



RESEARCH ARTICLE - ANTS

Positive Relation Between Abundance of Pericarpial Nectaries and Ant Richness in *Tocoyena formosa* (Rubiaceae)

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Abstract

Ants can interact with plants in several ways, being one of the most common visitors of extrafloral nectaries (EFNs). However, pericarpial nectars (PNs) may represent to the ant community a similar resource as EFN would do. Here, we investigate how an ant community interacts with PNs by an individual-based network, using *Tocoyena formosa* as a model. We hypothesized that plants with more PN's would present a higher ant richness in comparison to plants with less PNs and will occupy a central position in the network interaction. We observed 36 individuals of *T. formosa* and recorded both the ant species and abundances on each plant, as well as the number of active PNs. To test this hypothesis, we performed a linear regression between PNs and ant richness. A network analysis was performed to obtain both the specialization and centrality metrics of each plant, and we also conducted linear regressions between the number of PNs and both the specialization and centrality. The hypothesis was confirmed, the ant community was more rich in individuals of *T. formosa* with more active PNs, and these individuals were more central, being important for maintaining the interactions with ants. We believe that the coexistence between ants foraging is possible in *T. formosa* due to the seasonality and short time prevalence of the PNs, whose dominant ants do not have able time to master the resource and exclude the others, allowing different species to use the same plant.

Introduction

There are several cases of mutualism regarding plants and ants (Del-Claro et al., 1996; Heil & McKey, 2003; Mondal et al., 2013), ranging from opportunistic to mandatory interactions (Heil & McKey, 2003; Duffy & Hay, 2010; Byk & Del-Claro, 2011; Mayer et al., 2014). Such interactions are very common among plants having extrafloral nectaries (EFNs), a nectar secretory structure that is not involved in pollination (Koptur, 1994; Fiala & Linsenmair, 1995). Ants are attracted to EFNs due to the presence of nectar, a highly energetic and nutritious substance, being composed mainly of sugar (Byk & Del-Claro, 2011). In the Cerrado, there is a high incidence of EFNs (Fiala & Linsenmair, 1995; Ratter et al., 1997; Cogni & Freitas, 2002; Del-Claro & Marquis, 2015), and previous studies have reported a mutualistic interaction mediated by EFNs, having the ant acting as a protector against herbivores while searching for nectar in plants (Del-Claro et al., 1996; Oliveira, 1997).

Pericarpial nectaries (PN), a similar structure to EFNs, occur when the corolla falls and the nectariferous disc remains active throughout the fruit development (Del-Claro et al., 2013a). PNs are often seen as a type of EFN due to their morphological and functional similarities (Del-Claro et al., 2013a; Paiva, 2009). Despite their analogous importance in mutualistic ant-plant interactions, PNs are often underexplored structures in studies that consider mutualistic effects on ant-plant interactions (Del-Claro et al., 2013a).

Mutualistic interactions present distinct characteristics in comparison to other types of interactions. For instance, previous studies already identified that mutualistic facultative networks have a particular organization, which is called a nested pattern (Bascompte et al., 2003). It means that facultative networks have specialist species interacting mostly with generalist species, and these generalists are responsible for the network cohesion, acting as interconnectors among the network (Bascompte et al., 2003; Guimarães et al., 2006).



According Dattilo et al. (2014), it is possible to compare the individual-based networks of ant-plant interactions with the species-area model proposed by MacArthur and Wilson (1967), with each plant acting as an “island of resources”. In this model, it is expected an expansion of ant richness throughout the plant ontogeny due to an increase of resource availability (Campos et al., 2006). High quality resources provided by plants are more susceptible to receive a greater ant visitation, which in turn is related to a better ant protection against herbivores (Heil & McKey, 2003). EFNs, however, are not simply related to the number of ant visits, but also on how these visits are organized in time, meaning that plants with EFNs present differences between day and night visiting ant fauna, whereas plants without EFNs do not (Anjos et al., 2017).

