Extrafloral-nectaries and interspecific aggressiveness regulate day/night turnover of ant species foraging for nectar on Bionia coriacea

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Abstract Plants bearing extrafloral nectaries (EFNs) vary the secretion of nectar between day and night, which creates turnover in the composition of interacting ant species. Daily variation in the composition of ant species foraging on vegetation is commonly observed, but its mechanisms are poorly understood. We evaluated the daily variation in nectar availability and interspecific aggressiveness between ants as possible regulatory mechanisms of the turnover in ant–plant interactions. We hypothesized that (i) plants would interact with more ant species during periods of higher secretion of nectar and that (ii) aggressive ant species would compete for nectar, creating a daily turnover of species collecting nectar. We tested this hypothesis by measuring the production of nectar during the day and night and by experimentally removing EFNs of Bionia coriacea (=Camptosema coriaceum) (Nees & Mart.) Benth. (Fabaceae: Faboideae) plants in a Brazilian savanna (Cerrado). We then compared the abundance and composition of ant species between those treatments and during the day. Our results indicate that more ant workers forage on plants during the day, when nectar was sugary, while more ant species forage at night, when aggressiveness between ant species was lower. We also detected a day/night turnover in ant species composition. Ant species foraging for nectar during the day were not the same at night, and this turnover did not occur on plants without EFNs. Both dominant ant species, diurnal Camponotus crassus (Hymenoptera: Formicidae) and nocturnal Camponotus rufipes (Hymenoptera: Formicidae), were the most aggressive species, attacking other ants in their specific periods of forage while also being very aggressive toward each other. However, this aggressiveness did not occur in the absence of nectar, which allowed non-aggressive nocturnal ant species to forage only during the daytime, disrupting the turnover. We conclude that extrafloral-nectar presence and interspecific aggressiveness between ants, along with other environmental factors, are important mechanisms creating turnovers in ants foraging on plants.

Key words: Brazil, Cerrado, niche partition, resource competition, species coexistence.

INTRODUCTION

Many plant species have evolved mutualistic interactions with ants by offering nectar as a food reward in exchange for ant protection against herbivores (Koptur et al. 1998; Lange et al. 2013), which often results in increased reproductive outputs (Rosumek et al. 2009; Nascimento & Del-Claro 2010; Heil 2015). Nectar is highly energetic and nutritious for ants, increasing the weight and size of workers and enhancing colony growth (Byk & Del-Claro 2011). Production of extrafloral nectar varies temporally along with plant ontogeny (Quintero et al. 2013), plant phenology (Lange et al. 2013; Vilela et al. 2014), leaf development (Calixto et al. 2015), herbivore pressure (Heil et al. 2000) and climatic seasonality (Lange et al. 2013; Del-Claro et al. 2016). Nectar secretion is related to hydric stress, intensity of sunlight, humidity, air temperature and herbivory (Pleasants 1983; Heil 2011). This variation in nectar offered can affect the recruitment and coexistence of ant species on the plants, since various ant species can collect nectar from the same individual plant (Dáttilo et al. 2014; Del-Claro et al. 2016), sharing or competing for nectar according to resource availability and foraging costs (Blüthgen et al. 2004; Fagundes et al. 2016). This temporal variation in nectar availability can explain most of the variation in foraging of tree-dwelling ants (Díaz-Castelazo et al. 2004; Schoeder et al. 2010; Lange et al. 2013; Vilela et al. 2014).

Patterns of temporal variation in ant foraging on plants and interaction with extrafloral nectary
(EFN)-bearing plants have been observed in tropical and temperate ecosystems both seasonally (Buckley 1982; Rico-Gray et al. 1998a, b; 2008; Heil et al. 2000; Díaz-Castelazo et al. 2004; Rico-Gray & Oliveira 2007; Lange et al. 2013) and daily turnover of ant species (Oliveira & Brandão 1991; Cogni et al. 2000; Hossaert-McKey et al. 2001; Oliveira & Del-Claro 2005; Holland et al. 2010; Baker-Méio & Marquis 2012; Dáttilo et al. 2014; Falcão et al. 2014). Seasonal turnover of ant species interacting with EFN-bearing plants is frequently related to extrafloral nectar availability (Rico-Gray et al. 1998a; Blüthgen et al. 2000; Díaz-Castelazo et al. 2004; Lange et al. 2013). However, the same hypothesis has not been tested for day/night variation, although ants seem to be more frequent during the period of the day in which plants secrete more nectar (Holland et al. 2009; Dáttilo et al. 2014; Falcão et al. 2014).

