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Does edge effect and patch size affect the interaction between ants and *Croton lachnostachyus* in fragmented landscapes of Chaco forest?

Mariana Pereyra · Rodrigo G. Pol · Leonardo Galetto

Abstract Little is known about the role of ants visiting extrafloral nectaries (EFNs) of plants in fragmented forests of South America. The aim of this work was to determine whether patch size and edge effect affect the composition and frequency of ants that visit the EFNs of *Croton lachnostachyus*, and how these changes may alter the reproductive success of plants in a fragmented landscape of the Chaco forest, Argentina. Data were analyzed considering patch size and edge effects—as indicators of fragmentation—on ant assemblages visiting plants and on plant reproductive success through a field experiment. Ant species composition differed between the edge and interior of fragments, but not among fragments of different sizes. Dolichoderinae species and some bigger ants as *Camponotus mus* (Formicinae) were more abundant at the edges, whereas Myrmicinae ants dominated the interior of

fragments. Foliar damage was higher in plants located at interior than at edges of fragments. The ant-exclusion experiment showed that seed mass, germinability, and foliar damage did not differ between control and ant-excluded plants. In contrast, fruit (year 2011) and seed production (years 2010 and 2011) was higher in control plants. We highlight the importance of studying ant–plant interactions combining different attributes of biodiversity (composition, structure, and function) to better understand ecological processes in fragmented landscapes.

Keywords Extrafloral nectaries · Ant–plant interactions · Forest fragmentation · Foliar damage · *Croton*

M. Pereyra (✉) · L. Galetto
Instituto Multidisciplinario de Biología Vegetal (CONICET-UNC), Vélez Sarsfield 1611, CC 495, 5000 Córdoba, Argentina
e-mail: mariperey@hotmail.com

R. G. Pol
Desert Community Ecology Research Team (Ecodes), IADIZA-CONICET, Casilla de Correo 507, 5500 Mendoza, Argentina

R. G. Pol
Facultad de Ciencias Exactas y Naturales, Universidad Nacional de Cuyo, Mendoza, Argentina

L. Galetto
Departamento de Diversidad Biológica y Ecología, Facultad de Ciencias Exactas, Físicas y Naturales, Universidad Nacional de Córdoba, Córdoba, Argentina

Introduction

Destruction and fragmentation of natural habitats are two of the main processes contributing to the extinction and loss in species diversity (Ehrlich 1988; Noss and Cooperrider 1994; Fahrig 2003; Hobbs and Yates 2003). Habitat fragmentation is usually defined as a landscape-scale process by which habitat loss results in the division of large, continuous habitats into smaller, and more isolated remnants (Wilcove et al. 1986; Fahrig 2003; Didham et al. 2012). In consequence, fragmentation of natural landscape leads to a reduced patch area, increased edge effects, altered patch shape, increased patch isolation, and altered matrix structure (Didham 2010).

The reduction and division of continuous habitat into small fragments and increases in edges play a fundamental role in determining the abundance and diversity of insect fauna in fragmented ecosystems (Hunter 2002). Ants are particularly sensitive to habitat fragmentation. Many studies across a wide range of environments have shown

that fragmentation is usually associated with a decrease in species richness and population abundance and changes in species composition (Bestelmeyer and Wiens 1996; Suarez et al. 1998; Carvalho and Vasconcelos 1999; Brühl et al. 2003; Underwood and Fisher 2006; Crist 2009). These shifts in ant communities are often related with alteration of abiotic conditions (Dunn et al. 2007), the availability of food and nest sites (Braschler and Baur 2003), and the abundance of mutualists and competitors that results from the reduction in fragment size and the increase in edge effect (Crist 2009; Wiescher et al. 2012).

Reproductive success of many species of plants largely depends on ant interactions. Extrafloral nectaries (EFNs) are nectar-secreting organs not directly involved in pollination, present in approximately 2–4 % of flowering plants (Weber and Keleer 2013). Although EFNs attract a variety of nectar-feeding insects (Koptur 1992), ants are by far the most frequent visitors to EFN-bearing plants both in temperate and tropical habitats (Oliveira and Brandão 1991). In the past two decades, a number of experimental field studies have shown that ant–EFN interactions may increase plant fitness by deterring leaf, bud, and flower herbivores and seed predators (Rosumek et al. 2009; Trager et al. 2010). Other studies, however, have found no apparent benefit to the plant from ant visitation (O'Dowd and Catchpole 1983; Whalen and MacKay 1988; Pereira and Trigo 2013), or that ant-derived benefits may change among habitats (Barton 1986), seasons and years (Tilman 1978), supporting the idea that ant–plant mutualisms mediated by EFNs are in many cases facultative and non-specialized (Schemske 1983; Thompson 1988; Bronstein 1998).

Ant-derived benefits and the net outcome of ant–EFN-bearing plant interactions depend on multiple factors, such as the abundance and species composition of ants and herbivorous insects that visit plants (Rico-Gray and Oliveira 2007), the aggressiveness of ants (Horvitz and Schemske 1984; Oliveira et al. 1987; Rico-Gray and Thien 1989), as well as the capacity of herbivores to avoid ants (Koptur 1984; Heads and Lawton 1985; Freitas and Oliveira 1996). Therefore, the reduction in fragment size together with increases in edge effects may influence the outcome of ant–plant interactions, since changes in environmental conditions may have a great influence in the diversity and activity patterns of ants (Crist 2009; Brandão et al. 2011; Wiescher et al. 2012).