Several studies have focused in finding attributes of the ant-plant interaction that can predict the organization of such mutualistic associations (Chamberlain & Holland, 2009; Dáttilo, 2012; Gomez & Perfectti, 2012; Dáttilo et al., 2014, Dáttilo et al., 2017). Commonly used metrics are species degree (k), specialization (d), centrality betweenness (CB) and centrality closeness (CC). Species degree (k) and specialization (d) may be seen as a predictor of nestedness and are commonly used in order to investigate non-random patterns in networks (Okuyama & Holland, 2008, Blüthgen et al., 2008, 2015). It is believed that species having a high CC have a potential to affect many other species, while species with high CB are important to the maintenance of the network cohesion (González et al., 2010). Both metrics (CC and CB) indicate the importance of a species to the network as a whole, in which species with high values of CB and CC will likely be key species for the maintenance of mutualistic interactions, such as the plants with in EFNs (Gomez & Perfectti, 2012).

In the present study, we used as a model *Tocoyena formosa* (Rubiaceae) (Cham. & Schlecht.) K. Schum 1889, a typically shrub from Cerrado, which presents PNs, to measure the importance of such resource in the mutualistic interactions among ants in a Brazilian Cerrado area. Specifically, we tested the hypotheses that i) the number of PNs available influences the ants search for this resource ii) and plants with more active PNs are more visited by ants (richness and abundance).

Material and Methods

Study Area

We carried out the study at Serra de Caldas Novas State Park (PESCAN, 17 ° 47'13 '' S / 48 ° 40'12''O), in the municipality of Caldas Novas, GO, Brazil. The region is at an average elevation of 1,000 m above ground level and we collected the data in a plateau area, a typical *stricto sensu* Cerrado. The climate in the region is Aw according to the classification proposed by Köppen (Dias Cardoso et al., 2014).

Studied Species

Tocoyena formosa is a shrub from the Rubiaceae family, with 1.7 m of height on average (Carlos-Santos & Del

Claro, 2001). It mostly occurs on savanna-like vegetation, dry forests, thorn-scrub vegetation and humid rain forests (Silberbauer-Gottsberger et al., 1992). The inflorescence has yellow flowers with long corolla tubes. The blooming occurs from October to December, but there are reports of blooming in January as well (Silberbauer-Gottsberger et al., 1992). After the pollination, the corolla falls, but the nectariferous disk located at the chalice remains active (Carlos-Santos & Del Claro, 2001). The persistence of the nectariferous disk gives rise to the pericarpial nectary (Fig 1).

Study Design

We conducted the field work in November 2016, during the rainy season, coinciding with the flowering period of *T. formosa*. We used a track of 1km as transect, where individuals were screened for up to 5 m on each track side. We selected 36 individuals under the same conditions of luminosity, with measurements ranging from 1.50 to 1.70 high. We counted all active PNs of each selected plant and considered an active PN when the corolla had already fallen, leaving the nectariferous disk exposed. Plants that had any ant resources other than PNs (e.g. hemipteran honeydew) or had no active PNs or ants at all, were disregarded. These conditions were stipulated to evaluate only the effect of active EFNs, minimizing confounding factors (e.g. plant size, mutualistic interactions with other animals). Observations were taken from 08:00 to 11:30 am, and we collected the visitors from the nectaries of each plant. In order to determine which ant species in fact were visiting the PNs, we observed each branch inflorescence for about five minutes. We counted the effective visitors and two individuals of each species were collected for a more accurate identification after the field procedures. The identification was performed with the collaboration of a specialist and based on the published listings of the park's myrmecofauna.

Network Analysis and Statistics

We conducted all data analyses in the R software version 3.3.2. (R Core Team, 2016). We performed a rarefaction curve to investigate whether a satisfactory sample of ant richness visiting *T. formosa* was obtained. We performed a simple linear regression to investigate the relationship between the number of PNs and ant richness.