Competition between ant species for high quality nectar also could be an important mechanism for structuring day/night turnover of ant species (Dáttilo et al. 2014). In fact, ant activity and dominance of resources are higher during periods of increased nectar secretion (Holder & Polis 1987), so the benefits of the abundant nectar justify the costs of foraging and defending such a food source (Davidson 2004). In this context, daily variation in nectar secretion may cause high recruitment of ant workers in response to nectar offering, while nectar with more sugar content may increase interspecific competition, favouring the most aggressive ant species. Therefore, a daily turnover of interacting species may emerge from a temporal partitioning of nectar between aggressive species, although daily variation in environmental conditions, such as temperature and humidity, also may be important (Levings 1983; Fellers 1989; Cerdá et al. 1998; Rico-Gray & Oliveira 2007). Either way, the daily variation in the availability of nectar and foraging activity of ants has important consequences for the outcomes of mutualisms (Dáttilo et al. 2015), since plant protection is related to the identity of the interacting ant species and their abundance on plants (Novotný et al. 1999; Del-Claro & Marquis 2015).

In this study, we evaluated the hypothesis that daily variation in nectar availability and interspecific competition between ants for nectar regulate the day/night turnover in ant species foraging on plants bearing EFN due to temporal partitioning of extrafloral nectar between aggressive ant species. First, we predicted that ant species recruit more workers during the period of the day in which plants secrete nectar with greater volume and sugar content, although the number of ant species coexisting during this period might be lower due to increased aggressiveness for better quality nectar. Second, we predicted that the variation in nectar offerings creates a daily turnover between ant species due to interference between aggressive species for nectar access. Last, we test the prediction that the daily turnover of ant species foraging on plants would disappear in the absence of EFNs due to a reduction in ant recruitment and aggressiveness in response to the absence of an advantageous resource or that the turnover would be maintained by other factors that create foraging turns, such as temperature and risk of predation.

**METHODS**

**Study system**

We conducted this study from 21 to 25 August 2014 in a Cerrado area (Brazilian savanna) belonging to the Parque Estadual do Rio Preto (hereafter, PERP), near São Gonçalo do Rio Preto, Minas Gerais State, Brazil (18°11’13”S, 43°20’37”W). The PERP is in the basin of the Jequitinhonha River, located in the complex of the Espinhaco Range, with elevations ranging from 750 to 1825 m a.s.l. The PERP is covered by Cerrado and Rupestrian Fields. The climate consists of a mean annual temperature around 19°C and annual rainfall around 1500 mm. We studied B. coriacea (=Camposoma coriaceum) (Nees & Mart.) Benth. (Fabaceae: Faboideae), a small shrub (1.7 ± 0.4 m mean ± standard deviation, n = 20) that bears EFNs distributed along the raceme of the inflorescence (52 ± 6 EFNs per plant, n = 20), present only during the reproductive period in the transition between the dry and wet seasons (July to December; Pires 2015). Biomia coriacea is a typical and abundant woody shrub of the Cerrado (Pires 2015), allowing easy application of the experiments required to test our hypothesis.

Although B. coriacea is common in rupestrian fields (Jacobi & Carmo 2012), the only published study on the interactions between this plant and ants indicated the ant species Camponotus crassus Mayr 1862 as the only interact species (Guerra et al. 2016). Camponotus crassus is a numerically and behaviourally dominant species in Cerrado vegetation (Lange & Del-Claro 2014; Del-Claro et al. 2016) and Rupestrian Fields (Fagundes et al. 2016; Guerra et al. 2016), interacting with plants bearing EFNs, Hemiptera producing honeydew and caterpillars secreting sugary solutions. However, this species is predominantly considered a daytime forager (Dáttilo et al. 2014; Guerra et al. 2016), and there may be other species to replace it overnight, such as Camponotus rufipes (Dáttilo et al. 2014). Camponotus rufipes is also numerically and behaviourally a dominant species in Cerrado (Lange et al. 2013; Del-Claro et al. 2016) and Rupestrian Fields (Fagundes et al. 2016) but forages mostly at night (Dáttilo et al. 2014).