The aim of this work was to determine whether patch size and edge effect affect the composition and frequency of ants that visit the EFNs of *C. lachnostachyus*, and how these changes alter the reproductive success of plants in a fragmented landscape in Chaco forest (Central Argentina). The specific questions we addressed were: (1) does the reduction in patch size of remnant forest alter the composition and frequency of ants visiting the EFNs of *C. lachnostachyus*? (2) does the alteration of abiotic and biotic conditions between edge and interior of forest patches change the composition and frequency of ants visiting the EFNs of *C. lachnostachyus*? (3) can these changes be related to a different level of foliar damage between plants in edge and interior of forests? (4) do these alterations affect reproductive success of plants (fruit and seed production, seed mass, germinability, and foliar damage) in a forest fragmented landscape?

Materials and methods

Study site

The Semiarid Chaco is one of the regions more threatened by the advance of the agricultural borderline and deforestation in Argentina. Within this region, the Chaco forest of Córdoba has been particularly affected by human impact. This ecosystem is currently reduced to fragments of different size as a result of disturbances during the last century (Cabido et al. 1991). Moreover, the rapid expansion and intensification of crop production (mainly maize and soybean) during the last 30 years has increased the rate of deforestation and habitat loss, leading to a 94 % reduction in woodland cover (Gavier and Bucher 2004; Zak et al. 2004).

This study was conducted in a fragmented landscape of the Chaco forest located in the eastern foothills of the Sierras Chicas, Córdoba, Argentina. The study site is located between 31°11′19″S; 64°16′02″W; and 31°13′05″S; 64°15′55″W; elevation is between 500 and 600 m.a.s.l. (Fig. 1). Mean annual precipitation is 750 mm and occurs between October and May (Grilli et al. 2013). The characteristic vegetation is a low and open woodland, with tree layer (8–15 m tall) dominated by *Aspidosperma quebracho-blanco* Schltdl., *Prosopis* spp., *Zanthoxylum coco* Gillies ex Hook. f. & Arn. and *Lithraea molleoides* (Vell.) Engl.; shrubs (1.5–3 m height) dominated by *Celtis ehrenbergiana* (Klotzsch) Liebm. and *Acacia* spp.; herbs and grasses (0–1 m) and many vines and epiphytic bromeliads (Grilli et al. 2013). The landscape is characterized by forest remnants in an agricultural matrix of soybean or corn in summer and wheat and soybean in winter–spring (Grilli et al. 2013).

Study species

The monophyletic genus *Croton* comprises at least 800 species in the tropics and subtropics (Webster 1993; 1994). Members of Euphorbiaceae, including *Croton*, commonly have two nectary types, floral and extrafloral nectaries

Fig. 1 Study site in the Chaco forest of central Argentina. In *black*, the forest fragments and continuous forest selected. The number for each site correspond to: small fragments, *1* (S1): 1.48 and *2* (S2): 1.72 ha; medium fragments, *3* (M1): 3.91 and *4* (M2): 3.93 ha; big fragments, *5* (B1): 14.26 and *6* (B2): 16.38 ha; continuous forest *7* (CO): >400 ha. Image obtained from Google Earth
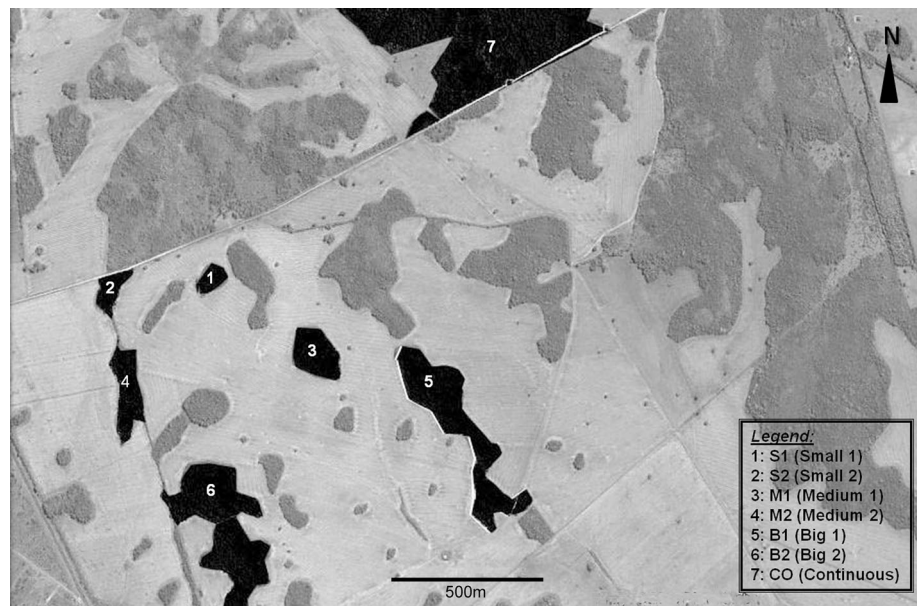
(EFNs), that are usually morphologically different and with distinct evolutionary origin (Bernhard 1966; Webster 1994). *C. lachnostachyus* Baill. is a diclinous monoecious shrub common in Argentina with a distribution from Jujuy to Córdoba provinces, growing between 250 and 1300 m.a.s.l. (Croizat 1941). This species fruits from November to June and display floral, EFN, and post-floral nectaries (Freitas et al. 2001). EFNs are found on different plant parts: 2–8 glands are usually located on the adaxial surface of the petiole distal portion, while there are 2–4 glands on the stipules. EFNs on the leaf margins vary from none to more than 10 glands per leaf. Both stipule and leaf EFNs are diminutive and produce small amounts of secretion. Petiolar EFNs are patelliform, stalked, and vascularized. All nectary types produce small amounts of highly viscous nectar (c. 1 μl, concentration: 60–80 %) (Freitas et al. 2001). In a previous work, Freitas et al. (2001) recorded a total of 20 insect species on the petiolar EFNs and the postfloral nectaries of *C. lachnostachyus*: three Coleoptera species (Coccinellidae, Curculionidae, and Dasytidae), one Diptera species (Sarcophagidae), and 16 Hymenoptera species (five wasps: one Braconidae, Encyrtidae, and Vespidae, and two Pteromalidae; and 11 ant species: Formicidae).

