *M. brandaoi* overlapped 94% with *E. trilobata* (Fig. 3). On the other hand, *E. trilobata* overlapped 94% with *M. gonzalezae* in host sizes selected and 65% with *Ap. vicosae* (Fig. 3).

We found no evidence for strong segregation using different host sizes by phorids attacking *Acromyrmex*, nor a tendency for greater overlap as found in *Atta* phorids (for the parasitoid species *M. crudelis*, *M. catharinensis*, *M. cristobalnesis*, *M. longipalpis*, *Ap neivai*, *Lucianaphora folgaraitae*; Czechanowski observed index = 0.46, mean simulated index = 0.49, P = 0.10). This was true even when only examining *Ap. neivai* and *M. cristobalensis,* the two species with the highest potential for resource overlap due to shared hosts and high abundance (Czechanowski observed index = 0.63, mean simulated index = 0.65, P = 0.40).

4. Discussion

We explored host segregation axes for these parasitoids by studying a community with exceptionally high host species richness, together with a community with lower species richness. In contrast of suggesting strong segregation by time, host species, or a differential use of nests or host sizes, our data showed either no difference with a random use of resources or more overlap (i.e. superposition of species along particular niche axis). In addition, our results suggest that host-parasitoid systems involving *Atta vollenweideri* or *Acromyrmex* species hosts differed as resource for parasitoids, since the parasitoids that attack *Atta* showed more overlap at several axes, whereas the parasitoids using *Acromyrmex* seemed to be mainly unsaturated (*sensu* Rohde, 2005).

In general, our data did not show significantly less overlap than expected by chance of these phorid parasitoids over different seasons or times of the day, suggesting that there is no strong segregation of the parasitoid community along these niche axes. However, temporal resource segregation could be relevant for *L. folgaraitae* because we recorded this species attacking ants only in winter, when the abundances
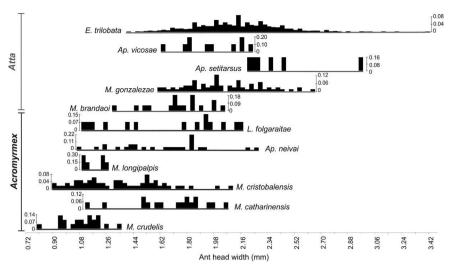
hispidus and only 5 individuals were recorded with LPC).

In very few occasions other species also attacked at refuse piles. *Apocephalus neivai* attacked ants mainly at foraging trails, and in few cases at refuse piles (only 3% of the individuals collected with APC, and none with LPC), and in all these instances there were no parasitoids of other species at foraging trails of the same nest. Only during the summer *M. cristobalensis*, a parasitoid species that searches for host at foraging trails, also attacked *Ac. lobicornis* at refuse piles. However, those attacks at refuse piles, both by *Ap. neivai* and *M. cristobalensis*, occurred when no other species was attacking at that foraging trail or even when there were other species at the refuse piles.

Fig. 3. Host ant sizes (ant head width range in mm in the *x*-axis and frequency in the *y*-axis) used by parasitoid species that attack *Atta* (first five species) and *Acromyrmex* (six last species).

of other phorid species were low. A higher tolerance to low temperatures may have given this parasitoid an advantage to exploit hosts when other species are hardly present. Similarly, those species ovipositing at night might have an advantage by avoiding interference competition with females of species that only attack during daylight hours.

Phorid species that oviposited *Atta* did not have the opportunity to segregate by host species in our study system, although it does not seem to happen elsewhere because it is infrequent that more than two species of this genus coexist at the same locality (L.E. unpublished results). However, segregating by using different host species neither seemed to be important for parasitoid species using *Acromyrmex*. Instead, we found some evidence of significantly greater overlap in host use in the low richness locality, where parasitoids had more limited host choices. The only two possible examples of segregation by using different hosts were in the high richness locality, with *M. catharinensis* over *Ac. heyeri* and *M. longipalpis* over *Ac. hispidus*. In the case of *M. catharinensis* at the low species richness locality, where there are fewer parasitoid species (e.g. *M. cristobalensis* is not present), this parasitoid species also uses other *Acromyrmex* species as hosts (Elizalde et al., 2018). Thus, it is possible that *M. catharinensis* may have been affected locally by negative interactions with another very abundant species (such as *M. cristobalensis*; Elizalde et al., 2018). However, other mechanisms need to be explored such as different micro-climatic tolerances (since host species used different micro-sites for nesting), host preferences, etc. In contrast, *M. longipalpis* was only recorded attacking *Ac. hispidus* at all localities were it was sampled (Disney et al., 2006), and thus it is not able to segregate by host species.

Using different nests does not seem to be a way to reduce the overlap over hosts by these phorid parasitoids, neither for those using *Atta* or *Acromyrmex* as host. For *Atta vollenweideri*, in fact, the six phorid species showed a greater tendency than expected by chance to overlap on nests. Almost all nests of this species had at least two parasitoid species, and in several cases, the number of species per nest was greater than three. However, we found that the abundance of one parasitoid species (*Ap. vicosae*) showed negative correlations with other two parasitoid species, suggesting that this species might segregate from the nests when the other two parasitoid species are present. In contrast, for *Acromyrmex* parasitoids there were many empty nests, and in those nests with parasitoids we found low co-occurrence of parasitoid species at both localities. This low co-occurrence was not different from that expected by chance. In addition, no pair of parasitoid species showed negative correlations of their abundances over nests. The co-occurrence for parasitoids of both *Atta* and *Acromyrmex* was higher using LPC than APC, as expected, because it represents a wider temporal window, but

the results lead to the same conclusion.