**Experimental design**

**Daily pattern of ant forage**

For all experimental procedures, we used 30 shrubs of similar height (1.7 ± 0.4 m) to prevent effects of foraging area,
We measured the production of nectar on 24 August 2016, spaced 10 m apart to maintain some degree of independence between sample units by reducing probability of an ant colony foraging on more than one sample plant (Dattilio et al. 2014). Plants in the area were sparse and poorly connected by branch contact or vegetation bridges. We tested the effect of the nectar presence creating the day/night turnover by a manipulative experiment of EFN removal. We treated 15 plants by using a needle to remove the EFNs from treatment plants (hereafter plants without EFNs; see details in Baker-Méio & Marquis 2012). We used the other 15 plants as a control group (hereafter plants with EFNs), and EFNs of these plants were just touched by a needle. Plants were distributed along a transect of 300 m and were assigned as a control or treatment in a paired design. For all experiments, we considered daytime the period between 06:00 h and 18:00 h and nighttime between 18:00 h and 06:00 h. We designed our samples according to those of previous studies concerning day/night turnover of ant foraging (Del-Claro & Oliveira 1999; Hossait-McKey et al. 2001; Holland et al. 2010; Dattilio et al. 2014, 2015), in which sampling occurred in one or 2 days due to the logistical problems of sampling at night, such as the isolation of the sample areas, the necessity of several researchers working simultaneously and the difficulties of moving in the dark without causing disturbances, since the use of artificial lights can alter ant behaviour. We quantified the number of ant species (interactions) and the number of individuals per species (recruitment) visiting the 15 shrubs with EFNs (sample units) and 15 shrubs without EFNs (sample units) in a 24-h period on 22-23 August 2014. We counted all ants foraging on the whole plant in a single survey. We repeated the observations once every 2 h, totalling 12 consecutive observations (repeated measures) starting at 07:00 h. We observed plants in the same sequence in all observations, which occurred under similar weather, with no rain or strong winds during the day or night. At night, there was no other source of light but the moonlight and the headlamp used for counting, which was covered by a red plastic filter and turned on only during the observation period. We deposited vouchers of the ant species in the zoological collection of the Departamento de Biodiversidade, Evolução e Meio Ambiente da Universidade Federal de Ouro Preto, Ouro Preto, MG, Brasil.

Mechanisms creating the turnover

We measured the production of nectar on 24 August 2016, immediately after we evaluated ant foraging. First, we quantified the daily variation in nectar volume and sugar concentration in all 30 shrubs. For this, we randomly selected one branch on every shrub. We bagged these branches for 6 h during the day (08:00–14:00 h) for accumulation of nectar. At the end, we collected the nectar with microcapillary tubes to measure volume. We collected nectar from two EFNs per branch for standardization. We transferred the nectar to an analog refractometer to measure the nectar sugar concentration. At night (20:00–02:00 h), we selected another branch from each plant and repeated the same procedure. We also evaluated the climatic variation between the day and night measuring the air temperature and relative humidity near the shrub every 2 h during the day and night. It was not possible to measure nectar hourly because the volume secreted was too low to be collected. Nectar features were measured only in plants with EFNs.

Second, we evaluated interspecific aggressiveness between ants collecting nectar. We used three complementary experiments. (i) We quantified the number of interspecific aggressions performed during the counting of ant species recruitment on plants with and without EFNs during day and night periods. We did this on 23-24 August 2016. On each plant with and without EFNs, we localized the ants foraging on the plant, then we observed each ant worker for 3 min, one observer per ant. We identified which ant species acted aggressively or passively. In each observation, we quantified the following behaviours: threats with open mandibles, formic acid release and attacks with bites to identify the behaviourally dominant species (e.g. Santos et al. 2005). We repeated the observations once every 2 h, totaling 12 consecutive observations (repeated measures) starting at 07:00 h. (ii) We used termites to measure ant aggressiveness against a neutral intruder to remove effects of interspecific variation in aggressiveness between ant species. We performed this experiment on 24–25 August. First, we removed all ants from the plant. We then glued one termite near the EFNs to a randomly chosen apical branch of each shrub for plants with and without EFNs (Campos & Camacho 2014). Shrubbs received termites during the day and night, on different branches. We quantified the number of termites removed by diurnal and nocturnal ant species to compare the defense effectiveness of ants during both periods. The observations lasted until all termites were found by the ants. We identified which ant species found and removed each termite and which species found but ignored the termites to determine the degree of aggressiveness and displacement of intruders. Thus, it was considered a success when the ant found and removed the termite and failure when the ant found but did not remove the termite. Even after the ant ignored the termite, we continued the observations until the ant left the plant. Although termites are commonly used to simulate herbivores, we just intended to evaluate the behaviour of different ant species toward a similar intruder. (iii) Once we had identified the diurnal and nocturnal species (see data analysis), we evaluated the interference between them by assessing their behaviour in paired encounters between workers of two different focal species each time (e.g. Espírito-Santo et al. 2012), because this two species do not encounter each other naturally in the field. We used 10 workers of the numerically dominant diurnal species and 10 workers of the dominant nocturnal species. We collected one worker of C. crassus and one worker of C. rufipes from each shrub on 25 August. Ants were captured at twilight, when both species could be found on the plant at the same time. This was necessary to avoid keeping the ants in nighttime or daytime captivity for long until the clash, as both would have been under stress during the same period. In this last experiment, we only used shrubs with EFNs because, at the sample time, we found few ants foraging on plants without EFNs, making it difficult to perform the experiment. Each pair was placed in a Petri dish (10 cm diameter) immediately after being
We evaluated the variation in the number of ant workers captured. We quantified all behaviours presented by each ant for 15 min.