Study design

For this study, we selected six forest fragments ranging from 1.5 to 16.4 ha, and a continuous forest (ca. 400 ha) >50 m apart from each other (Fig. 1). All fragments have comparable south-east orientation and vegetation stratification and are included within the same biogeographic

region (Chaco Serrano Forest), thus sharing similar climatic conditions.

The effects of patch size on ant species composition and frequency of ants that visit the EFNs of *C. lachnostachyus* were assessed during the reproductive season (spring–summer) of years 2010 and 2011, whereas the edge effects on the same variables were evaluated on year 2011 (Fig. 2). On the other hand, the effect of ants on the reproductive success of *C. lachnostachyus* was evaluated through an ant-exclusion experiment performed during the summer of years 2010 and 2011(Fig. 2).

For all these purposes, we chose focal adult individuals of *C. lachnostachyus* with similar morphological features (i.e., height ca. 1 m, number of branches approximate eight) that were located at least 5 m apart from each other in every condition (interior or edge of each fragment). Individuals or pairs of individuals at edges were selected in the first 3 m from the matrix, whereas those at interior were >15 meters inside from the edge (Fig. 2). Since there is no available quantification of edge effects in the Chaco, we arbitrarily defined edge as the belt of vegetation growing up to 10 m into the fragment in concordance with Christianini and Oliveira (2013).

Ant surveys

To determine the composition and frequency of ants visiting EFNs of *Croton lachnostachyus*, we chose three individuals in each of the forest fragments and continuous forest, selected in summer 2010. In summer 2011, we repeated ant sampling in other three individual plants at forest interior and at three individual plants at edges of
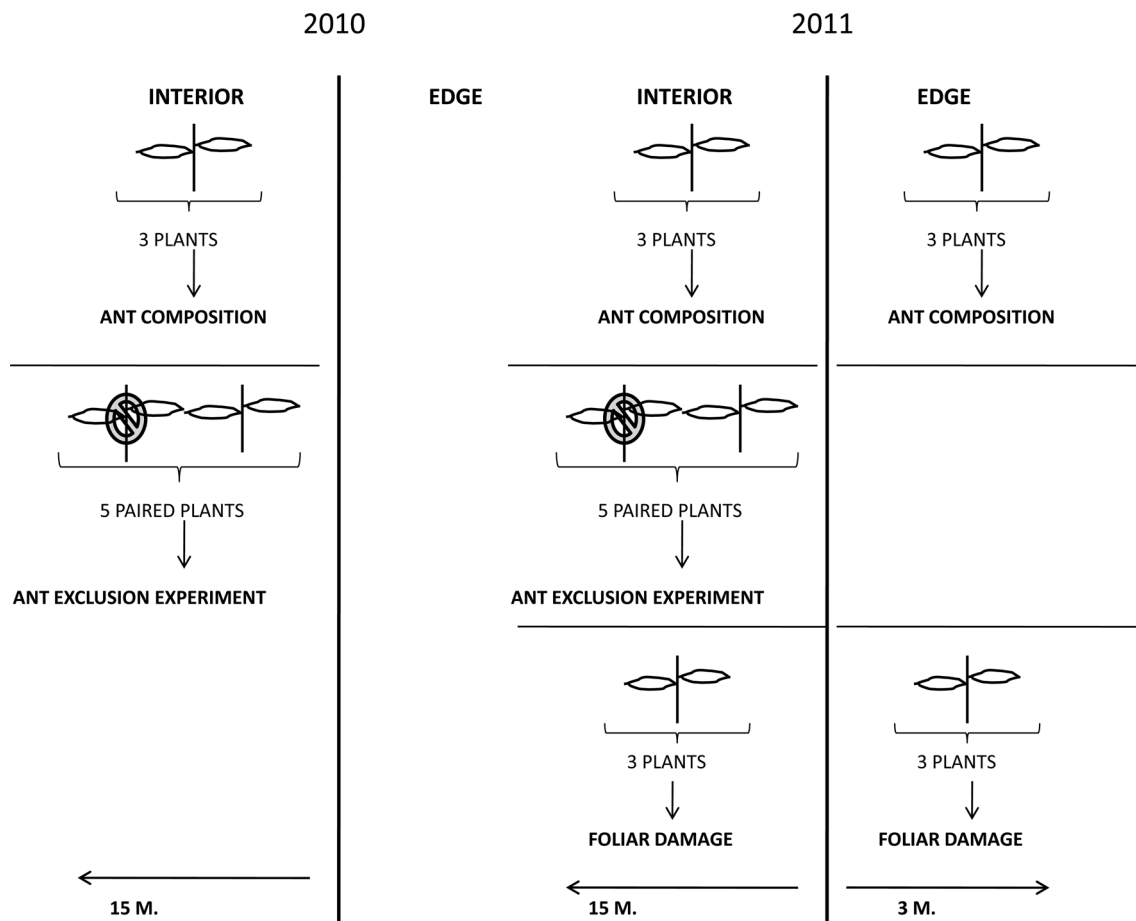
Fig. 2 Experimental design performed to analyze ant–plant responses along a forest fragmentation gradient including a continuous forest ($n = 7$) in Córdoba, Argentina. Number and location of plants of *Croton lachnostachyus* selected in two consecutive reproductive seasons (2010 and 2011). See details on the text for each of the variables considered in the study

fragments (Fig. 2). We registered the number and species of ants visiting EFNs during 2 min in two randomly selected branches of each plant. Samplings were carried out early in the morning (6–8 h) and before midday (10–12 h) in sunny days. For each plant, we calculated the frequency of ant visits using morning and midday data. Ant specimens that could not be identified in the field were collected and determined in the laboratory using current available taxonomic keys.