An individual-based network was performed using the package bipartite (Dormann et al., 2008). Network analysis shows associations (links) between species (nodes) in a community, and is a commonly used approach in order to study how interactions are organized (Dáttilo et al., 2014; Blüthgen et al., 2015; Del-Claro et al., 2016). Several studies have been using this method for either community networks (Lange et al., 2013; Maruyama et al., 2014, 2015) or individual-based ones (Baker-Méio & Marquis, 2012; Gomez & Perfectti, 2012; Dáttilo et al., 2014). This analysis provides a great number of metrics and, for our aim, we selected six: complementary specialization ($H2'$), species specialization (d'), degree (k),



Fig 1. A) A view of *Tocoyena formosa*. B) Inflorescence with some active pericarpial nectaries (PNs). C) *Camponotus* sp. foraging on a recently available PN and D) *Camponotus* sp. foraging in a PN with a growing fruit.

closeness centrality (CC) and betweenness centrality (BC). H_2' is an index that measures network the specialization for quantitative matrices ranging from 0 to 1; 0 means no

specialization and 1 depicts a perfect specialization (Blüthgen et al., 2006; Dormann et al., 2009). Species specialization (d') is characterized by the sum of links per species, which is the richness of ants that interact with an individual plant. Degree (k) shows each species specialization based on a randomly selected partners discrimination. Closeness centrality (CC) describes the centrality of a species in the network by counting the number of links to other nodes. It indicates that the higher the closeness, the biggest the effect to other nodes would be; and, at least, betweenness centrality (BC) that describes the centrality of the species in a network analysis. It also takes as a reference the position of a species in relation to other nodes, as well as the importance of this node as a connector to other parts of the network. (Dormann, 2011). Our matrix has each individual of *T. formosa* in the rows, while ant species are placed at the columns, with cells expressing the abundance of each ant species. We tested all these indices in a general linear model (GLM), having the number of PNs acting as a predictor variable to check if they are a good predictor to organizing ant-plant networks. We conducted a null model with 1000 randomizations using the function "oecosimu" from the package Vegan (Oksanen et al., 2007) in order to investigate if the pattern of the network and its metrics were not randomly obtained. We used the method r0 to maintain the *T. formosa* frequencies (row) and to fill presences anywhere on the row disregarding the ant species frequencies (column) (Oksanen et al., 2007).

Results

We found a total of 818 PNs (Min = 2; Max = 86; Mean \pm SD = 22.72 ± 23.11) in the 36 individuals of *T. formosa* and 610 ants distributed in four sub-families, from 13 species (Table 1). The rarefaction curve showed a degree of stabilization, which supports that most ant species that interact with *T. formosa* were sampled, indicating a strong robustness of the network (Nielsen and Bascompte, 2007) (Fig 2).

The simple linear regression between PNs and the ant richness was statistically significant ($R^2 = 0.11$; $F_{(1,34)} = 5.19$; $b = 10,857$; $p = 0.03$), showing that the increased resource is accompanied by an increased number of links in the analyzed network (diversity). In the network analysis, we have a high specialization ($H_2' = 0.799$), without compartment. With the GLMs, we found a negative relationship of PN abundance with the specialization (d') ($R^2 = 0.08$; $F_{1,34} = 4.14$; $b = -7.61$; $p = 0.05$) and a positive relationship between the network's betweenness ($R^2 = 0.09$; $F_{1,34} = 4.41$; $b = 123.99$; $p = 0.04$) and closeness ($R^2 = 0.39$; $F_{1,34} = 23.45$; $b = 3473.48$; $p < 0.01$) (Fig 3). The relationship between species degree (k) and number of PNs was not significant ($R^2 = -0.03$; $F_{1,34} = 0.02$; $b = -2.39$; $p = 0.88$). The randomizations showed that the model did not represent a random organization ($p < 0.01$) which supports the non-random pattern of mutualistic networks found in other studies.

Table 1. List of ant species found in *Tocoyena formosa* plants with the total number of observed individuals.