**Data analysis**

We evaluated the variation in the number of ant workers interacting with shrubs of *B. coriacea* during a 24-h period using a general linear mixed model (GLMM). We considered the number of ants per shrub as the dependent variable, the hours of the day as a repeated measure (12 levels) and the experimental group (with or without EFNs) as a fixed factor. Each shrub represented a random block.

We tested the difference between air temperature and humidity between the day and night using a U-test and the correlation with ant abundance using Spearman’s correlation coefficient. Data did not present a normal distribution of residuals when tested by GLMM.

We tested the existence of day/night turnover in the interaction between ants and plants using a seriation analysis algorithm based on work of Brower and Kile (1988). In this analysis, the species were reorganized along the diagonal of a presence/absence matrix with vertices ‘a,’ where ‘a’ is the presence of ant species ‘i’ foraging at the time ‘j.’ We then calculated the seriation criterion based on species turnover over a hypothetical gradient (day/night turnover). The program created 30 random matrices by Monte Carlo simulation and calculated the seriation criteria for each. A gradient of species turnover exists when the observed criterion is greater than that expected by chance. Additionally, we performed an analysis of similarities (ANOSIM), considering the similarity in ant species composition between plants for day and night periods according to the Raup–Crick index of similarity. Raup–Crick is an index for presence/absence data that compares the number of observed co-occurrences with the distribution of 1000 random replicates from the pool of samples (Raup & Crick 1979). We used the day and night periods as fixed factors and the 15 shrubs as sample units and performed one ANOSIM for plants with EFNs and another for plants without EFNs.

We used a Friedman ANOVA to compare nectar volume and sugar concentration (dependent variables) between day and night (fixed factors) using individual plants as random blocks. Data did not exhibit a normal distribution of residuals when tested by GLM. We corrected the Brix value using air temperature according to a conversion table provided with the refractometer’s manual of instructions.

We compared the number of workers displaying aggressive behaviour (dependent variable) on plants with EFNs and without EFNs (fixed factor) and between day and night (fixed factor) using a GLMM with a binomial distribution (1 = attacked other ant, 0 = did not attack). We used GLMM with a binomial distribution (1 = captured, 0 = ignored) to compare the displacement aggressiveness (rate of termite removal) of diurnal and nocturnal ant species (fixed factor) on shrubs with and without EFNs (fixed factor). For both analyses, we considered the shrub a random block.

We also compared the frequency of aggressive behaviours performed by the two dominant species using the Friedman test. For this, we used the results of the contests between diurnal and nocturnal dominant species in paired encounters (*n* = 10 trials) as samples. We considered the number of aggressions per fight as a dependent variable, the species as fixed factors and paired encounter as a random block.

Data did not exhibit a normal distribution of residuals when tested by GLM.

We performed a seriation analysis using PAST software v3.0 (Hammer et al. 2001). We performed all remaining analyses using Action software v2.9 (Estatcamp 2014), a package based on the R platform (R Core Team 2014).