Plant reproductive success and foliar damage

To evaluate the effect of ants visiting EFNs on the reproductive success of *C. lachnostachyus*, we chose five pairs of plants in each fragment and continuous forest in summer 2010 and 2011 (Fig. 2). One plant of each pair was randomly selected for ant-exclusion treatment, while the other served as un-manipulated control. Ants were excluded using a sticky repellent resin (Tanglefoot Co., MI, USA) applied at the base of the plant on an adhesive tape. Ants

found on treated plants were removed before resin application. Stem and grass bridges that could allow ants to climb onto treatment plants were also removed. Ant-exclusion experiment was carried out between January and February on 2010 and January and March on 2011. Plants and repellent barriers were checked every week, until the fruit ripening.

For each plant, we estimated fruit and seed production, seed mass, and seed germinability. We marked three inflorescences by plant, which were checked periodically until fruit ripening. When they were mature, we harvested and stored every inflorescence in labeled bags for later analysis. In the laboratory, we counted the well-formed fruits (without signals of damage or herbivores attack) and seeds per inflorescence. To assess differences in seed mass between treatments, we weighed 10 randomly selected seeds per plant, and we estimated the average seed mass for each plant.

To evaluate seed germinability, we placed 10 seeds of each plant in labeled Petri dishes inside a germination

chamber with controlled temperature (24.5 ± 0.5 °C) and a light–dark cycle of 12 h. Labeled Petri was watered every day. We recorded the number of germinated seeds every week during 2 months; afterwards, we calculated the mean percentage of seeds germinated per plant.

We estimated the foliar damage caused by herbivores in plants excluded and non-excluded for ants. For this, at the beginning of the experiment (January 2010, 2011), we chose and marked five undamaged leaves of similar size in each treated and control plants. On February (2010) and March (2011), marked leaves were collected. In the laboratory, we scanned the leaves and estimated the average foliar damage per plant as the percentage of total area attacked by herbivores using Adobe Photoshop CS3. We considered leaf damage when parts of the lamina were missing, including holes, scraped-off areas, or incomplete leaf margins. Most of this damage corresponds to chewing insects and/or the combined effect of pathogens (Ruiz-Guerra et al. 2010). Additionally, following the same procedure described above, we estimated the foliar damage in plants located on the edge and the interior of the fragments and continuous forest, where we randomly selected five leaves in each of the three plants described above (Fig. 2).

Nutrient content and light availability could affect EFNs traits (e.g., their number, production of nectar) and, therefore, have an effect on the nectar rewards available for ants (Agrawal and Rutter 1998; Heil et al. 2001; Heil 2008). In order to test the assumption that number of EFNs did not varied between plants of the edge and interior of fragments, we counted the number of EFNs in five leaves of five randomly selected plants located at edge and interior of the same seven forest fragments studied (two small, two medium, two big, and one continuous) (Fig. 1). As we expected, the mean ($\pm$SD) number of EFNs per leave was very similar between plants from edge and interior of fragments (interior 2.42 ± 0.83; edge: 2.75 ± 1.04; $\chi^2 = 7.41$, $P > 0.05$).

Data analysis

Differences in the frequency of ants visiting EFNs between fragments of different size at interior (2010, 2011) and edge (2011) were assessed using a generalized linear model (GLM) conducted in R v.2.12.0 (R Core Development Team 2012). The variable "fragment size" was considered as categorical. We analyzed the mean number of visits per plant with Poisson error structure and log, and when the overdispersion parameter was >2, quasipoisson error structure and log was used as detailed in Zuur et al. (2009).

To analyze foliar damage between plants located at the interior and the edges of fragments, we conducted a one factorial permutation ANOVA, with "fragments" nested in "position" of the plant in the fragment (interior or edge). Permutation ANOVA analysis was conducted using the lmPerm package within R v.2.12.0 (R Core Development Team 2012) and the aovp () function.

We examined the reproductive success (fruit and seed production, seed mass, germinability, and foliar damage) of ant-excluded and control plants of *C. lachnostachyus* considering fragments of different size. The variable "fragment size" was considered categorical, as described above. Each response variable was analyzed with generalized linear mixed models (GLMM) in R v.2.12.0 (R Core Development Team 2012) with ant-exclusion treatment and size of fragment as fixed effects, including the interaction term. Generalized mixed-effect models use the concept of random effects to emulate the randomness inherent in the data (Millar and Anderson 2004). "Pair of plants" term was nested within ''forest fragments'' and specified as a random effect to avoid spatial pseudo replication. Variables fruit and seed production and seed mass were fitted with a Poisson error structure and log. For the variables germinability and foliar damage, we used Binomial error structure and logit. The significance of model terms was assessed by examining changes in the deviance using Chi-square test.

Results

Ant species composition

A total of 21 species were found visiting EFNs of *C. lachnostachyus*, representing nine genera and four subfamilies (Table 1). In the interior of the forest fragments, most of the species occurred in more than one fragment. The total number of species registered per fragment ranged from two (M2, B2) to five (S2) in 2010 and from one (B1, B2, CO) to three (S2, M1) in 2011.