Phorid were not using different nest microsites, i.e. foraging trails or refuse piles, as a way to reduce the overlap with other parasitoid species. For example, *Acromyrmex* parasitoids *M. crudelis* oviposited ants mainly at the refuse piles where it could hardly find an already oviposited ant (ants that engage in removing wastes generally do not perform foraging activities, Hart and Ratnieks, 2001; Ballari et al., 2007) but this species also attacked ants at the foraging trails, even when parasitoid species such as *Ap. neivai* or *M. cristobalensis* were also attacking at trails. In addition, *M. longipalpis* attacked *Ac. hispidus* at refuse piles, but this was the only species that frequently attacked that host. However, as *Ac. hispidus* foraging trails were mainly buried, the use of refuse sites could also be related to host availability and location (L.E. pers. obs.). Another host location microsite was the cutting site, but the parasitoid species using it can potentially find a host already parasitized by a phorid species that attacked ants at foraging trails, especially since leaf-cutting ants from *Atta* and *Acromyrmex* tend to use the same foraging trail several times throughout days to get to cutting sites (Shepherd, 1982; Elizalde and Farji-Brener, 2012). However, it is likely that this effect is reduced because ants cutting leaves generally stay at cutting sites the whole day (Röschard and Roces, 2003). We found only one species attacking *At. vollenweideri* at cutting sites (*Ap. setitarsus*).

Host segregation by using different ant sizes does not seem to be an important mechanism since we found greater overlap than expected by chance for the sizes used by all parasitoid species attacking *Atta* and no difference from chance for the sizes used by the parasitoids that attack *Acromyrmex*. However there were differences in the sizes used by *Atta* phorids when considering central tendency measures (Elizalde and Folgarait, 2011; Guillade and Folgarait, 2011). In addition, the overlap on host sizes chosen by some phorid species attacking *Atta* was asymmetrical. This is probably a benefit for species with low overlap, or for those that are able to oviposit over a great range of ant sizes (such as *E. trilobata*) because they are able to use sizes which are not used by other parasitoid species.

In conclusion, host resource segregation does not seem to be a strong force structuring these two host–parasitoid communities. In fact, *Atta* parasitoids showed overlap in most axes evaluated. A high niche overlap may reflect intense actual competition for shared resources, or in turn it can indicate a surplus of resources and the absence of competition (Glasser and Price, 1988). Although mean percentage of parasitism in phorids that attack leaf-cutting ants in these localities is generally low (circa 4%; Elizalde and Foglarait, 2011), it can be very high in some particular situations (near 35% of foragers attacked;

Elizalde and Folgarait, 2011). It is possible that in these rare instances some parasitoid species that show flexibility in host use are able to adjust the combination of niche dimensions in order to avoid overlapping with other species. For example, the ability to change the temporal frame of host use by attacking hosts at night might help some *Myrmosicarius* and *Apocephalus* to avoid exclusively diurnal parasitoids; or by using a different host species when other preferred species is highly used by other parasitoid species for generalist *Acromyrmex* parasitoids; or by attacking hosts at refuse piles when many other parasitoids are at foraging trails; or by adjusting host size for parasitoid species that use a wide array of host sizes, such as *E. trilobata* attacking *Atta*. In fact, this flexibility is predicted by the "variable environment" hypothesis, that poses that animal populations are rarely at carrying capacity suggesting that competitive effects are important only during occasional "resource crunches" (Wiens, 1977). Moreover, our results highlight that the host parasitoid system involving *Atta* and *Acromyrmex* seem to differ regarding that flexibility, since *Atta* parasitoids appear to be more flexible in the sizes of host used, taking advantage of the great size polymorphism in *Atta* workers (Hölldobler and Wilson, 1990). On the other hand, *Acromyrmex* parasitoids are more prone to use different host species or microsites within the nest as to avoid interacting with other phorid species. More data is needed to register changes in host use when parasitoids are searching for hosts alone or with other parasitoid species.

Our findings are highly relevant when considering these parasitoids for the biological control of leaf-cutting ants, and promising for targeting at more than one parasitoid species, which is desirable when planning a control strategy. The proposed flexibility in the use of host resources by parasitoids, in the light of the presence of overlap over some niche axes, can aid in choosing appropriate species combinations in order to avoid species that are not flexible and can overlap given an increase in parasitoid abundance. However, our results should be considered only as a guide for pre-selecting parasitoid species combinations as experimental tests between selected parasitoid species should be carried out, including their interaction at different resource levels and when parasitoids increase in abundance as it occurs after applying biological control strategies.

Author's contributions

LE and PF designed the study. LE collected and analyzed data. LE and AG wrote the article. All authors read and approved the article.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.actao.2018.10.005.

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