**RESULTS**

**Daily pattern of ant forage**

We quantified 606 ants from 14 species foraging on *B. coriacea* (Table 1). Considering daily variation, 314 workers (52%) of seven species interacted with plants during the day, 136 workers (22%) of eight species interacted at night and 156 (26%) of two species interacted during both periods (Table 1). Considering EFN presence, 511 ants (84%) from 14 species foraged on plants with EFNs, while 95 ants (16%) from eight species foraged on plants without EFNs (Table 1). The mean abundance of ants foraging on plants was higher during the day than at night (ANOVA factor: *F*₁₁,₃₀₈ = 6.18, *P* = 0.0001; Figs 1,2). However, this difference occurred only for shrubs with EFNs (ANOVA interaction: *F*₁₁,₃₀₈ = 3.6, *P* = 0.0001; Fig. 2). Ants recruited more workers for plants with EFNs than for plants without EFNs, regardless of the day period (ANOVA factor: *F*₁,₂₈ = 14.1, *P* = 0.0001; Fig. 2). We found no difference between day and night air temperature (day = 24.5 ± 7.9°C, night = 20.1 ± 4.9°C, Wilcoxon: *W* = 27, *n* = 12, *P* = 0.18) or air humidity (day = 50.1 ± 19.1%, night = 58.7 ± 8.8%, Wilcoxon: *U* = 10.5, *n* = 12, *P* = 0.27), although the temperature dropped from a mean of 24.5°C during the day to 20.3°C at night and to 15°C between 03:00 and 05:00 h. Variation in ant abundance throughout the day and night was not correlated with air temperature (*r*ₐ = 0.48, *P* = 0.87) or relative humidity (*r*ₐ = 0.05, *P* = 0.88).

We observed a pattern of ant species turnover between day and night periods in *B. coriacea*. This pattern was consistent for shrubs with EFNs (seriation: observed = 0.82, expected = 0.56 ± 0.04, *Z* = 6.22, *P* < 0.001; Table 1a) but not for shrubs without EFNs (seriation: observed = 1.16, expected 6.01 ± 12.17, *Z* = 0.39, *P* = 0.69; Table 1b). *Camponotus crassus* was the most abundant (169 ants, 41%) diurnal species. *Camponotus rufipes* was the most abundant (120 ants, 63%) nocturnal species (Fig. 1). These two species exhibited the same pattern of daily turnover in shrubs without EFNs;
although recruitment was 10-fold less (Table 1). Moreover, in plants without EFNs, five species reduced nocturnal foraging and increased diurnal activity (Table 1). The composition of ant species foraging on plants during the day differed from that at night for both plants with EFNs (ANOSIM: $R = 0.52$, $P < 0.001$) and plants without EFNs (ANOSIM: $R = 0.6$, $P < 0.001$).

### Mechanisms of daily turnover in ant forage

Nectar secreted during the day ($31.6 \pm 16.5\%$ Brix) showed higher concentration of sugar than nectar secreted at night ($8 \pm 3.5\%$ Brix; Friedman $\chi^2 = 11.3$, $P = 0.001$, d.f. = 1; Fig. 1). The mean volume of nectar per EFN was not different between the day ($0.07 \pm 0.07 \mu$L EFN$^{-1}$ h$^{-1}$) and night ($0.07 \pm 0.09 \mu$L EFN$^{-1}$ h$^{-1}$; Friedman $\chi^2 = 0.11$, $P = 0.74$, d.f. = 1; Fig. 1).

Ants were more aggressive against each other on plants with EFNs than without EFNs (GLM$_{\text{factor}}$: Wald = 3.9, d.f. = 1, $P = 0.04$; Fig. 3) for both periods of the day (GLM$_{\text{interaction}}$: Wald = 2.1, d.f. = 1, $P = 0.15$). We also observed more workers engaging in interspecific aggression during the day compared to night (GLM$_{\text{factor}}$: Wald = 5.4, d.f. = 1, $P = 0.02$). *Camponotus crassus* was the more aggressive diurnal species (41% of total aggressions, $n = 54$), although *C. crassus* was aggressive only on plants with EFNs. *Camponotus rufipes* was the more aggressive nocturnal species (17%) on both plants with EFNs and without EFNs, but aggression was two-fold less than *C. crassus*. The diurnal species *Camponotus sericeiventris* (14%)...
and *Cephalotes* sp1 (12%) were less aggressive, but they engaged in confrontations regardless of EFN presence. Species active day and night *Cephalotes attratus* (8%) and *Crematogaster* sp1 (8%) occasionally displayed interspecific aggressiveness. The other nine species were not aggressive in interspecific encounters (Fig. 4).