From the 21 species collected, nine (43 %) were found exclusively in the interior, seven (33 %) on the edges of fragments, whereas only five (24 %) species occurred in both sites. In the interior of fragments, we found only one specie representing the Dolichoderinae family, whereas five species, including the invasive ant *Linephitema humile*, were collected at the edges in fragments of different sizes (Table 1). Ants from the subfamily Myrmicinae were usually present at interior of fragments. That is, from the seven species registered, five were found exclusively at interior, no one was exclusively found at edges of fragments, and two of them were present in both sites.

Table 1 Ants found visiting EFNs of *Croton lachnostachyus* at the interior and edges along a forest fragmentation gradient (n = 7) including a continuous forest in Córdoba, Argentina in years 2010 and 2011

Subfamily	Species	2010 S1 I	S2 I	M1 I	M2 I	B1 I	B2 I	CO I	2011 S1 E	S1 I	S2 E	S2 I	M1 E	M1 I	M2 E	M2 I	B1 E	B1 I	B2 E	B2 I	CO E	CO I
Dolichoderinae	*Dorymyrmex* sp. 1																				•	
	Dorymyrmex sp. 2																				•	
	Dorymyrmex thoracicus										•				•						•	
	Linephitema gallardoi	x	x	x			x							x								
	Linephitema humile								•				•		•		•					
	Linephitema sp.1								•													
Formicinae	*Brachymyrmex patagonicus*				x		x		•	x			•					x	•			
	Brachymyrmex sp. 1			x		x	x					x										
	Camponotus mus																		•	x		
	Camponotus punctulatus												•									
	Myrmelachista nodigera		x																			
Myrmicinae	*Crematogaster cisplatinalis*	x	x	x		x	x					x										
	Crematogaster sp. 1									x	•		•	x			x					
	Pheidole radoskowsky				x																	
	Pheidole sp. 1	x	x																			
	Pheidole sp. 2													x								
	Pheidole sp. 3				x		x						•		•	x					•	x
	Wasmannia sulcaticeps				x																	
Pseudomyrmecinae	*Pseudomyrmex denticollis*											x									•	
	Pseudomyrmex gracilis		x																			
	Pseudomyrmex pallidus																				•	

Fragments abbreviations: *S1* small 1, *S2* small 2, *M1* medium 1, *M2* medium 2, *B1* big 1, *B2* big 2, *CO* continuous. Plant location: *E* Edge (dot) *I* Interior (x)

Ant visitation frequency

The frequency of ants visiting the EFNs of *C. lachnostachyus* differed among fragments of different size during summer 2010 ($F = 14.64$, $P < 0.05$; Fig. 3a) and 2011 ($F = 30.43$, $P < 0.05$; Fig. 3b). Nevertheless, differences were not consistent with the fragmentation gradient or between years (Figs. 3a, b).

In contrast, the frequency of ants visiting the EFNs of *C. lachnostachyus* differed among the edge and interior of fragments ($F = 36.52$, $P < 0.05$; Fig. 3c), but again differences were not consistent with the fragmentation gradient.

The most frequent visitor species at the interior of fragments were *Crematogaster cisplatinalis*, *Brachymyrmex patagonicus*, and *Pheidole* sp. 3 (Fig. 4) and at edges of fragments (in 2011) were *B. patagonicus* and *Camponotus mus* (Fig. 4).

Foliar damage

Foliar damage was higher in plants located at the interior than in those located at the edge of forest fragments ($F = 3.75$, $P < 0.05$; Fig. 5). Even though most plants had low levels of hervibory, significant differences were obtained for all the fragment sizes and continuous forest, except fragment S2 (Fig. 1). At the interior of fragments, foliar damage ranged between 1 and 14 % and at the edges plants nearly did not suffer leaf damage (0–1.5 %).

Fig. 3 *Box plots* for the number of ant visits per plant of *Croton lachnostachyus* per fragment in Córdoba (Argentina): **a** and **b** at the interior of the forest fragments and continuous forest for two plant reproductive seasons, 2010 and 2011, respectively, and **c** at the edges of the fragments and continuous forest for one reproductive season, 2011. Fragments: *S1* small 1, *S2* small 2, *M1* medium 1, *M2* medium 2, *B1* big 1, *B2* big 2, *CO* continuous forest. (Generalized Linear Model test): **a** (Interior 2010): $F = 14.64$, $P = 2.763e^{-0.8}$; **b** (Interior 2011): $F = 30.43$, $P = 1.684e^{-12}$; **c** (Edge 2011): $F = 36.52$, $P = 1.169e^{-13}$)
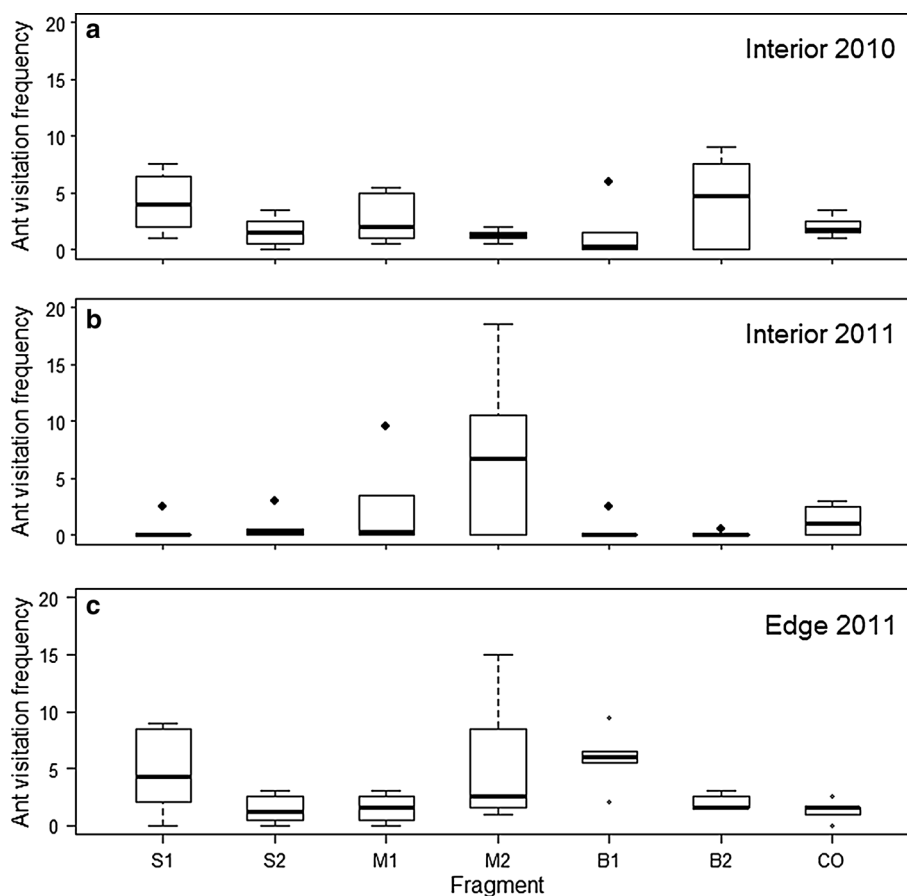
Ant-exclusion experiment