Sub-family	Species	Abundance
Formicinae	<i>Brachymyrmex</i> sp1	63
	<i>Camponotus blandus</i>	113
	<i>Camponotus senex</i>	203
Myrmicinae	<i>Cephalotes atratus</i>	3
	<i>Cephalotes pusillus</i>	4
	<i>Cephalotes</i> sp1	8
	<i>Crematogaster</i> sp1	27
Ectatomminae	<i>Ectatomma brunneum</i>	120
	<i>Ectatomma</i> sp1	12
Pseudomyrmecinae	<i>Pseudomyrmex</i> sp1	13
	<i>Pseudomyrmex</i> sp2	14
	<i>Pseudomyrmex</i> sp3	6
	<i>Pseudomyrmex gracilis</i>	24

Discussion

The main hypothesis of our study was confirmed, indicating that PNs are indeed capable of influencing an individual-based ant-plant mutualistic network. We found a positive relationship between PNs abundance and ant richness. This result agrees with Lange et al. (2017), where they find a positive relation between the amount of nectar produced and ant richness in many plants with EFNs in a Cerrado area. Lange et al. (2013) also found that a greater availability of EFN may increase the co-occurrence of ants in plants. This may occur as interactions of ant-plant with EFNs are mostly opportunistic and ants rarely monopolize the nectar produced by these plants (Floren & Linsenmair, 2000). For example, Blüthgen et al. (2000), studied ant-plant interactions in Amazonian forest, found a co-occurrence of different ant species in plants having EFNs, which was not the case for plants with aphids. They argue that EFNs are visited by both aggressive and non-aggressive ants, with more than one foraging species on the same plant. This occurs because the nectar is not continually produced throughout the year, thus, it may be difficult for ants to be exclusive users of a single plant species having EFNs, as plants are periodically generating and interrupting the nectar production. Conversely, honeydew is a constant resource, allowing the “loyalty” of the ants. On the other hand, the PNs are located in the fruits, the most distal parts of the plants (Del-Claro et al., 2013a; Alves-Silva et al., 2015). This might provide a sufficient spatial segregation among ants, which can minimize the encounters between ants and, consequently, the conflicts. Nectar quality may be influencing the results if we consider a trade-off between protection and ant exclusion, happening only if the reward (EFNs), such as the energy provided by nectar, is high enough (Blüthgen et al., 2000; Davies et al., 2012). All these factors may be contributing to the pattern found in *T. formosa* about the ant richness correlated with PNs.

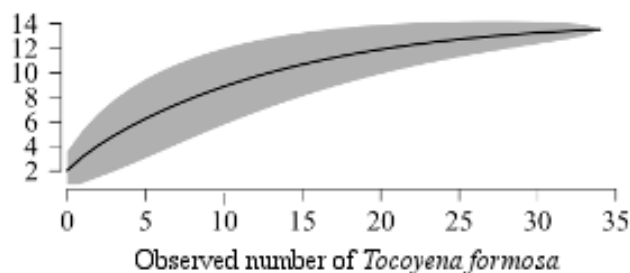


Fig 2. A rarefaction curve with each individual of *T. formosa* as a sample in the x axis and the number of ant species in the y axis.

Unlikely our findings, Dáttilo et al. (2014) did not find a clear correlation between ant richness and EFNs, even when analyzing three plant-bearing EFN species. They indicated two alternative not mutually exclusive hypotheses, though: 1) that EFNs has been monopolized by few dominant ant species that have competitively excluded other ant species or, 2) that the tree height in Cerrado are relatively low, which eases the resource domination by few ant species. However, the second justifications presented by them is not extrapolated to our system, because *T. formosa* is a shrub, according to the arguments used by them, and yet we found this relation. Our results are in accordance with other studies (Blüthgen et al., 2000; Lange et al., 2013; Lange et al., 201), indicating that an increase in EFNs abundance is followed by an expansion of the ants richness, having an association with the resource type and maybe with ant aggressiveness (Blüthgen et al., 2000). Because of that, we consider that future works should take into account the ant aggressiveness factor.