Ants captured more termites during the day (19 termites, 63%, *n* = 30) than at night (13 termites, 43%; GLM<sub>factor</sub>: \(\chi^2 = 3.84\), d.f. = 1, *P* = 0.04; Fig. 4), regardless of the presence of EFNs (GLM<sub>interaction</sub>: \(\chi^2 = 0.15\), d.f. = 1, *P* = 0.7; Fig. 4). The percentage of captured termites was higher in shrubs with EFNs (25, 83% of termites) than those without EFNs (5, 17%; GLM<sub>factor</sub>: \(\chi^2 = 6.9\), d.f. = 1, *P* = 0.01; Fig. 4) in both daytime periods. Only eight species removed termites: three diurnal species...
(C. crassus, C. serisceiventris and Cephalotes sp1), three nocturnal species (C. rufigenes, Camponotus melanoticus and Pheidole sp1), and two full-time species (Crematogaster sp1 and Cephalotes atratus). Camponotus crassus captured termites on 11 (58%) plants during the day, while C. rufigenes captured termites on 8 plants (61%) during the night. When confronted in the laboratory, both diurnal and nocturnal dominant species were highly aggressive toward each other and displayed different behaviours. Camponotus rufigenes performed 6.7 ± 4.1 attacks (n = 10) against C. crassus, while C. crassus performed 2.8 ± 3.2 attacks against C. rufigenes. Camponotus rufigenes was more aggressive than C. crassus (Friedman $\chi^2 = 6.4, P = 0.01$, d.f. = 1). The aggressiveness of C. crassus consisted mostly of formic acid release (abdomen rubbing underneath the thorax) toward C. rufigenes (13 events, 25% of behaviours, n = 52 acts) and attacks with bites (13, 25%), along with non-aggressive behaviours of touches with antennae (21, 40.3%) and grooming (3, 6%). Camponotus rufigenes reacted to threats with open mandibles (31, 29%, n = 108) followed by bites (31, 29%), along with non-aggressive behaviours such as grooming (20, 19%) and touches with antennae (21, 40.3%). Camponotus crassus performed few threats (2, 5%), whereas C. rufigenes performed few displays of acid release (5, 6%).

**DISCUSSION**

In this study, we confirmed our hypothesis that ant species interacting with EFN-bearing B. coriacea plants during the day differed from species interacting at night and that this pattern occurred only with plants bearing EFNs. We observed that sugar concentration was higher in nectar secreted at day. In daytime, there were more ant workers and species foraging on the plants and the aggressiveness between ant species and also against termites was higher during the day than at night time. Therefore, ant aggressiveness may occur even in the absence of nectar, albeit at lower levels, indicating that intrinsic aggressive behaviour could be stimulated rather than created by nectar resources. The most abundant diurnal species, C. crassus, and most abundant nocturnal species, C. rufigenes, showed high aggressiveness against other species but mostly on plants with EFNs during the daytime, which indicates context-dependent aggressiveness. Based on these results, we proposed three important factors creating the day/night turnover of ant–plant interactions: (i) the presence of extrafloral nectaries, (ii) the regime of
nectar secretion and (iii) the interspecific aggressiveness among ants.

**Daily pattern of ant foraging**

Daily turnover in ant species composition foraging on vegetation was reported in several studies in tropical (Koptur 1984; Torres 1984; Oliveira & Pie 1998; Oliveira et al. 1999; Cogni et al. 2000; Dáttilo et al. 2014) and temperate (Hunt 1974; Savolainen & Vepsāliainen 1988; Fellers 1989; Hossaert-McKey et al. 2001) ecosystems. We added the case of daily turnover of interactions between ants and *B. coriacea* in Brazilian Cerrado. Turnovers in ant–plant interactions between day and night have been observed for the plants *Turnera ulmifolia* (Cogni et al. 2000), *Caryocar brasiliense* (Oliveira 1997; Oliveira & Pie 1998), *Ouratea hexaspermae* (Oliveira & Pie 1998), *Opuntia stricta* (Oliveira et al. 1999), *Hibiscus pernambucensis* (Cogni & Freitas 2002), *Chamaecrista desvauxii* (Baker-Méio & Marquis 2012), *Chamaecrista mucronata*, *Stachytarpheta glabra*, *Qualea grandiflora* (Dáttilo et al. 2014) and *Didymopanax vinosus* (Oliveira & Del-Claro 2005). Similar results also were observed in other mutualisms, such as those between ants and honeydew-producing hemipterans (Del-Claro & Oliveira 1993, 1999). All of these studies report that ant abundance is higher during the day than at night but that richness is similar, although none of the studies tested the importance of nectar availability and interference competition for nectar.