Fruit production was higher in control than in ant-excluded plants in 2011 ($\chi^2 = 14.9$, $P < 0.05$), but did not differ in 2010 ($\chi^2 = 97.5$, $P > 0.05$). In the year 2010, we found an effect related to the fragment size term ($\chi^2 = 22.54$, $P < 0.005$), but we did not detect any relevant interaction between fixed factors ("fragment size" and "treatment") neither in year 2011 nor in 2010 (Table 2).

Seed production differed between plant treatments and fragment size in summer 2011 ($\chi^2 = 28.4$, $P < 0.05$; $\chi^2 = 22.2$, $P < 0.05$, respectively) and 2010 ($\chi^2 = 44.5$, $P < 0.05$; $\chi^2 = 53.4$, $P < 0.05$, respectively). In both reproductive seasons, control plants produced more seeds than those excluded from ants (Fig. 6). Despite the term related to fragment size was significant, we did not find a consistent pattern related to fragmentation gradient (Fig. 6). The interaction term was marginally significant for summer 2011 data ($\chi^2 = 11.3$, $P = 0.08$) and significant for 2010 data ($\chi^2 = 43.5$, $P < 0.05$) (Table 2).

Seed mass, germination, and foliar damage data did not show significant differences for the terms involved in the model (fragment size, plant treatment, and the interaction between them) (Table 2).

Discussion

Does the reduction in patch size of remnant forest alter the composition and frequency of ants visiting the EFNs of *C. lachnostachyus*?

In contrast to the work published by Freitas et al. (2000) where they found 11 ant species visiting EFNs of *C. lachnostachyus*, we found 21 species visiting EFNs in all the fragments studied. A clear and consistent pattern related to size of forest fragments of ant composition associated to EFNs of *C. lachnostachyus* was not detected for plants located at forest interior. Although there are studies in which habitat fragmentation effects on ant communities were reported (Suarez et al. 1998; Vasconcelos et al. 2006), other works did not (Zschokke et al. 2000). For example, it is interesting to note that generalist and specialist ants vary in a similar way at tropical continuous forests and at fragments of different area (Bruna et al. 2005; Passmore et al. 2010). Unlike oceanic island systems, however, species–area relationship across habitat patches frequently did not show clear patterns (Ewers et al. 2007), particularly when there is an overriding influence of external variables, such as context dependence in the effects of the
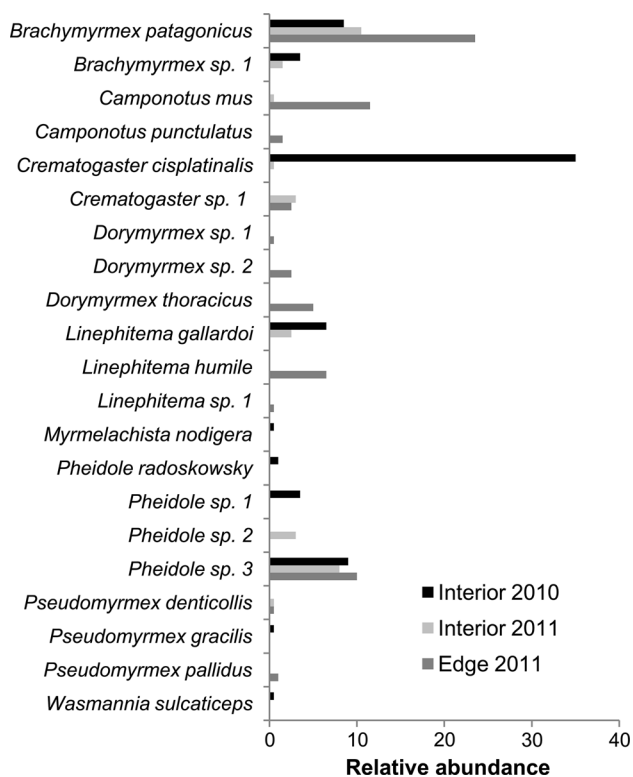
Fig. 4 Abundance of ants' species found visiting EFNs of *Croton lachnostachyus* at forest interior (years 2010, 2011) or edges of the six forest fragments and continuous forest selected (year 2011) in Córdoba, Argentina. Abundance was calculated as the total number of ant species recorded at each site (interior or edge) of the forest fragments and continuous forest
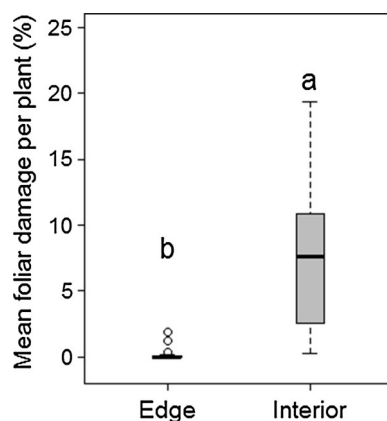
Table 2 GLMM outputs of the five variables used to characterize the reproductive success of *Croton lachnostachyus* in control and ant-excluded plants along a forest fragmentation gradient (n = 7) including a continuous forest in Córdoba, Argentina