The absence of modulation in the network is commonly found in facultative mutualistic networks, which suggests a homogeneous and cohesive network (Díaz-Castelazo et al., 2013; Dáttilo et al., 2014). In our system, the non-modularity may be explained by the short period that PNs are active during a year, preventing the emergence of restrictive relations with some species of ants. It also occurs because ants do not usually show foraging fidelity in the same group of plant individuals (Dáttilo et al., 2014), which is indicated by the low specialization that we found in *T. formosa* individuals with high numbers of PNs. These two results (modularity and specialization) are complementary and reinforce the idea of seasonality acting on the organization of these interactions. PNs are associated with betweenness and closeness (centrality metrics), as indicated in the results of the present study. Centrality is a metric related to the plant fitness in pollinator-plant interactions and indirectly to ant-plant interaction, as shown by Gomez and Perfectti (2012), which found that centrality is positively related to the number and richness of visitant pollinators in individual-based networks. According to Santos and Del Claro (2001), the main function of PNs in *T. formosa* is to protect their fruits, meaning that when fruit herbivory is reduced, *T. formosa* produces larger fruits with more seeds. In this way, we may admit that for *T. formosa*,

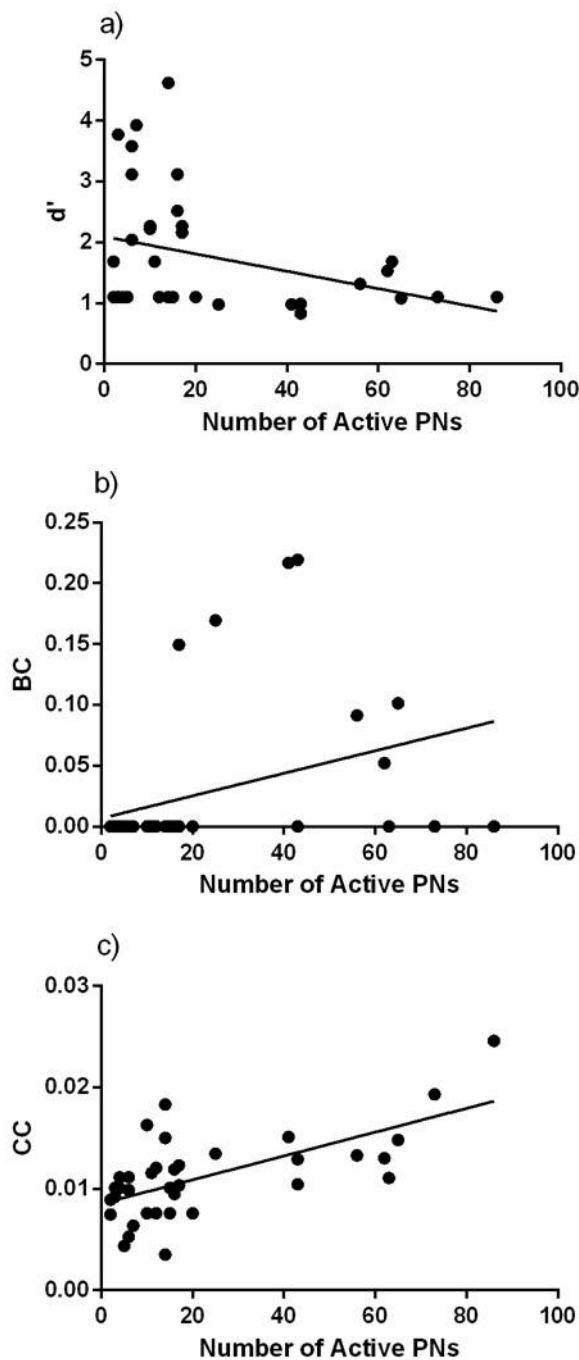


Fig 3. Linear regression between PN's and A) specialization (d'); B) betweenness and; C) closeness.

centrality and PNs may also be studied as a fitness measure, since individuals with higher values of centrality were correlated with the increase in PNs. We also found a negative relationship between PNs and individual specialization, indicating that the increasing PNs may also expand the ant richness in this plant-model. This pattern is supported by the literature, where betweenness is associated with generalist species. Martín González et al. (2010) found in pollination networks that high generalist and central species can act as keystone in a network, interacting with most other species and connecting subgroups.

On the present study, we state that mutualistic interactions between ant community and *T. formosa* plants having pericarpal nectaries (PNs) can be strongly influenced by the abundance of resource. We believe that this pattern might be preserved at other plants of the Rubiaceae family, which also have PNs.

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