We observed a turnover between the diurnal dominant *C. crassus* and the nocturnal *C. ruﬁpes*, with few ant species coexisting with *C. crassus* and several others coexisting with *C. ruﬁpes*. In the Brazilian savanna, these two species are dominant in ant–plant interactions (Oliveira & Brandão 1991; Lange et al. 2013; Dáttilo et al. 2014), where *C. crassus* is restricted to foraging during the daytime but *C. ruﬁpes* can forage during both periods (Oliveira & Brandão 1991; Oliveira & Pie 1998; Cogni et al. 2000; Tavares et al. 2008; Stefani et al. 2015). These two species were also dominant and exhibited daily turnovers when associated with *T. ulmifolia* (Cogni et al. 2000), *C. brasiliense*, *O. hexasperna* (Oliveira & Pie 1998), *S. glabra* and *C. mucronata* (Dáttilo et al. 2014). Daily turnovers between *C. crassus* and *C. ruﬁpes* were also observed for ants interacting with the honeydew-producing hemipteran *Guayaquila xiphias* in the Brazilian savanna (Del-Claro & Oliveira 1999).

**Mechanisms of daily turnover in ant forage**

Daily variation ant foraging occurred only on plants bearing EFNs. The recruitment of ants to plants with EFNs was five times higher compared to plants without EFNs, with which several species did not interact. Several studies show reduced numbers of ants interacting with plants naturally lacking EFNs compared to plants bearing EFNs in the Brazilian savanna (Oliveira 1997; Rico-Gray & Oliveira 2007; Schoereder et al. 2010; Maravalhas et al. 2011; Baker-Méio & Marquis 2012). Our results conﬁrmed this scenario since *Camponotus* spp., *Cephalotes* spp. and *Pseudomyrmex* spp., which are typically tree-nesting species (Silvestre et al. 2003), were observed on plants with and without EFNs, while *Ectatomma* spp. and *Pheidole* spp., which are typically ground-nesting species (Silvestre et al. 2003), were observed only on plants with EFNs. The largest number of ants foraging during the day may have resulted from increased availability and attractiveness of nectar and also the higher number of diurnal species in the area. Although there was greater amount of foraging ants during the day, the number of species was similar between day and night. There was only a change in species composition. On plants without EFNs, there was no great difference in ant abundance between day and night, despite a reduction in richness to only two nocturnal species. The reduced number of ant species on plants without EFNs reﬂects a lack of interest from opportunistic species and low attractiveness to the plants, since only restricted arboreal species may keep foraging for prey on vegetation without nectar (Hossaert-McKey et al. 2001). The reduced numbers of ant species foraging on plants without EFNs at night is due to a change in foraging period for most nocturnal species that became diurnal after EFN removal.

In our study, ants exhibited daily turnover in foraging activity only when interacting with plants with EFNs. The presence of EFNs was also the determinant of the day/night turnover of ants foraging on *C. desvauxii*, which did not occur in plants with a low supply of nectar or plants with inactive nectaries (Baker-Méio & Marquis 2012). Aggressiveness between ant species mediated by nectar availability was an important factor behind the observed daily turnover in ant foraging. Nectar was more concentrated with sugar during the day when ants were more aggressive, especially *C. crassus*. At night, *C. ruﬁpes* was the most aggressive species but was less aggressive than *C. crassus*. Moreover, ants were very aggressive when foraging on plants with EFNs, but almost no conﬂicts were observed on plants without EFNs. Although *C. crassus* remained diurnal and *C. ruﬁpes* remained nocturnal on plants without EFNs, all submissive ant species showed nocturnal foraging on plants with EFNs but diurnal foraging on plants without EFNs, or the ants left the plant, breaking the turnover. Perhaps these submissive species climb the plant to collect nectar and can forage...
at any time of the day but are restricted to nighttime by dominant ant species on plants with EFNs. One explanation could be that the amount of sugar in nectar is so high in the day that it compensates the foraging costs of submissive species in a highly aggressive environment, which did not happen overnight nor on plants without EFNs. However, some submissive ant species could not deal with so much aggressiveness in the day, or could foraging at night without environmental stress, and chose to foraging at night when the diurnal dominant species occurred, as our results showed. Additionally, some dominant arboreal ant species may tolerate a specific group of associated submissive species that are allowed to forage with them (Majer et al. 1994), and so, this day/night turnover might reflect the submissive species of C. rufipes and C. crassus. Nevertheless, the high number of ant species foraging during the day, when aggression is high, and the specific mechanisms causing the turnover of specific ant species, is an issue that deserves more specific studies on natural history of ant species associated with EFN-bearing plants.