	2010			2011		
	χ^2	Df	P	χ^2	Df	P
Fruit production						
Treatment (T)	97.5	7	0.203	14.93	7	0.037*
Fragment (F)	22.54	12	0.032*	14.06	12	0.297
T × F	91.31	6	0.166	29.94	6	0.809
Seed production						
Treatment (T)	44.45	7	<0.0001*	28.42	7	0.0002*
Fragment (F)	53.42	12	<0.0001*	22.2	12	0.035*
T × F	43.5	6	<0.0001*	11.33	6	0.079
Seed mass						
Treatment (T)	0.002	7	1	0.003	7	1
Fragment (F)	0.02	12	1	0.01	12	1
T × F	0.0006	6	1	0.003	6	1
Germination						
Treatment (T)	3.76	7	0.807	6.19	7	0.518
Fragment (F)	8.77	12	0.723	11.77	12	0.465
T × F	2.2	6	0.9	6.07	6	0.416
Foliar damage						
Treatment (T)	0.33	7	0.999	0.38	7	0.999
Fragment (F)	0.66	12	1	1.22	12	1
T × F	0.2	6	0.999	0.24	6	0.999

* Means significant relationships ($P < 0.05$)

Fig. 5 *Box plots* for the mean foliar damage per plant (%) for those *Croton lachnostachyus* plants located at the interior (I) and edges (E) of the six forest fragments and continuous forest selected in Córdoba, Argentina. Different *letters* indicate significant differences between zones (Permutation ANOVA; $F = 3.75$, $P = 0.002$)

surrounding landscape matrix on the ability of species to invade and occupy small patches (Didham 2010). Considering this, it would be interesting to find out the reproductive and dispersal abilities of the ants found in this

study. On the other hand, several ant species that feed on the extrafloral nectar have alternative sources of food as insects (Fisher et al. 1990); thus, this ant functional group is not exclusively nectarivorous, as opposed to myrmecophyte ants, which in general depend exclusively on what plants give to them (Fonseca 1993, 1999).

Does the alteration of abiotic and biotic conditions between edge and interior of forest patches change the composition and frequency of ant visiting the EFNs of *C. lachnostachyus*? Can these changes in ant composition be related to a different level of foliar damage between plants in edge and interior of forests?

Previous studies reported that an increased solar radiation on habitat edges favors dominant Dolichoderinae species, whereas generalized Myrmicinae and opportunist species can be negatively affected (Holway et al. 2002, Andersen and Majer 2004). Accordingly, we found five species from the Dolichoderinae subfamily at forest edges (*Dorymyrmex* sp. 1, *Dorymyrmex* sp. 2, *Dorymyrmex thoracicus*, *L. humile*, *Linephitema* sp. 1), whereas only one (*L. gallardoi*) at interior of the fragments. Myrmicinae species, in turn, were
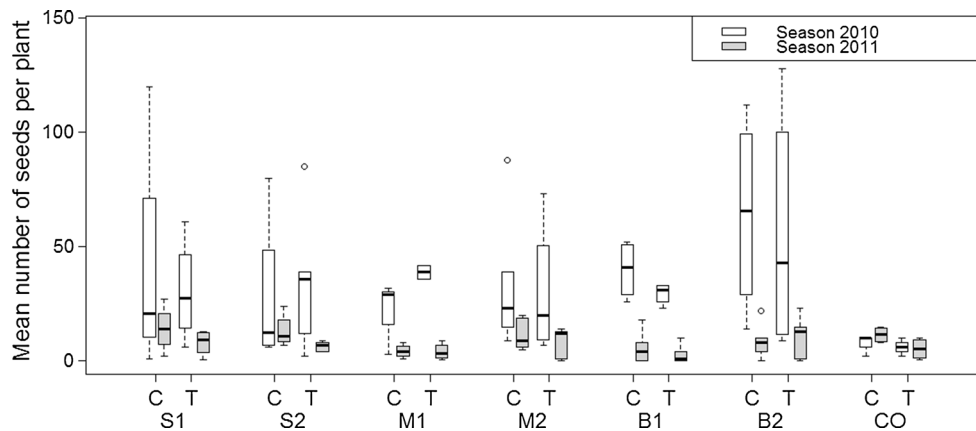
Fig. 6 *Box plots* for the mean number of seeds per two branches selected per plant for control and ant-excluded plants of *Croton lachnostachyus* located at six forest fragments and continuous forest selected in year 2010 and 2011 in Córdoba, Argentina. Abbreviations for fragments: *S1* small 1, *S2* small 2, *M1* medium 1, *M2* medium 2, *B1* big 1, *B2* big 2, *CO* continuous (see Fig. 1). Experimental paired plants: *C* control, *T* treatment

more abundant at interior of fragments: Five out of the seven species were only collected at interior of the fragments (Table 1).