In the Cerrado, C. crassus is commonly observed to be active at daytime (Tavares et al. 2008; Stefani et al. 2015) and remains as such when interacting with EFN-bearing plants (Oliveira & Pie 1998). However, C. rufipes, Ectatomma tuberculatum and C. atratus are also seen as daytime foragers (Tavares et al. 2008), but they appeared as nighttime foragers when interacting with plants bearing EFNs (Oliveira & Pie 1998) or hemipteran producers of honeydew (Moreira & Del-Claro 2005; Rico-Gray & Oliveira 2007). This indicates that several ant species can forage both day and night, but the aggressiveness of the dominant C. crassus, powered by the presence of high quality nectar, can change the foraging behaviour of another species. Alternation of species over time was observed for Crematogaster brasiliensis avoiding C. melanoticus foraging for Passiflora spp. nectar (Hossaert-McKey et al. 2001), C. rufipes avoiding C. blandus on T. ulmifolia (Cogni et al. 2000), Camponotus planatus avoiding Camponotus abdominalis on O. stricta and C. rufipes avoiding C. crassus tending to the hemipteran G. xiphias (Del-Claro & Oliveira 1999).

Ants captured termites even in the absence of nectar, though their displacement aggressiveness was enhanced during the day, when nectar had higher sugar concentration. Oliveira (1997) found ants attacking termites on plants lacking EFNs in the Cerrado, but such aggressiveness was five times higher in nectaried C. brasiliensis, especially during the daytime. Ants are also more aggressive during the daytime in tropical forests (Springate & Basset 1996; Novotny et al. 1999; Basset et al. 2001). Ants were ineffective in removing termites from C. desvauxii that were secreting low volumes of nectar or lacking EFNs, and these plants suffered high leaf herbivory (Baker-Méio & Marquis 2012). These results have implications in ant defense of plants against herbivory since EFN presence and nectar production could enhance the effectiveness of ant protection by attracting aggressive ants, as termite removal by ants is positively correlated with a reduction in leaf herbivory (Jeanne 1979). However, we must consider a method error causing ants to capture termites in plants without EFNs, since this activity might be remnant of ant forage trails prior to EFN removal.

We conclude that daily variation in nectar volume and concentration is correlated with the variation in the number of foraging ants on the plant. Therefore, nectar availability could be an important mechanism regulating ant foraging on plants, although manipulative experiments are required to confirm this. We observed that the occurrence of day/night turnover of ant–plant interactions is also correlated with the presence of nectar as well as the variation in aggressive interactions between ant species. Thus, we added daily variation in quality and quantity of the nectar resource and interspecific interference for high quality nectar as mechanisms of the daily turnover. However, daily turnover is a complex process involving many factors, such as intrinsic physiology constraints of ant species (Hossaert-McKey et al. 2001) and parasitoid abundance (Orr 1992), and thus requiring more investigation. Our results corroborate the assumption that the demand for nectar should increase competition during the daytime (Blüthgen et al. 2000), generating a temporal partition of resources (Lange et al. 2013). We also call attention to the temporal complexity of these mutualisms, mainly to the importance of studying mutualisms at different scales and considering species behaviour idiosyncrasies that could affect protective effectiveness and mutualism stability. Mutualism outcomes are linked to the behaviour of the ant species interacting (Koptur 1992; Del-Claro & Marquis 2015) and to plant investment in nectar reward (Lange & Del-Claro 2014), more than to the total number of interactions performed with ants (Koptur 1984; Rico-Gray & Thien 1989; Del-Claro & Marquis 2015). Indeed, interaction with ineffective species can lead to lack of defense regardless of plant investment in nectar (Barbosa et al. 2015), and the plant must ensure continuous presence of the best protector ant species. We suggest that future studies investigate the consequences of day/night turnover of ant species for plant defense against herbivores. This is the next step in understanding temporal variation in ant–plant interactions.

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