This evidence suggests that ant composition associated with to EFNs of *C. lachnostachyus* differs between plants located at interior and edges of forest fragments, which in turn may change the ecological patterns or processes due to modifications of the ant–plant interactions. In accordance, foliar damage was higher in plants located at the interior than at the forest's edge in six out of the seven fragments evaluated. This pattern could be related to differences in ant composition, since Dolichoderinae ants were more abundant at the edges and are usually dominant and more aggressive species (Bestelmeyer and Wiens 1996; Holway et al. 2002; Andersen and Majer 2004; Crist 2009). On plants located at edges, we found *Camponotus mus* frequently visiting EFNs. This species is bigger than all the ants registered visiting EFNs at the interior of fragments. Usually, bigger ants offer a better protection against herbivorous insects than smaller ones (Schemske 1982; Horvitz and Schemske 1984; Oliveira et al. 1987; Koptur and Lawton 1988; Rico-Gray and Thien 1989). However, it would be interesting to experimentally test this hypothesis to dismiss other factors that also can be related to this pattern.

In addition, increased edge habitat in fragmented landscapes may facilitate invasions by non-native ant species, which may alter the composition of native ant communities (Crist 2009; Savage et al. 2009; Savage and Rudgers 2013). In our work, *L. humile* was detected only at edges. Thus, the invasion of non-native ant species together with environmental changes (as changes in temperature) could strengthen the replacement of native species. These changes in ant composition may modify interactions between

ants and plants, as for example in the South African shrublands, the invasion by the Argentine ant (*L. humile*) leads to a shift in composition of the plant community, owing to a disproportionate reduction in the densities of large-seeded plants (Christian 2001). On the contrary, Christianini and Oliveira (2013) found that edge effects decrease ant-derived benefits to seedlings of a shrub, because of changes caused in ant composition that remove the seeds in edge and interior of the Cerrado, with better seed dispersers at interior. Considering these previous works, it would be interesting to continue studying the ant assembly that visit EFNs of *C.lachnostachyus*, to find out whether the presence of *L. humile* affects it. And ultimately the interaction between the plant and the ants that visit them.

Do changes in ant composition and frequency affect reproductive success of plants (fruit and seed production, seed mass, germinability, and foliar damage) in a forest fragmentation gradient?

The mechanism behind the hypothesis of ant protection for *C. lachnostachyus* was that differences in the frequency of visits and composition of ant species in plants located in fragments from different sizes will result in a higher (or lower) efficiency in the defense against herbivorous insects, increasing plant reproductive success. Our results partially support this hypothesis. On the one hand, seed mass, germinability, and foliar damage of *C. lachnostachyus* did not show a consistent pattern between ant-excluded and control plants across a wide range of fragment sizes, a tendency that was consistent between years. On the other hand, fruit and seed production per plant were significantly higher in control than in ant-excluded plants

in at least one of the two reproductive seasons. These variables are particularly relevant for plant fitness, due to the number of fruits and new propagules summarizing the capability for plant population dynamics in the fragmented landscape (Aguilar et al. 2006). But these results did not show a consistent pattern with the area of fragments, that is, there was no relationship with the fragmentation gradient, and these results vary between years (Fig. 6).

This positive effects of ants on the reproductive success of *C. lachnostachyus* contrast with those previously reported by Freitas et al. (2000), since they did not find differences in reproductive variables for plants located within a continuous forest. Such inconsistent results suggest that this particular type of ant–plant interaction may be diffuse (no extreme specialization), largely fortuitous, and context dependent or facultative (Schemske 1983; Rico-Gray and Oliveira 2007). Thus, experiments performed in many sites would be necessary to detect some general patterns on ant–plant interactions and plant reproductive consequences.

An increase in the reproductive and/or vegetative component of the plants can depend on the species composition of the ant assemblage (Ness 2003a, b; Mody and Lisenmayer 2004). Usually, bigger ants offer a better protection against herbivorous insects than smaller ones (Schemske 1982; Horvitz and Schemske 1984; Oliveira et al. 1987; Koptur and Lawton 1988; Rico-Gray and Thien 1989). The four species that visit with major frequency EFNs of *C. lachnostachyus* at fragment interiors do not exceed 5 mm and are smaller than the majority of herbivorous insects that visits this plant (González 2014). On the other hand, Heil et al. (2001) found that the effects of protection by ants varied notably between studies of short and long periods. In accordance, foliar damage of *C. lachnostachyus* was higher in the experiment of long (3 months) than in the short period (1 month) (Online Resource 7). The pattern registered for seed production seems to be stronger during the second year where the ant-excluded plants were isolated for a longer period (Fig. 6).

Conclusions

Most studies related to habitat fragmentation in Latin America are focused on patterns and not in processes (Grez and Galetto 2011). There exist only a few well-conserved habitats in our changing world and nowadays lots of landscapes are fragmented, so understanding how the interactions between different species in these contexts are, would be increasingly important in order to conserve modified habitats (Grez and Galetto 2011). In this study, we found that forest fragmentation affected ant species composition, but this effect was only found when we compared edges versus interior of the fragments selected

and not between fragments of different size. Ants from the Dolichoderinae subfamily were more abundant at fragment edges, while ants from the Myrmicinae subfamily were more abundant at interior of fragments. Despite of these changes, we did not find a consistent pattern related to ant visitation frequency and forest fragmentation either in the edges or at the interior of fragments. The results of the ant-exclusion experiment partially support the hypothesis that ants protect the plant from herbivorous insects; we found a higher seed production in control than in ant-excluded plants in the 2 years considered, and a higher fruit production in the second year. However, we did not find meaningful differences when we considered seed mass, germinability, and foliar damage. When we compared foliar damage between plants located at edge and interior of fragments, we detected much greater foliar damage in plants located at interior of fragments than at edges. The results of this study suggest that this particular type of ant–plant interaction may be diffuse, largely fortuitous, context dependent or facultative.

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